M. Lanzetta · J.M. Dubernard *Eds.*

Hand Transplantation

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"to my wife Mariagrazia and
my sons Matteo, Michael and Pietro,
for sharing all my dreams,
and
to Valter, Gianni, Domenico, heroic patients,
for their trust in me and my trust in them"

Marco Lanzetta

"to my grand children
Louis, Hugues, Alexis, Émilie, Céline, Elsa ...
dreams might come true"

Jean-Michel Dubernard

Preface

The story of this book is quite simple: after the first few hand transplants, we felt the need to have regular meetings so that our clinical experiences would be beneficially shared and serve as a basis to draw some guidelines for the future. In so doing, we soon realised that a huge amount of original clinical data and information was becoming available as we were proceeding with our operations and closely following our patients' progress and functional outcomes. Some of these data were reported in scientific articles, but to group them in a comprehensive book seemed the best way to provide a complete review of this pioneering work.

Since starting our hand transplantation programmes, we often felt we were exploring a new area of surgery without the possibility or comfort of referring to already published work to support our own decisions. Indeed, some aspects of hand transplantation required prompt decisions based solely on personal interpretation of presenting clinical scenarios. New techniques were introduced to address different needs, such as the analysis of brain remodeling using functional magnetic resonance imaging or the use of a sensory glove to precondition the patient or accelerate the recovery of sensibility. Original work included creation of a specific consent form for patients, psychological tests for candidates and a comprehensive scoring system for assessing clinical outcome. There was the need to establish a grading system to evaluate acute skin rejection and clear criteria to select ideal candidates. From a legal point of view, there were no criteria for dealing with persons carrying two different sets of fingerprints. From an insurance perspective, there were no criteria as to how the disability of these patients would be revaluated after the hand transplant.

Clinically, the most challenging issues have been identifying the best immunosuppressive drug regime and understanding how acute rejection develops and how to reverse it. Use of an additional distant skin island allograft resulted in better rejection monitoring, at the same time avoiding the need to take multiple biopsies from the hand. Nerve regrowth and excellent sensibility/motor recovery was one of the most important results in hand transplantation, and leading experts in peripheral nerve regeneration suggest a scientific explanation. Rehabilitation of the transplanted hand has been very demanding for both therapists and patients, and specific protocols had to be implemented.

As the hand is only one of the composite tissues currently transplanted in order to correct disabilities or deformities, we include chapters on all the other types of non-life-saving allografts, including the face, knee joint, uterus, abdominal wall and larynx.

We are honoured to have such a great number of internationally renowned personalities share their experience and knowledge in this book; their contributions have made it the most complete work available on hand and other composite tissue allografts. We hope it will serve as a useful guide for those desiring to launch their own composite tissue transplantation programmes or to those who simply wish to read about the current state of the art of this new and exciting field.

Marco Lanzetta
Jean-Michel Dubernard

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1. Historical Remarks

Section 1-a

Cosmas and Damian Revisited

Bruce W. Conolly, Mario Benanzio

Introduction

Transplantation – that is, the transfer of tissue from one location to another – as a surgical procedure dates as far back into human history as cave paintings. As long ago as 8000 BC, domestic animals were castrated, initiating the physical juxtaposition of donor and recipient necessary for transplantation. The oldest surgery recorded in humans from the prehistoric archaeological records from the Bronze Age showed skulls from this time being subject to trephination: To relieve intracranial pressure, a circular disc of bone was removed from the calvaria and later replaced as an orthoptic autograft.

Myths and legends from Egypt, China, India and early Christendom illustrate transplantation, the oldest record being 2000 BC in Egypt. One Hindu text (700 BC) explained the procedure of nasal reconstruction in which, after a man's nose had been cut off or destroyed, the doctor took a leaf of a plant the size of the destroyed parts, measured a piece of the cheek of the same size, and replaced the nasal defect. On this surgical wound would be placed powder of sapanwood, liquorice root and barberry covered with cotton. As soon as the skin had grown together with the nose, the connection with the cheek was cut.

From the earliest times, medical practitioners have sought divine help in their healing endeavours. In the early Christian era, there were four patron saints of medicine, all from Asia Minor and all educated in the Greek medical tradition. They were the apostle Luke, Saints Cosmas and Damian and Saint Panteleimon. Through the centuries, they have served as worthy role models for the physicians who have invoked their aid [1].

At the dawn of the Christian era, there was the popular custom of "incubation" [2]. Sick people would visit the temples of Aesculapius to pray that the gods would heal them as they slumbered. The temple attendants would apply oil and wax to the afflicted body parts and perform surgery if necessary. This was the background of the third-century "miracle of the black leg" (Fig. 1). In the third century AD, St. Cosmas, a physician, and St. Damian, a surgeon, removed the malignant and gangrenous limb of an aged sacristan of the church. While the sacristan slept, these two doctors successfully transplanted the leg of a recently deceased Ethiopian Moor to the leg stump of the dreaming patient [3]. Whilst one brother removed the diseased leg with a saw, the other went to the pagan gladiator graveyard on Vatican Hill at the Circus of Nero where St. Peter's Cathedral now stands, exhumed the body of a recently buried Ethiopian man, procured one of his legs and returned to the church. The saints joined the Ethiopian's leg to the dying man's stump. The sacristan, on waking, discovered he had a new, healthy, although black, leg. The sacristan who lost his leg found this old leg later in the Moor's grave. Although it is unlikely that the Saints Cosmas and Damian legend was derived from historical fact, it is clear that exper-

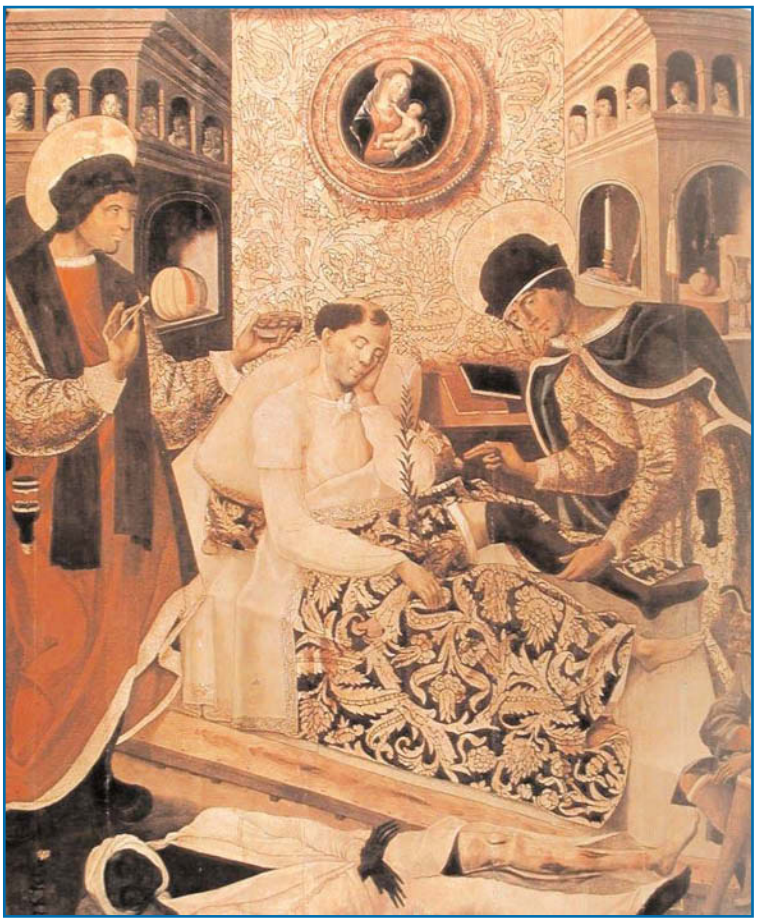

Fig. 1. Saints Cosmas and Damian, the martyred twin physicians, shown in a sixteenth-century painting attributed to Fernando del Rincon performing their most famous posthumous miracle: the replacement of a sacristan's gangrenous leg with that of a dead Negro. The Prado, Madrid

imentation with various types of transplants did persist in following centuries. There are archaeological records from Egypt, North and South America, Greece, Rome and China showing teeth transplants as early as 1000 AD.

Fifteenth to Nineteenth Centuries

Skin graft prognosis and techniques increased considerably during the fifteenth century. A popular hero of transplantation is Gaspare Tagliacozzi, a sixteenth-century surgeon who restored noses. He is a symbol of the glorious Renaissance after the darkness of the Middle Ages. The Italian poet Calenzio wrote that some slaves donated their own noses to their masters. In the eighteenth century, John Hunter [4], Scottish anatomist and surgeon (founder of experimental surgery) and the so-called father of British surgery, reported effective allografts of chicken testes and Achilles tendon autografts in other species. In 1804, Baronia performed free tendon allografts between sheep, and by 1880, stable corneal transplants in both humans and animals were recorded. By the nineteenth century, free grafts were documented of the following tissues: skin, tendons, nerves, cartilage and corneas.

Twentieth Century

Substitution of a healthy organ for a sick or damaged one has always been the dream of surgeons. Such techniques need to unite the transplant with the patient's system of blood vessels. This obstacle was overcome by French surgeon Alexis Carrel [5] who, in 1902, introduced his vascular suture method, but the initial wave of enthusiasm ran into a basic problem: the guest organ would be rejected by the host organism. The body's immunity defence mechanism react-

ed against such foreign cells and killed them. The efforts of Carrel and American surgeon Charles Guthrie is described in their work on the "transportation of veins and organs", and this served as a foundation of both vascular surgery and organ transplantation [6]. In 1905 at the University of Chicago, Carrel and Guthrie performed the first cardiac transplant in animals. The early theory regarding the mechanism of rejection was malnutrition of the grafting tissue, suggested by Paul Ehrlich in 1906 [7].

In the early 1960's cadaveric donations were thought to be impracticable and impossible and living donors were the only available source of organs for transplantation.

In 1910, Carrel noted that the physiological disturbances in transplanted organs were likely caused by biological factors. Soon after, the Viennese pathologist K. Landsteiner discovered the ABO blood grouping system that eventually led to the introduction of clinical blood transfusion. Sir Peter Medawar, in World War II, transplanted skin on badly burnt soldiers in London [8]. He understood that rejection was not due only to surgical and technical mistakes but mostly to the immune response. Without biological compatibility between the donor and the host, the transplanted organ would undergo failure. He was awarded the Nobel Prize for his pioneering work.

In 1914, the fact that lymphocytes infiltrated grafts was recognised, but it was many years before the molecular basis of T-lymphocyte activation was known as the cause of acute rejection. This knowledge of immunology led to the first successful kidney transplant between identical twins in 1954, and this initial work in the field began with the recognition that organ allografts may be transplantable. In this vein, skin grafts performed by Medawar during World War II for burn victims were successful only when performed between identical twins.

Twentieth-Century History of Organ Transplantation in Humans

In 1954, the first living related kidney transplant was performed by Joseph Murray between identical twins in Boston [9]. Over the next 25 years, discoveries in immunosuppression paved the way for a 10% manipulation of the recipient's immune system. The first successful cadaveric diseased kidney transplant was performed in 1962; in 1963, the first successful lung transplant was performed; also in 1963, the first successful liver transplant was performed by Thomas Starzl on a 3-year-old child in Denver, USA; and in 1967, the first successful heart transplant was performed. Following its discovery by Jean-François Borel in 1972 [10], cyclosporine was introduced to prevent rejection of transplanted organs by suppressing the body's immune system, which increased survival rates of transplanted organs.

History of Hand Transplantations

In 1964, a hand transplant was attempted in South America with primitive immunosuppressive agents. The transplant was rejected at 2 weeks. On 23 September 1998, the first hand transplant with early success was performed on a New Zealand patient in Lyon, France. However, the hand was later amputated in the United Kingdom in February 2001 at the patient's request. It was reported that the patient failed to follow the correct antirejection treatment and physiotherapy. In January 1999, the Louisville hand and microsurgery team in the USA carried out their first hand transplant. Their patient continues to do well, gaining strength, control and range of movement of his new left hand. In September 1999, two single hand transplants were reported from China, and since then there have been many more around the world.

Now Back to Saints Cosmas and Damian

Who were Cosmas and Damian, and why are they relevant to the history of hand transplantation? Cosmas and Damian are regarded as the most famous of all medical saints. They were Arabian twin brothers, the first children born in

a family of 7 boys in Cilicia, Turkey. Like their parents, they were strongly committed to the Christian faith. They were convinced that the Holy Spirit called them to study medicine. They practiced not only in the city of Aegea, now Ayash (Ajass), on the Gulf of Iskanderun in Cilicia, Asia Minor, where they attained a great reputation, but also travelled widely, tending not only to suffering humanity they encountered along the way but also to beasts of burden. They devoted themselves to healing rich and poor alike, accepting no payment for their medical services, thus earning their title of *anargyroi*, the "Silverless Ones" [11]. Their example established four tenets of proper conduct for the physician: to neglect no one suffering infirmity, to apply suitable treatment, to inflict no harm and not to demand excessive fees.

Artists portray them as youthful, beardless and wearing long, fur-trimmed robes and red caps (Fig. 2). They hold medicine boxes, urine glasses, mortars, salve spatulas and other items used by contemporary physicians living in the time of these artists. Although pictures of the saint healers representing Cosmas and Damian can be found in many churches, the oldest picture is said to be in the church situated in Densus. This picture is important because one of the saints holds a small portable medical pouch in one hand that seems to be made of wood, and in the other, he holds a spoon used to administer the medicine of the time, usually pills or bolus. Many saint healers have been painted in churches but they do not have such utensils.

So popular were the brothers that in antiquity, numerous Christian doctors took the names Cosmas or Damian. During the Crusades, a group of knights formed the Cosmas and Damian Order to assist ill pilgrims and to exchange prisoners of war. Among the cities selecting the brothers as patron saints are Florence, Prague, Salamanca and Essen, as well as the country of Bohemia. They became the patron saints of physicians, surgeons, barbers,

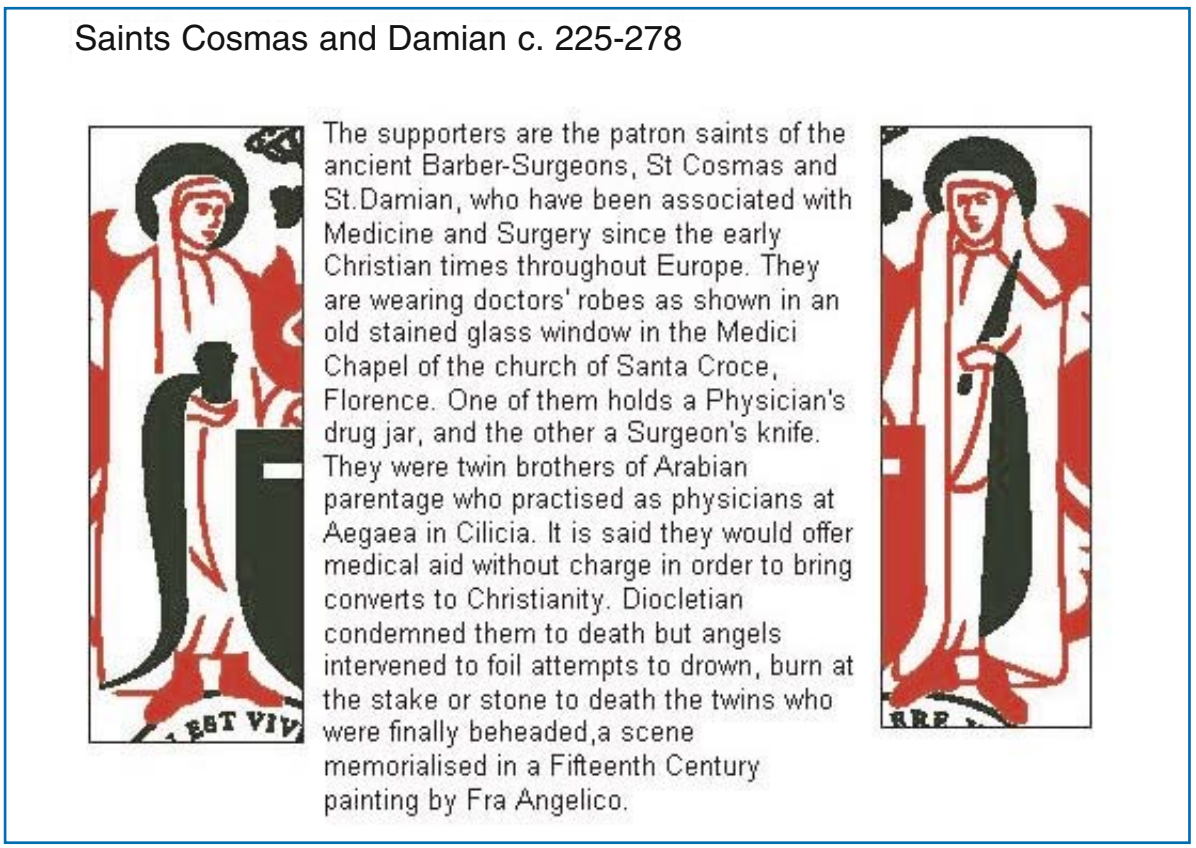

Fig. 2. Saints Cosmas and Damian from the Royal Society of Medicine Coat of Arms UK. From [3], used with permission

physicists, bakers and apothecaries, and representations of them are found in the coats of arms of medical bodies to the present day. Their numerous healing successes were regarded as miracles, and their example inspired many to embrace Christianity. The emperors Diocletian and Maximian were so concerned about the influence Cosmas and Damian were having against the ideas of the Roman Empire that when the saints refused to give up their faith, they were arrested by Lisia, the governor of Aegea, and put on trial in the court of Caesar and sentenced to death by three tortures. The first torture was being cast into the sea with their hands and feet bound. A miracle occurred as they became free, enabling them to swim to shore. The second torture was burning at the stake, but a second miracle occurred as the flames failed to burn them. The third torture was by flogging but the whips would not hit their mark. After a final demand that they renounce their Christian faith was refused, Cosmas and Damian were decapitated by the sword on 27 September, 287. Their death is depicted in the famous angelic panel in the monastery of St. Mark in Florence (Fig. 3). The holy doctors were buried in a magnificent tomb in Ciro, Syria, the home of Bishop Teodoreto, their first biographer, who eulogised them in the famous *Heroes and Glorious Martyrs* [12].

After the deaths of Cosmas and Damian, there was a continuous procession of pilgrims and sick people to visit their grave to petition them to intercede with God to heal their afflictions [13]. When Emperor Justinian (527–565) was healed of his grave and debilitating disease, he had an exquisite basilica erected at the grave site in Constantinople, and he fortified the entire city [14]. Pope Felix IV (526–530) established a church dedicated to Cosmas and Damian in the Roman Forum built by Vespasius in Constantinople [15]. It was

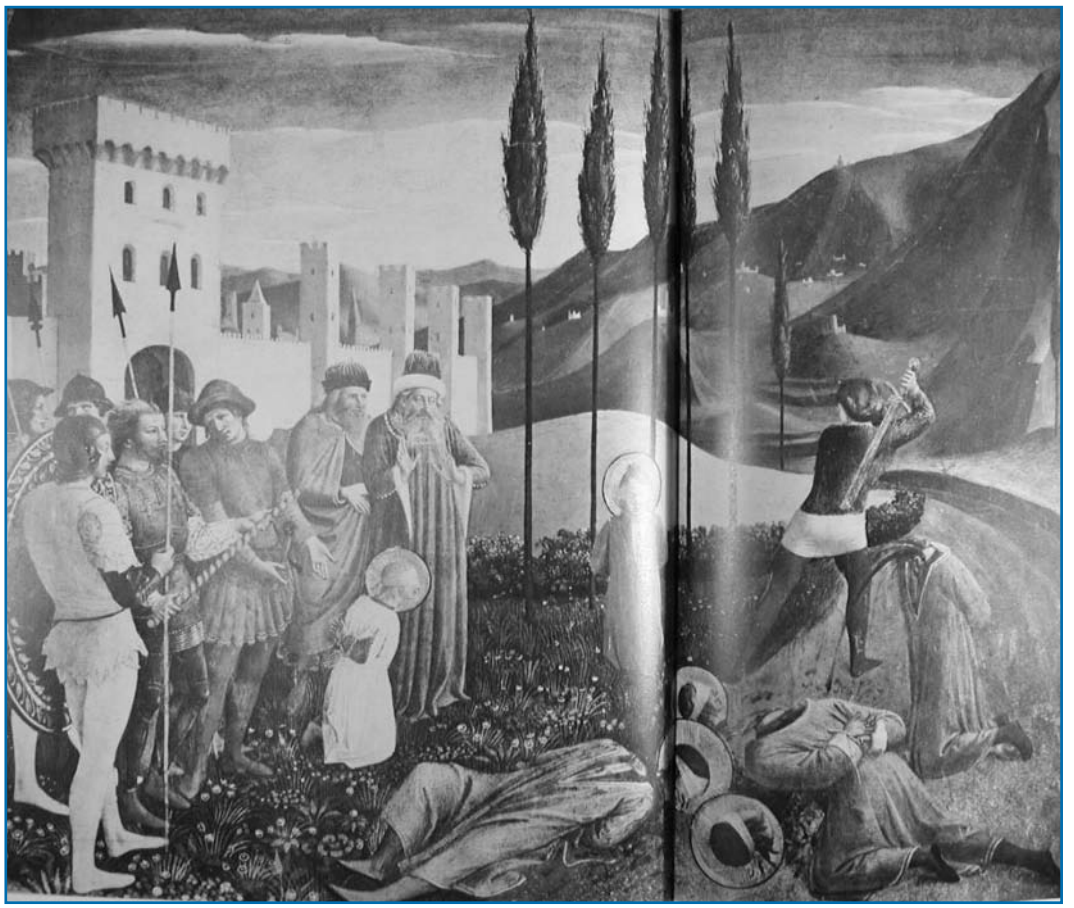

Fig. 3. The martyrdom of the sainted physicians Cosmas and Damian, as envisioned by Fra Angelico in a painting from the predella of the San Marco altarpiece (c. 1438-1440). The Louvre, Paris

here that Galen himself taught and wrote his commentaries on the Hippocratic text in 169 AD. The aps of the new basilica was decorated with mosaics that are now considered to be one of the most unique and best-preserved examples of Byzantine art. The inscription reads: "To the medical martyrs the hope for the salvation of the people". An eighth-century fresco in Santa Maria Antiqua in Rome also highlights Saints Cosmas and Damian. Hans Suss von Kulmbach, a late Middle Ages artist (1480–1522), depicts them as the patrons of doctors and pharmacists on two altar wings in Nuremberg, Germany. Fra Angelico (c. 1401–1455) painted their crucifixion as well as the famous leg transplant scene (Fig. 4). They also are shown as helpers during the plague in a painting by Titian in Santa Maria della Salute, Venice (Fig. 5). In Essen, woodcarvings of Saints Cosmas and Damian show them holding salve boxes and swords.

Cosmas and Damian are the patrons of the city of Gaeta in Italy, and it is believed, through their intercession, the city's population was spared during the eighteenth-century plague. Cosmas and Damian were also very important to the Medici family – they became the family protectors. They were more than personal symbols of the Medici because they fulfilled the purpose that all saints do for Catholics, serving as spiritual mediators or, in effect, representatives of the family in heaven. The Medici were concerned with exposing their souls to as many prayers as possible, and they had built the Medici chapel of Cosmas and Damian in San Lorenzo, Florence.

A close connection between religion and medicine existed amongst primitive peoples in early civilisations. With the spread of Christianity, medicine became the concern of the priestly cast, and one of the first principles of Christianity was the healing of the sick [1]. Investigation into natural causes of the diseased was discouraged. Treatment at the time consisted of being quiet and restful in a peaceful atmosphere, with intercession and prayer and the cult of healing saints. Churches and shrines dedicated to certain saints and martyrs became places of pilgrimage. A patron saint was regarded as having the power to relieve afflictions of a particular organ or part of the body. St. Agatha was concerned with diseases of the breast, Saints Sebastian and Roch were the saints of the

Fig. 4. Saints Cosmas and Damian, seen caring for an amputee in this late sixteenth-century painting by Ambrosius Francken the Elder, had numerous miraculous cures attributed to them and later became patron saints of the healing professions. Koninklijk Museum voor Schoen Kunsten, Antwerp

Fig. 5. Fra Angelico, in the mid-fifteenth century, depicts another miraculous cure by the martyred twin physicians in "The Healing of Palladia by Saints Cosmas and Damian". National Gallery of Art, Washington DC

plague, St. Blaze the patron saint of the throat, St. Apollonia the patron saint of toothache and Saints Cosmas and Damian the patron saints of barbers, surgeons and apothecaries. Later, the practice of surgery was forbidden to priests and therefore passed almost entirely into the hands of barbers and other uneducated men although there were always a few surgeons of high rank who attended to royalty and the nobility.

Cosmas and Damian, apart from being represented on the coats of arms of medical bodies, have hospitals, monasteries, schools and chapels dedicated to them around the world. The Society of Saints Cosmas & Damian is an Italian/American organisation formed in 1926 during a period of heavy immigration to America by many Europeans. A large group came from the beautiful coastal city of Gaeta in Italy and settled in Cambridge and Somerville in Massachusetts in the USA. These immigrants had a sense of community belonging, and a small group of women, encouraging this, met periodically to pray to these patron saints of their beloved city of Gaeta. Soon the group grew bigger and involved men, and it became a yearly festival to honour these saints in 1926. A chapel to house the life-size statues of the two saints, which had been brought from Gaeta, was built in 1940 in East Cambridge, and it underwent major renovations in 1995. The feast day of Cosmas and Damian is celebrated on 27 September.

Conclusions

The possibility of successful organ replacement has challenged men's minds through the ages: Greek Chimera, the Minotaur, the wings of Daedalus and Icarus, the dragon, the gryphon, the sphinx and the siren. The dream of the Ancients from time immemorial has been the junction of portions of different individuals, not only to counteract disease but also to combine

the potentials of different species. This desire inspired the birth of many mythical creatures, which were purported to have capabilities normally beyond the power of a single species. The modern world has inherited these dreams in the form of the sphinx, the mermaid, and the chimerical forms of many heraldic beasts [16]. The Hindu pantheon has become the object of surgical reinterpretation; the chimeric state of certain Asian gods, such as Brahma, is endowed with many arms as well as heads or faces.

It is unlikely that the legend of Cosmas and Damian derives from historical fact, but experimentation with the various types of transplants did persist in the following centuries. There is no way that the transplanted black leg could have survived when reattached to the aged sacristan. Cosmas and Damian would not have had the prerequisite vascular suture technique or immunosuppressive agents, but this legend is incorporated into transplantation medicine, if not as an actual precedent, at least as proof that the idea has existed for a long time. Transplantation existed first as a legend, but now surgery is about to make the dream come true with the advent of chemotherapy immunosuppression mercaptopurine (6-MP) in 1960, azathioprine by Joseph Murray in 1963 and cyclosporine A in 1976 [9].

Reference to Saints Cosmas and Damian even has a twentieth-century application. When a newspaper reporter asked if the Massachusetts General Hospital claimed priority for the restoration of a young boy's severed arm, the hospital spokesman referred the reporter to the Syrian surgical team of Saints Cosmas and Damian and the legend of the transplanted leg.

The dream of humankind is now being fulfilled [17]. Depicting themselves as the legitimate heirs of Saints Cosmas and Damian, fifteen centuries later surgeons of today can pursue the historiographical tradition of representing themselves as the new divine healers. Saints Cosmas and Damian are thus enrolled as allies.

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Section 1-b

Hand Transplantation as an Evolution of Microsurgery: A Logical Step Towards Better Reconstructive Options

Marco Lanzetta

Introduction

Microsurgery was developed in the twentieth century by combining techniques of vascular surgery with an operating microscope, fine instruments, microsuture and new operative strategy and techniques. The first vascular anastomosis was performed by J.B. Murphy in 1897, but Alexis Carrel [1] originated the method for triangulation of blood vessels to perform arterial and venous repairs in 1902. He performed an end-to-end anastomosis. It was in 1960 that Jules Jacobson [2], a vascular surgeon, described microsurgical anastomoses in vessels as small as 1.4 mm using an operating microscope.

A traumatic arm amputation was reattached surgically for the first time in 1962 when Malt and McKhann [3] described their experience with a ten-year-old boy, but microsurgical techniques were not used, as blood vessels were large enough to be repaired by conventional methods. In 1965, the first successful replantation of an amputated finger by microvascular technique was done by Komatsu and Tamai [4] in Japan. The first microsurgical transplantation of the great toe (big toe) to thumb was performed in April 1968 by Mr. John Cobbett [5] in England. In the 1970s, a number of surgeons, including Buncke [6], Daniel [7] and Taylor [8], opened the way to the routine use of free flaps to cover defects around the body.

Reconstructive Microsurgery Levels

Today's reconstructive microsurgery is a complex and more refined technique than the one that was employed only two decades ago. Until some time ago, microsurgery meant mainly covering a defect or replanting severed parts back where they belonged. Now, restoration of original function and aesthetic appearance are considered a must in planning any reconstructive procedure. Minimising morbidity at the donor site and selecting more sophisticated flaps for better matching with the tissues to be reconstructed is a mandatory choice. Lately, it is not unusual to take part in scientific panels or meetings about "aesthetic microsurgery". Hand transplantation, which can be included in the wider concept of composite tissue transplantation, represents one of the logical evolutions of microsurgery and seems both inevitable and scientifically justified by the initial clinical results.

Five different levels of reconstructive microsurgery can be identified, from easy to very difficult, depending on the complexity of the procedure, the reconstructive goal to be achieved and the provenience of the selected flaps. Fresh from the experimental lab, where he or she has been extensively training under the microscope to become accustomed with the likely clinical challenges, a young microsurgeon *in pectore* will be

eager to enter at least the entry level. Usually, he or she will be initially asked to deal with emergency situations needing revascularisation or replantation of body parts, so the job will be quite similar to that performed in the lab (Level 1). As the microsurgeon develops experience he or she will consider alternatives to replanting body parts back to where they originally were, as this strategy might not necessarily be ideal. In some cases, the amputated part needs to be altered, replanted in a different position or two or more parts combined to obtain a replantable unit (Level 2).

Moving to the next level will require more skills and anatomical knowledge, as flaps need to be harvested from a donor site and moved to a different body area with the intent of covering a defect or tissue loss caused by trauma or surgical removal of neoplastic lesions (Level 3). When just "covering a hole" is not enough but there is the necessity to reconstruct a specific function or restore the cosmesis of a body part as close as possible to the original appearance, then free flaps might require careful planning for selecting the most appropriate tissue(s) without leaving an unacceptable defect or unaesthetic scars. The concept of the body as a "bank" must be explored and the lost tissue/part replaced with the most similar and closest tissue/part available on the patient (Level 4). However, there is a limit beyond which it is not possible to achieve satisfactory restoration of function/appearance because the missing tissue has some unique characteristics that are not found anywhere else on the body. For instance, if the loss of a thumb can be successfully reconstructed by either using a simple flap, such as an osteocutaneous flap from the forearm, or in a much more functional way by transferring a toe, then the loss of the entire hand is not amendable with autologous tissue due to both its size and unique function. When facing these clinical situations, a microsurgeon is bound to admit defeat and accept the impossibility of using the usual strategy of thinking about the patient's body as a bank to carve out a vascularised free flap that can be moved across, restoring the initial situation. Some have been exploring the area of prefabrication or prelamination of flaps to assemble new and more versatile flaps according to needs, for example, when facing an ear or nose reconstruction, but we are aware that we are far away from perfection in this area. It is evident, therefore, that if a unique body part is needed but is either unavailable or impossible to be custom made, the most logical choice is to adopt the same strategy as that of a transplantation surgeon and consider harvesting the same part/composite tissue as an allograft from a donor (Level 5).

In Table 1, we offer a schematic representation of these five levels, showing their grade of difficulty, provenience and typical application (elective or trauma).

In the following section we present some clinical explanatory examples of the different levels of reconstructive microsurgery, from level 1 to level 5.

Table 1. Levels of reconstructive microsurgery

RECONSTRUCTIVE MICROSURGERY LEVELS			
	TYPE		COMPLEXITY
ORTHOTOPIC REPLANTATION	TRAUMA	OMOGRAFT	++
ETHEROTOPIC REPLANTATION	TRAUMA	OMOGRAFT	+++
COVERAGE FREE FLAP	TRAUMA ELECTIVE	OMOGRAFT	+
FUNCTIONAL FREE FLAP	TRAUMA ELECTIVE	OMOGRAFT	+++
TRANSPLANTATION FREE FLAP	ELECTIVE	ALLOGRAFT	++++

Level 1: Orthotopic Replantation

These procedures are performed in emergency situations; minor or major parts might need to be replanted according to the level of amputation (Figs. 1-3). They can be graded as medium complexity.

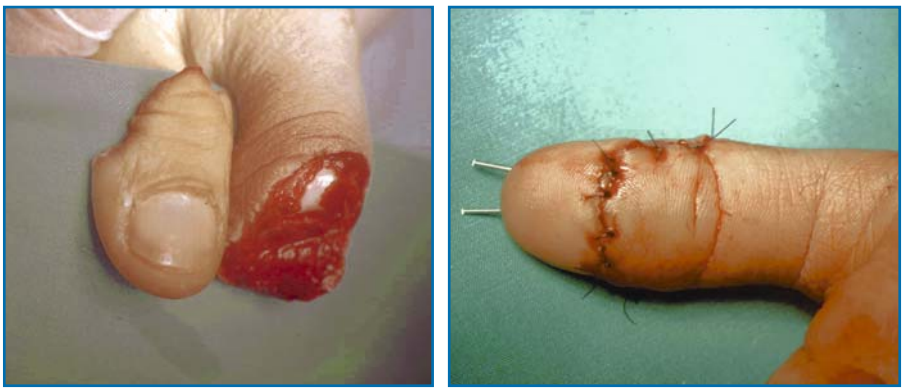

Fig. 1. Orthotopic replantation of a small peripheral part (thumb, distal phalanx)

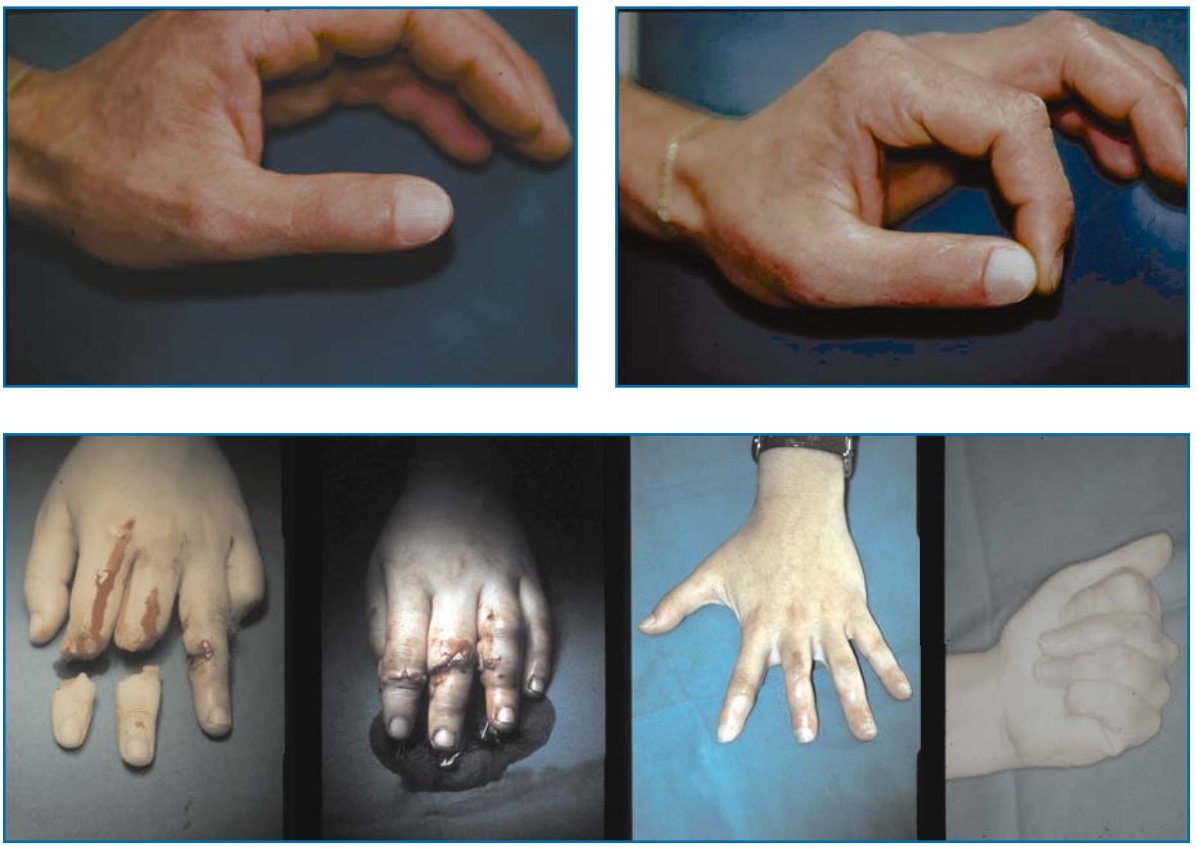

Fig. 2. Routine orthotopic replantation of two fingers and revascularisation of a third finger

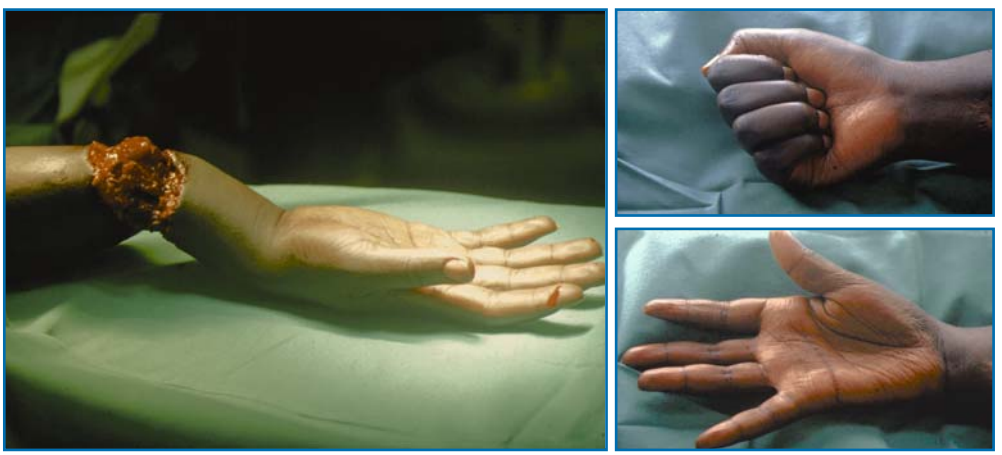

Fig. 3. Orthotopic replantation of a major body part (forearm)

Level 2: Heterotopic Replantation

These operations are carried out in emergency-only conditions, as those in Level 1, but present a higher degree of difficulty/complexity (Figs. 4, 5). Extra planning is necessary to achieve a satisfactory functional result and restore conditions close to those before the trauma.

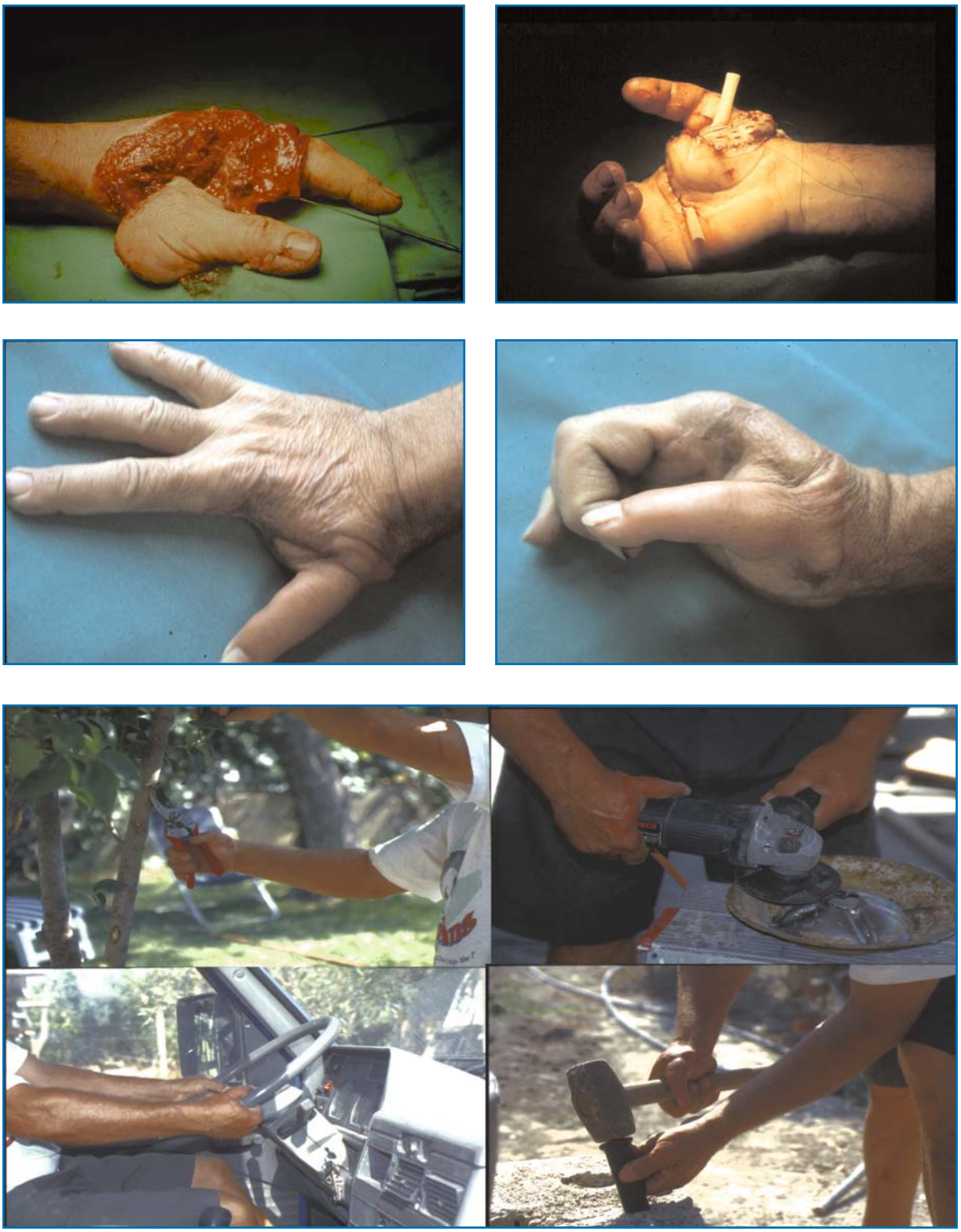

Fig. 4. Heterotopic replantation. The thumb cannot be replanted due to the traumatic avulsion. The index finger is replanted on the thumb position. The result is a functional hand, including pinch. This procedure can be called "replanted pollicisation"

Fig. 5. Bilateral amputation of the lower limbs in a 2-year-old boy. Combination of the two amputated parts into a replantable single lower limb. Restoration of deambulation using one combined replanted limb and one prosthetic limb

Level 3: Coverage Free Flap

From this level, microsurgical operations can be performed as an emergency procedure or, more likely, as an elective procedure (Fig. 6). Normally, all that is required is to reconstruct traumatic tissue loss (i.e. skin) or a surgical defect resulting from radical excision of a neoplastic (cancerous) area (Fig. 7). Several well-established flaps are available, and their use has become standard in the armamentarium of a competent microsurgeon.

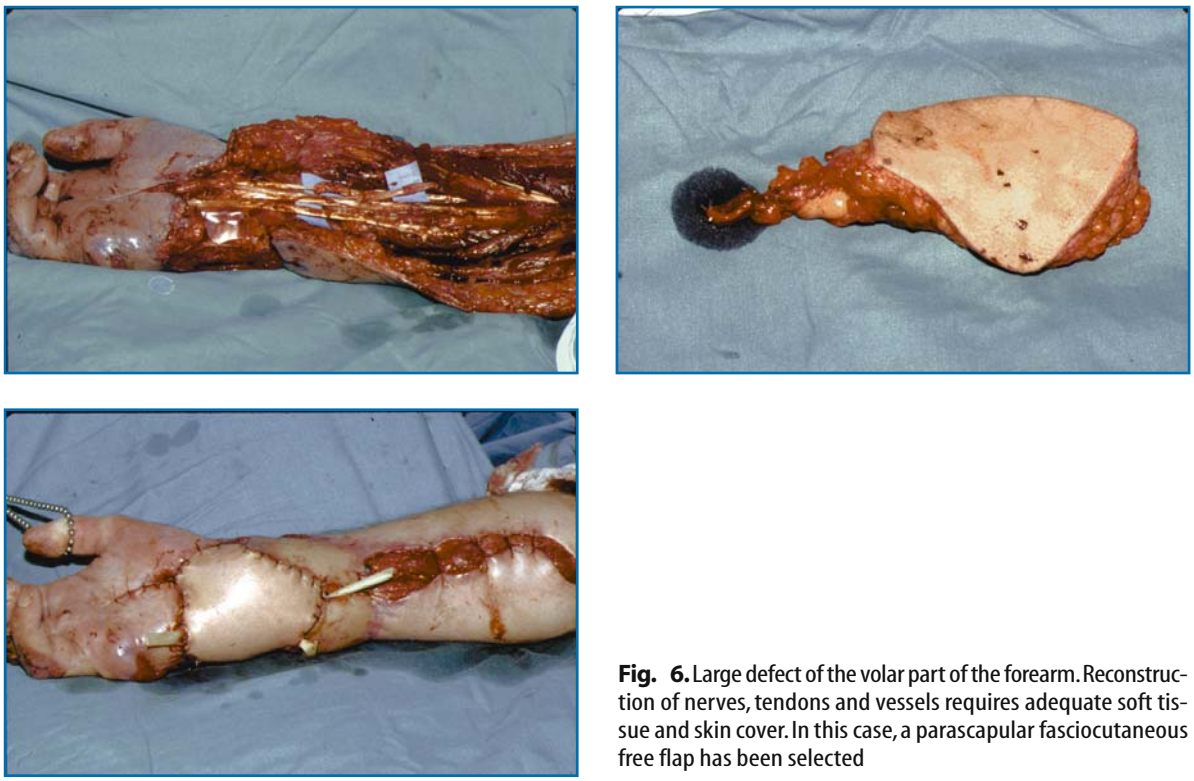

Fig. 6. Large defect of the volar part of the forearm. Reconstruction of nerves, tendons and vessels requires adequate soft tissue and skin cover. In this case, a parascapular fasciocutaneous free flap has been selected

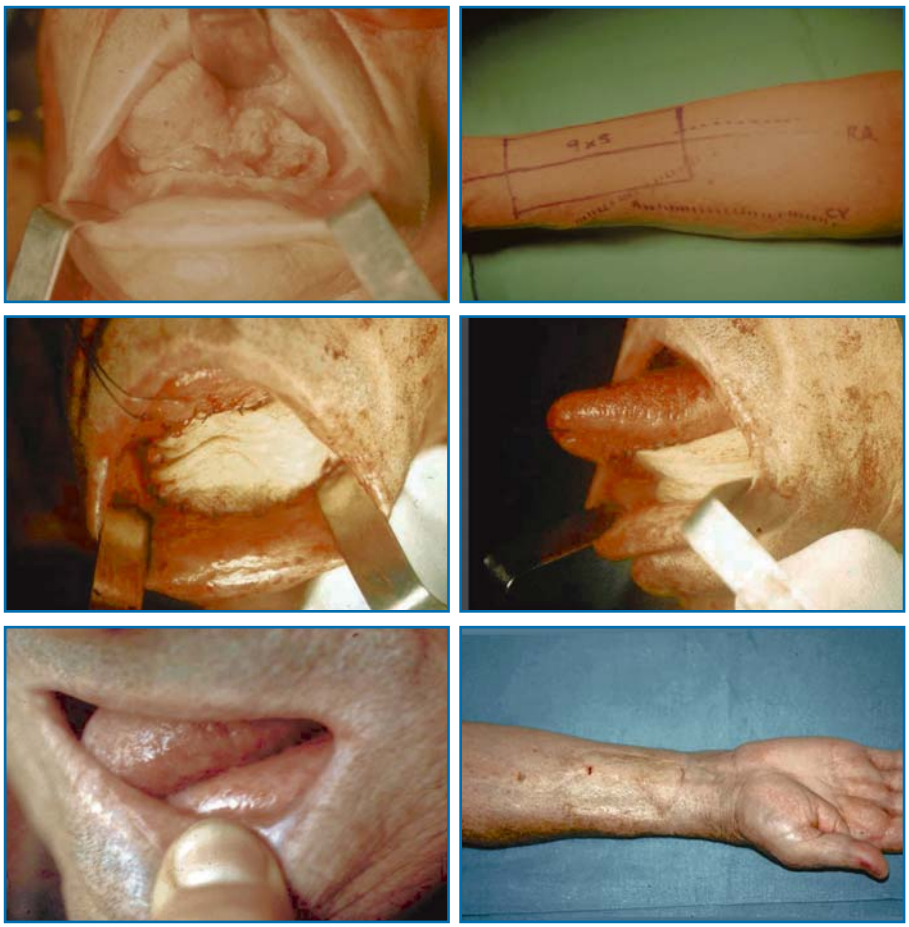

Fig. 7. Carcinoma of the oral mucosa. Radical excision and immediate reconstruction using a fasciocutaneous forearm free flap (Chinese flap). Continued contact with saliva allows for metaplastic change of the flap into a typical mouth floor lining. Donor site appearance is acceptable at 2 years follow-up

Level 4: Functional Free Flap

These microsurgical operations allow the restoration of a particular function that has been damaged or lost due to trauma or cancer (Figs. 8-13). These free flaps require a higher degree of planning as they are more complex. They must be selected and harvested according to specific functional needs. In specific situations, a flap can be prefabricated or prelaminated and subsequently harvested as a new composite tissue unit.

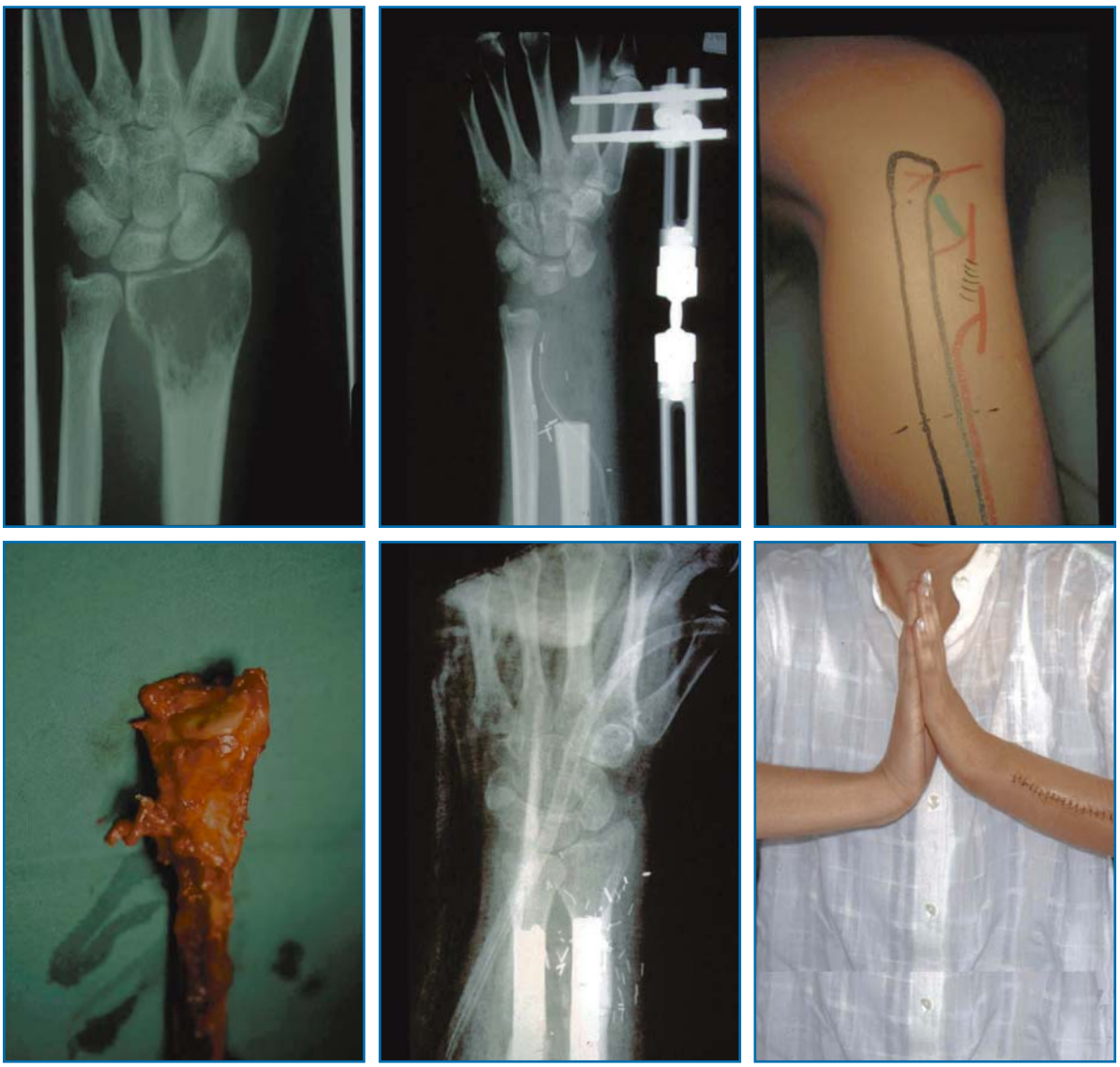

Fig. 8. Extensive giant cell tumour of the distal radius treated by excision, use of an external fixator device to maintain length while waiting for the pathology assessment and secondary reconstruction with a fibular bone flap, including the fibular head, which is similar to the distal radius. This is necessary to avoid wrist arthrodesis and conserve movement of flexion/extension at the wrist joint. A few days after the operation, the patient is already using her new joint

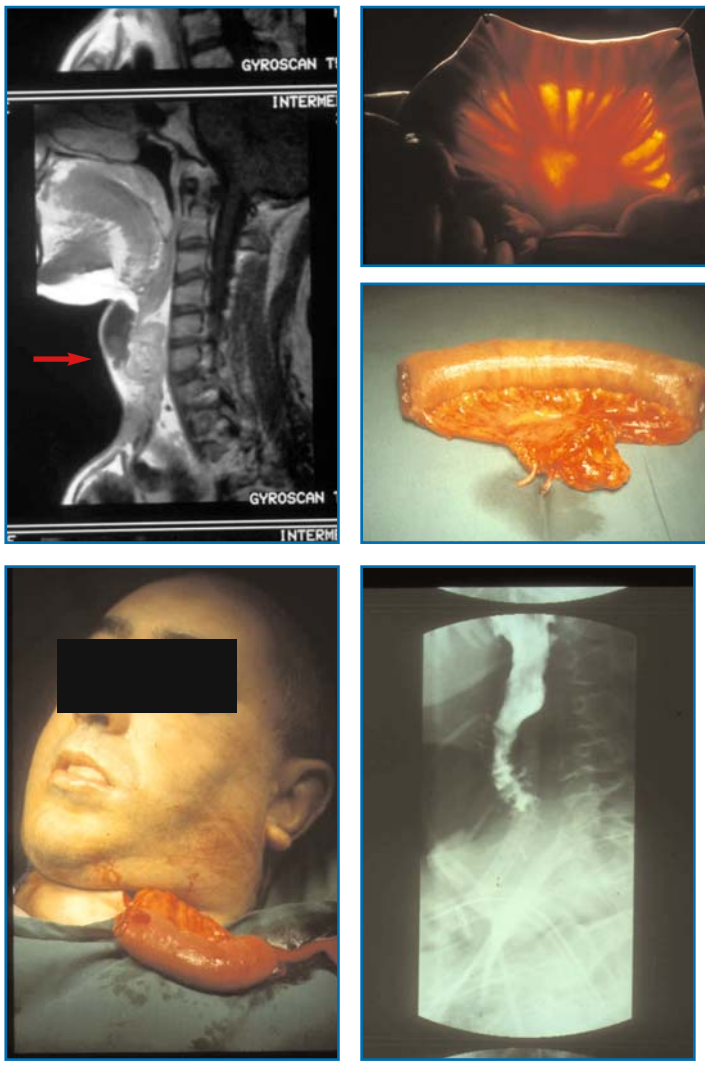

Fig. 9. Esophageal malignant tumor (*arrow*) requiring resection of the esophagus from the oral cavity to the stomach. In order to avoid the need for a transabdominal feeding catheter, a segment of jejunum is transferred as a free flap to restore the alimentary tract. Part of the flap is left externally for better monitoring of its viability. At 2 weeks postoperatively, contrast shows a patent neoesophagus, the external part of the flap is removed and the patient is allowed to feed normally

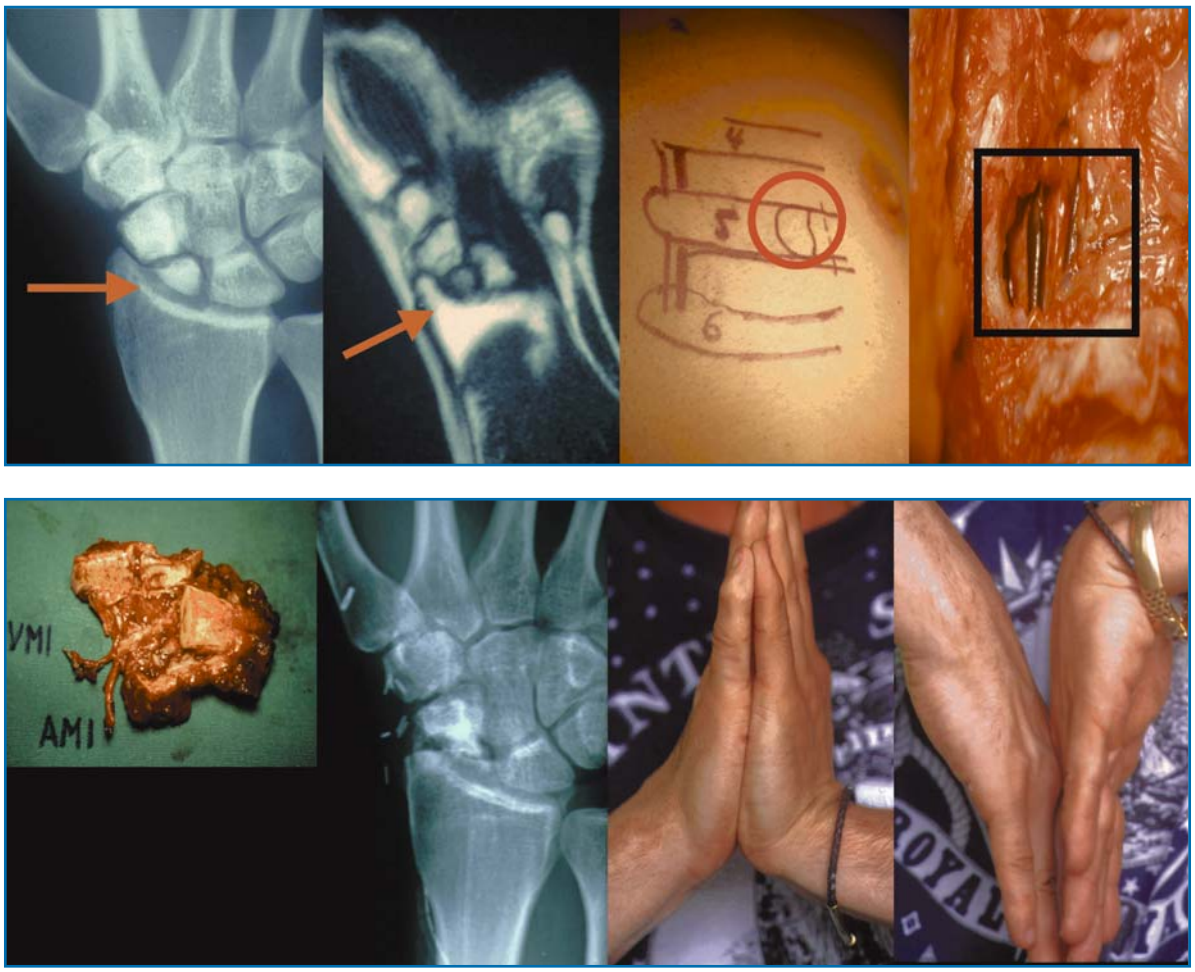

Fig. 10. Avascular necrosis of the proximal pole of the scaphoid following traumatic fracture and pseudoarthrosis (*arrows*). Free osteochondral flap from the fifth rib (circle) vascularised by the intercostal vessels (internal mammary artery and vein) (*rectangle*). This is a functional flap to restore wrist movement. *VMI*, internal mammary vein; *AMI*, internal mammary artery

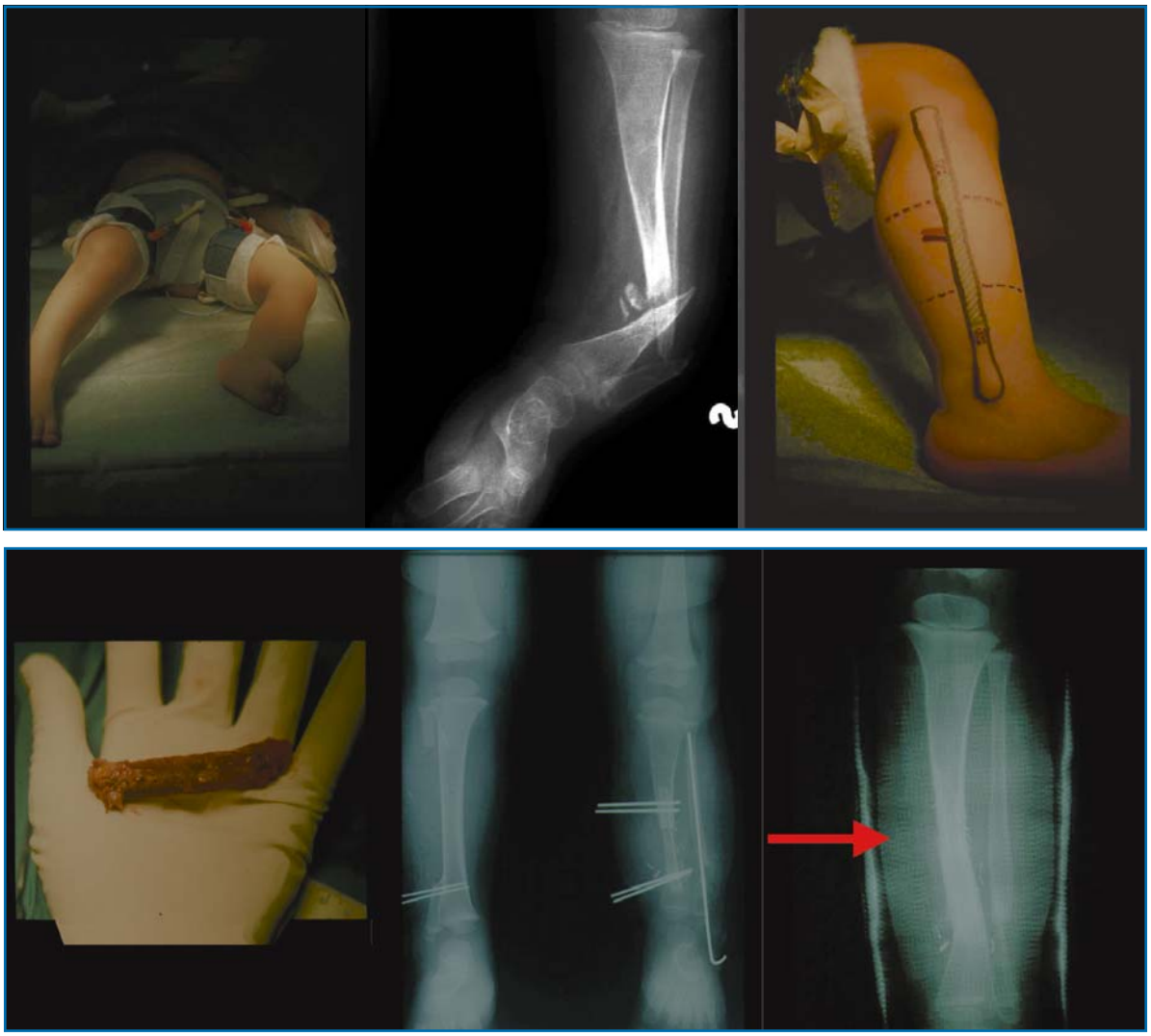

Fig. 11. Congenital pseudoarthrosis of the tibia. Reconstruction with a free fibular flap to allow walking. Note hypertrophy of the transferred bone at 2 years postoperatively (arrow), which acts now as a neotibia

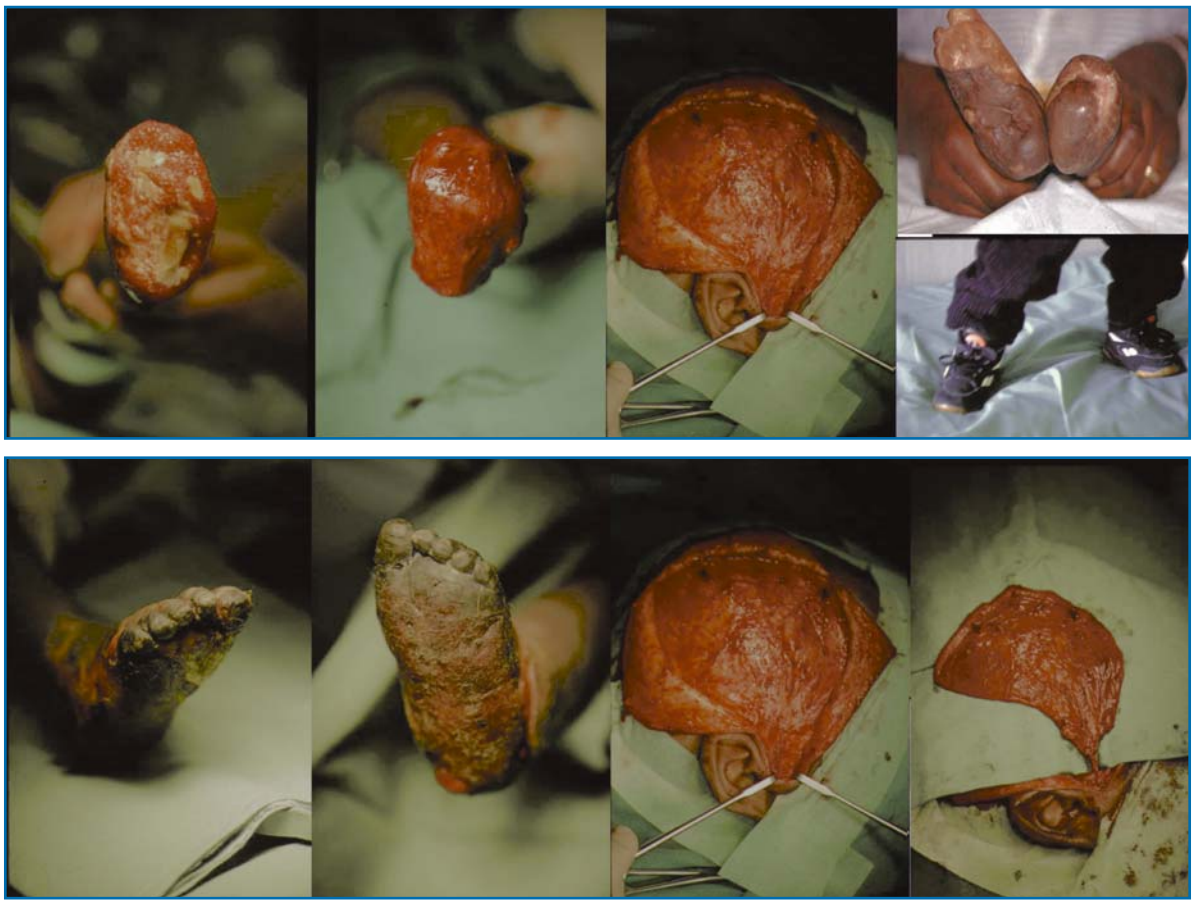

Fig. 12. Peripheral bilateral foot necrosis in an 18-month-old boy due to drepanocytosis. Temporal fascia free flap to avoid amputation of the left foot. Reconstruction of the weight-bearing surface of the foot by covering the bare bone, recreating the plantar fascia and therefore providing a viable surface for skin grafting and safe walking

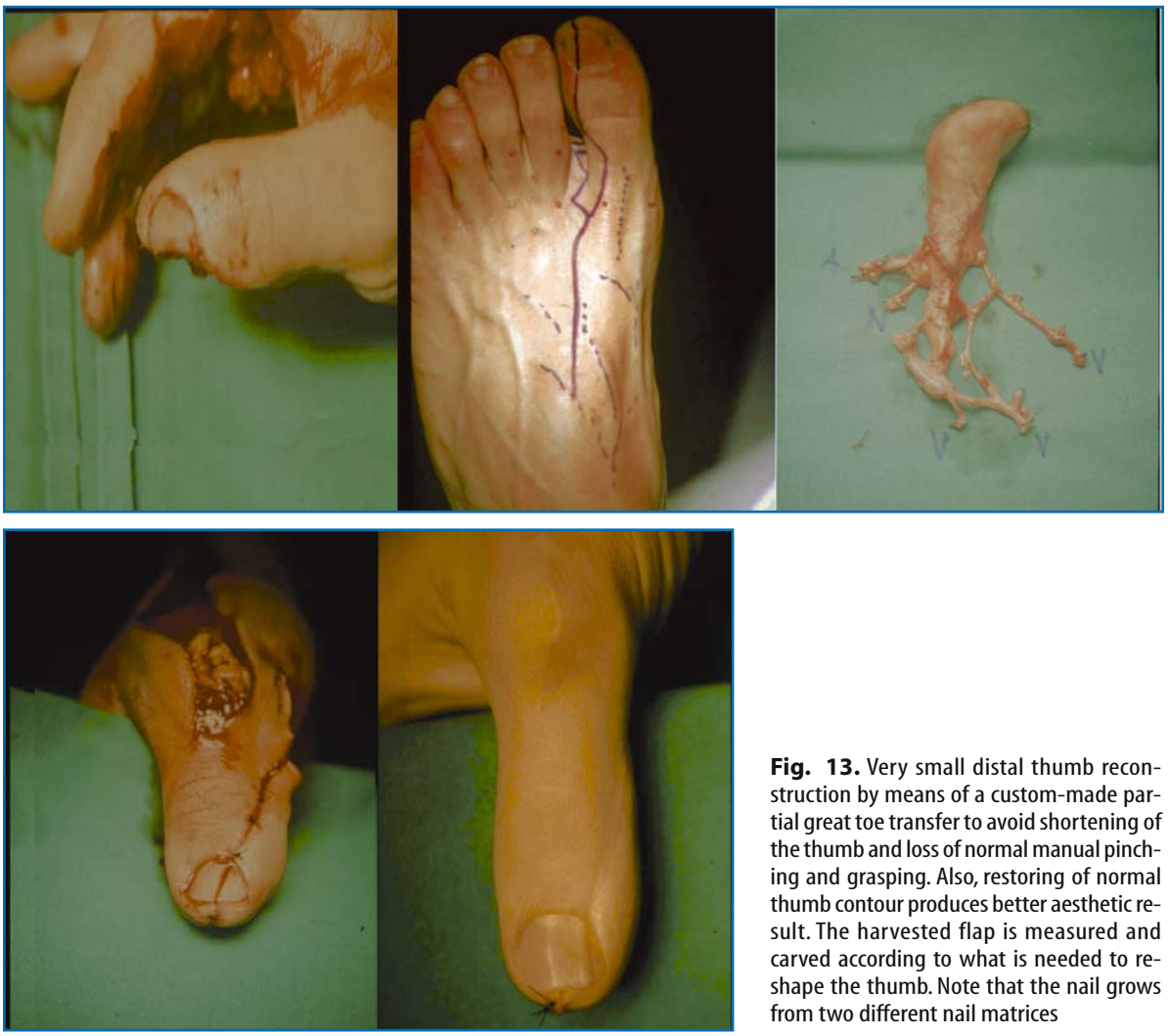

Fig. 13. Very small distal thumb reconstruction by means of a custom-made partial great toe transfer to avoid shortening of the thumb and loss of normal manual pinching and grasping. Also, restoring of normal thumb contour produces better aesthetic result. The harvested flap is measured and carved according to what is needed to reshape the thumb. Note that the nail grows from two different nail matrices

Level 5: Transplantation Free Flap

As the size of the lost/damaged part of the body increases and/or the unique features of the missing tissues do not permit selection of an autograft, conventional microsurgery no longer has a place. These patients are traditionally told there is no surgical solution to their disability and are invited to approach a prostheses centre if their defect concerns the upper or lower limbs. Some of them present very severe disabilities, and having tried the available prosthetic solutions, they find them highly unsatisfactory and therefore they have to live with their disability (Fig. 14).

Patients with other deformities/disabilities do not even have the opportunity to revert to some sort of prosthetic replacement of the lost body parts (i.e. face, abdominal wall, knee joint, larynx, uterus). It is quite logical that the only surgical solution for us as microsurgeons is to explore the issue of using new free functional flaps coming not from the same body but from a donor, a concept that is widely accepted in cases of solid internal organs, even in the case of non-life-saving situations. Currently, we can count 52 cases of composite tissue transplantations, and it seems inevitable that this field of surgery will continue to expand in the near future. The introduction of better and safer immunosuppressive drugs will allow us to push our limits even further. In the meantime, it is our duty to offer our patients possible alternatives to solve their disabilities.

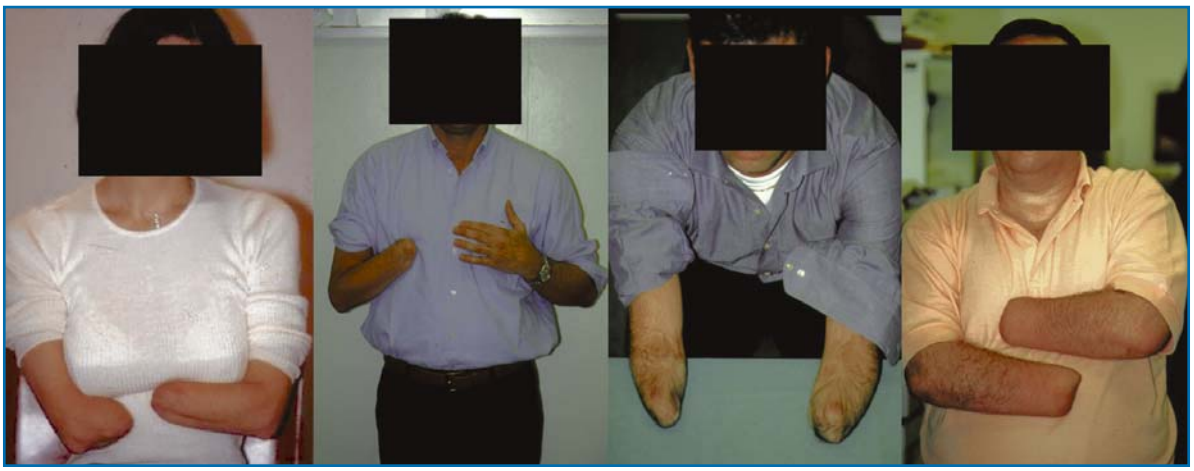

Fig. 14. Patients with a single dominant hand amputation or bilateral amputation cannot be treated with traditional free autologous flaps. If they cannot adapt to the available prostheses and they fulfill allograft the inclusion criteria, they should be considered for an allograft

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2. Experimental Limb Transplantation

Section 2-a

Hind-Limb Transplantation in the Rat: Surgical Technique, Anaesthesia and Early Postoperative Management

Martin Molitor, Takako Kanatani, Marco Lanzetta

Introduction

Human hand transplantation became a reality on 23 September 1998 when the first successful case was performed, with long-term transplant survival and promising functional recovery [1]. Twenty-four hand transplantations have been performed until the writing of this book (12 unilateral and 6 bilateral). However, there remains considerable debate over the ethical consequences of hand allotransplantation, and many questions must be answered before it becomes a routine procedure.

A functional primate model for hand transplantation does not exist, and the rat hind-limb allograft is the most widely used animal model for human hand transplantation research. Although hind-limb reimplantation on rats was first described in 1977 by Shapiro and Cerra [2], a precise description of entire operation has not been published. There are many research groups around the world performing limb transplantation on rats. Each group, however, uses its own surgical technique and type of anaesthesia. Surgery duration, animal survival rate and functional results vary, which makes it difficult to compare studies. However, we are convinced that optimal hind-limb transplantation with good functional outcome and safe, simple and easily controlled anaesthesia will make research more transparent.

In this section, we describe in detail a simple, quick and reliable surgical technique with excellent functional results. Using this technique, an experienced microsurgeon is able to perform hind-limb transplantation in a rat within 2 h. Further, we introduce safe, simple and easily controlled anaesthesia and an early postoperative management programme.

Hind-Limb Transplantation in Rats

Animals

The Animal Research Application Form for study must be approved by the institution's Animal Ethics Committee. All procedures using experimental animals must be carried out according to the Health and Medical Research Council's code of practice for the care and use of animals for scientific purposes. Ideally, animals are male rats between 10 and 16 weeks old and weighing between 250 and 400 g. Such animals are large enough for comfortable surgery and are young enough for long-term survive after transplantation, if necessary. For transplantation research, usually strong rejection is advisable. Therefore, in our research, we almost always use two inbred strains (Brown Norway and Lewis) because of their strong antigenic mismatch [3].

Anatomical Minimum

Rat hind-limb muscles can be divided into thigh, leg and foot muscles. Muscles of the thigh form

four groups: (1) anterior femoral muscles (thigh extensors), supplied by the posterior section of the femoral nerve; (2) medial muscles (adductors), supplied by the obturator nerve (*n. obturatorius*); (3) gluteal muscles, supplied by gluteal nerves; (4) posterior muscles (hamstrings), supplied by the sciatic nerve. Leg muscles are all supplied by branches of the sciatic nerve and are composed of three groups: (1) anterior (dorsal foot flexors), supplied by the peroneal nerve; (2) posterior muscles (plantar foot flexors), supplied by the tibial nerve; (3) the lateral group, supplied by the peroneal nerve (*n. peroneus*). Foot muscles are supplied by the ischiadic nerve (*n. ischiadicus*) and are divided into: (1) dorsal muscles (toe extensors), supplied by n. peroneus; (2) plantar muscles (flexors), supplied by the branches of the tibial nerve.

The saphenous nerve (*n. saphenus*) supplies sensory function to the medial surface of the lower leg and dorsal foot skin in the region of the first metatarsal. The skin of the dorsal distal third of the leg is innervated by the sural nerve. The lateral side of the lower leg is supplied by the peroneal nerve, and this nerve also supplies the dorsal area of the foot (except for the part supplied by the saphenous nerve). Terminal branches of the tibial nerve (*n. tibialis*) innervate the plantar area of the foot and toes. The femoral nerve is formed from 2–4 lumbar nerves and appears between the psoas minor muscle (*m. psoas minor*) and iliacus muscle (*m. iliacus*) and runs under the inguinal ligament together with external iliac vessels. Before entering the thigh, it divides into anterior and posterior sections. The anterior section innervates *m. iliacus* and pectineus muscle (*m. pectineus*) while the posterior division supplies quadriceps femoris muscle (*m. quadriceps femoris*). The third branch from the femoral nerve (*n. femoralis*) is the sensory saphenous nerve (*n. saphenous*) [4].

Surgical Procedure

Choosing the side of the animal on which to perform the surgery is the first step. For the right-handed surgeon, right hind-limb transplantation is more convenient. However, surgery time for left hind limb was found to take on average only 7 min longer. Moreover, from an ethical point of view, it is most desirable to use both hind limbs from one donor.

The surgical procedure begins on the donor. A circumferential skin incision of the donor hind limb is made at mid-thigh level. The inguinal fat flap with pedicle superficial epigastric artery (*a. epigastrica superficialis*) and superficial epigastric vein (*v. epigastrica superficialis*) and sensory nerve branch from *n. saphenus* is sharply dissected and after isolation of the pedicle flap is reflected distally. This flap can be retained in place, or after ligature of the pedicle, it can be removed (Fig. 1).

The saphenus nerve is prepared and transected proximally at the level of its branching from *n. femoralis*. The femoral artery and vein are then identified and skeletonised, and after ligature of all branches at mid-thigh level with 9-0 nylon, are ligated at the level of the inguinal ligament with a 6-0 nylon suture. The femoral artery is clamped distally from the ligature with a single microvascular clamp, transected closely distal to the ligature and cannulated using a 24-gauge intravenous (i.v.) polyurethane catheter. The artery clamp is removed, the femoral vein transected near the ligature and perfusion washout is performed with 4°C cold heparinised solution (1,500 UI heparin in 500 ml 10% dextran 40 i.v. infusion BP in 0.9% sodium chloride i.v. infusion). This perfusion is conducted by gravity at a height of 135 cm and continued until outflow from the femoral vein becomes clear. This procedure takes from 5 to 10 min, with volume range between 4 and 6 ml [5]. After perfusion, the catheter is gently removed, and the artery and vein are clamped with microsurgical single clamps. The thigh muscles are sharply cut approximately 1 cm distally from the level of the femoral nerve branching into the muscular branches and *n. saphenus*. This muscle dissection must be performed very carefully, as it is close to the branching of the femoral artery and vein into the saphenous and popliteal vessels. During muscle dissection, the sciatic nerve is solicitously protected when it emerges between thigh adductors and quadrate muscle (*m. quadratus femoris*) on the one side and

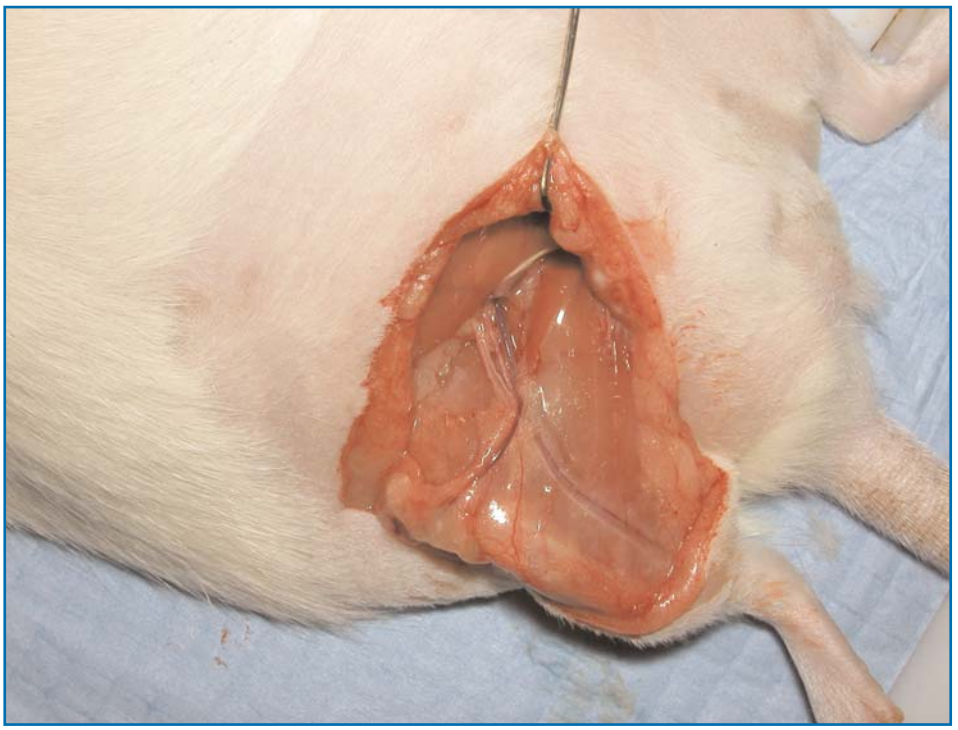

Fig. 1. Exposure of the femoral vessels and nerve

biceps muscle (*m. biceps femoris*) on the opposite side. This nerve is isolated and cut proximally. After skeletonisation of the middle third of the femoral bone, a hole is drilled in ventral–dorsal direction 3 mm distally from the mid-thigh level. The femur is then divided in the middle using a bone saw. During drilling and sawing, intensive cooling is performed by sprinkling with 0.9% sodium chloride solution. After amputation, donor-limb muscles, bone, vessels and nerves are meticulously washed down with 0.9% sodium chloride solution and the limb is wrapped with a wet gauze swab and stored at 4°C while the recipient animal is prepared.

The recipient limb is prepared using the same technique as for the donor limb except that the femoral artery is not cannulated. It, along with the femoral vein and both nerves, is transected more distally to obtain a sufficient length of vessels and nerves for tension-free anastomosis without need for shortening the femoral bone. Thigh muscles are transected more distally in order to preserve muscular branches of the femoral nerve and maximum functional capacity of thigh muscles. The hole in the femur is drilled 3 mm proximally from the midpoint.

The limb rejoining begins with femoral bone fixation, which is achieved by a combined technique using an intramedullary rod made from a 19-gauge stainless steel needle and osteosuture using 28 SWG monofilament stainless steel suture wire (Figs. 2, 3).

Muscles are then sutured with 4-0 nylon interrupted stitches with emphasis on precise adaptation of functional muscle groups. An epiperineurial suture of the sciatic nerve is performed en block either from anterior or posterior access under the operating microscope using 10-0 nylon before muscle suture completion (Fig. 4).

Suture of the nerve is of paramount importance for limb function and must be done perfectly. Femoral vessels are washed out with 0.9% sodium chloride solution, and the ends are precisely trimmed before anastomosis. Revascularisation begins with vein suturing. Both vessels are sutured under the microscope using 10-0 nylon single stitches. For the artery, from 8 to10 stitches are usually needed; the vein requires 10–12 stitches (Fig. 5).

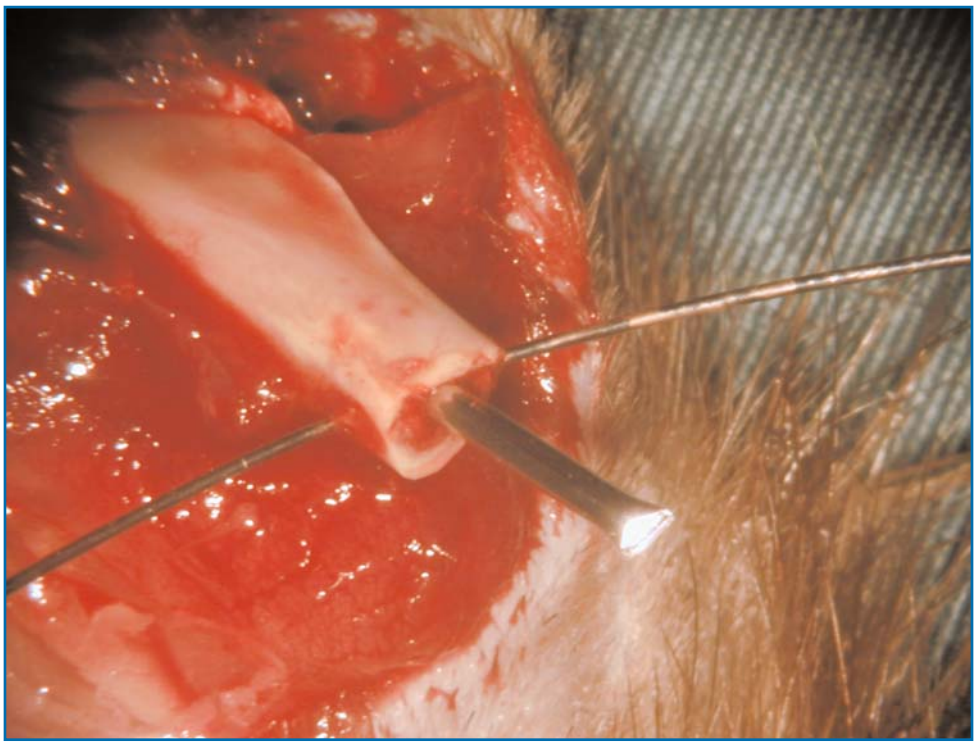

Fig. 2. Femoral bone osteosynthesis

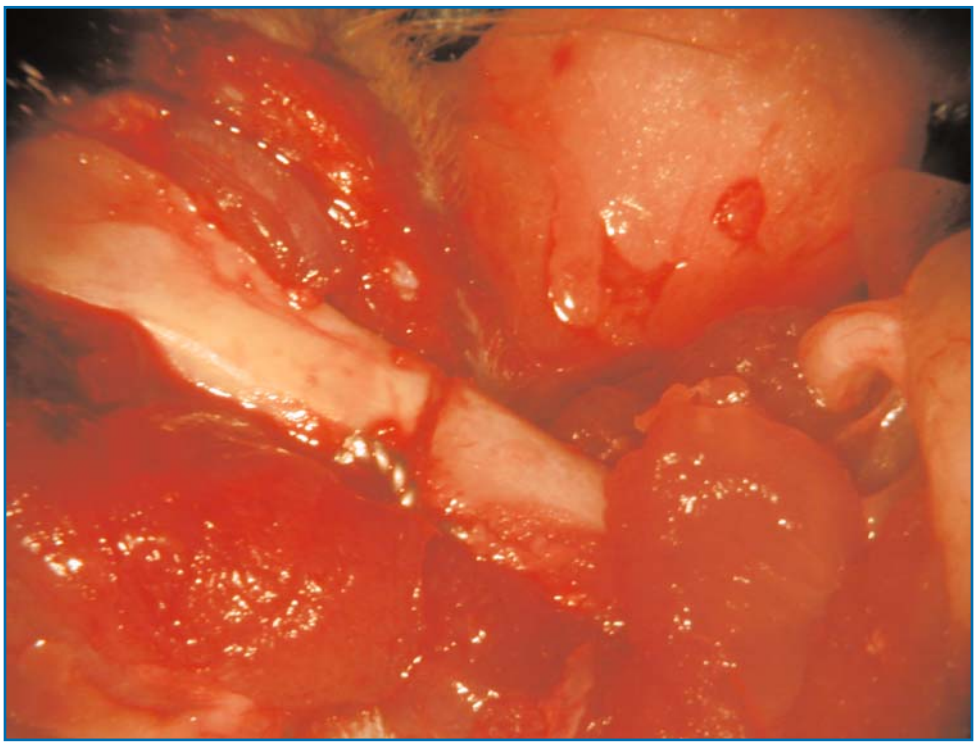

Fig. 3. Femoral bone osteosynthesis completed

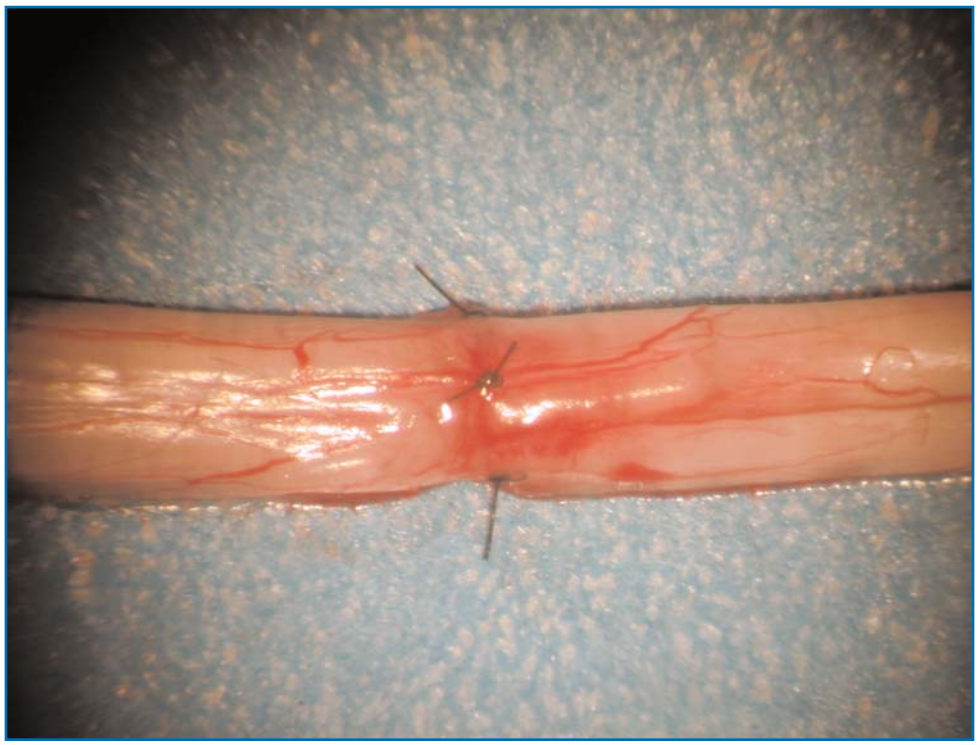

Fig. 4. Sciatic nerve suture

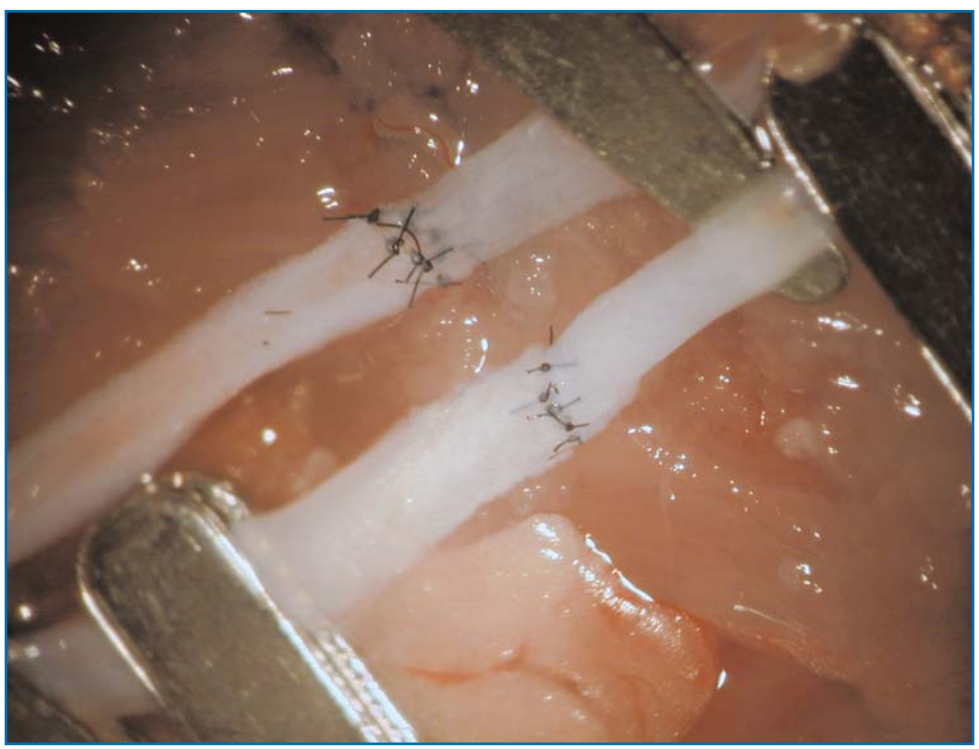

Fig. 5. Femoral vessels suture

All microsuturing is carried out with 10-0 nylon monofilament sutures (preferably black for better contrast) with a 135° curve MET 70 micron needle. Clamps are removed from the artery first, and after a few seconds, when the femoral vein begins to expand with blood, the clamps are removed from the vein. The distal vein clamp is removed first to allow dilatation of vein anastomosis, and then the proximal clamp is removed. The initial slight bleeding from both anastomoses stops after a few seconds of gentle compression with a wet gauze swab. After restoration of blood flow in the reconnected vessels, *n. saphenus* is sutured under the microscope using the same technique as for the sciatic nerve (Fig. 6).

Ample washout of the wound with 0.9% sodium chloride solution is performed, and the transplantation is completed by skin closure with running 4-0 nylon sutures. Note that it is important to include subcutaneous fat into this suturing to prevent bleeding from large vessels in the subcutaneous fat mainly in the hypogastric and inguinal area. After completion of the surgery, the skin suture is wiped down with povidone-iodine 10% solution, and no wound covering or limb splint is used.

The entire operation is performed under aseptic conditions, and antibiotic prophylaxis is administered using Clavulox (12.5 mg/ml clavulanic acid + 50 mg/ml amoxicillin) 0.1 ml/100 g subcutaneously (s.c.) at the commencement of the operation. Before skin closure, a single dose of Temgesic (buprenorphine hydrochloride 0.324 mg/ml) 0.15 ml/100 g s.c. is administered to continue analgesia. Fluid loss during the operation is compensated with 6–7 ml 0.9% sodium chloride solution given by intraperitoneal injection. No vasodilating or anticoagulating drugs are used except for limb perfusion.

Total operative time (from beginning of anaesthesia of the donor to cessation of anaesthesia after transplantation on the recipient) is on average 2 h. The duration of the operation on the recipient is usually only 1 h 30 min; and ischaemia time is round 1 h.

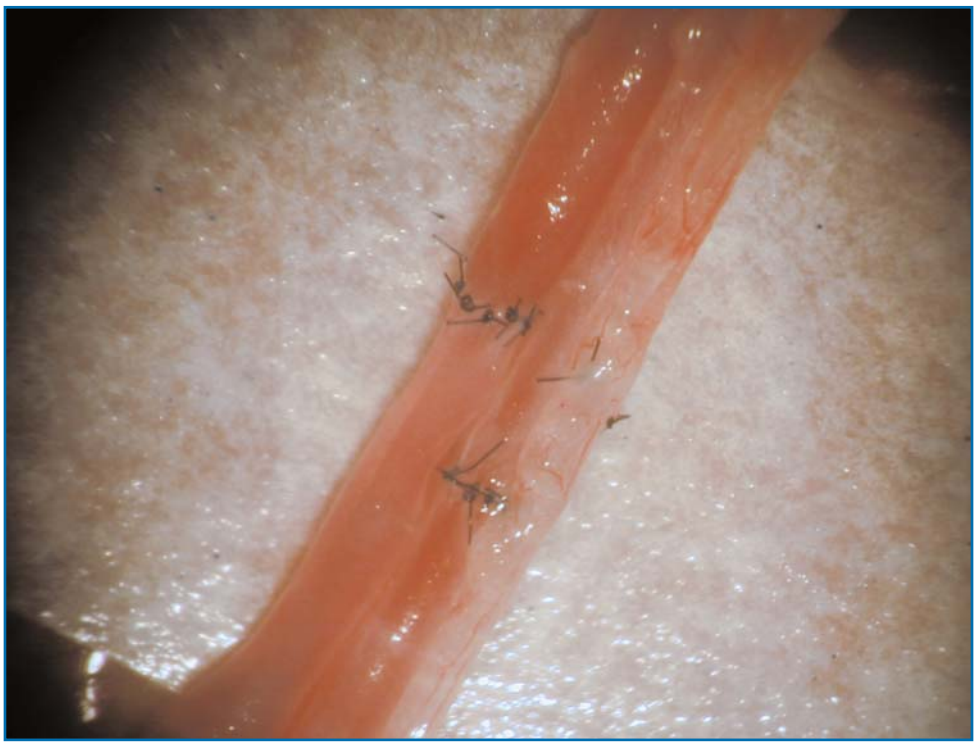

Fig. 6. Femoral vessels and saphenous nerve suture completed

Anaesthesia

The animals are placed into the induction chamber with anaesthetic gas inflow. Anaesthesia is induced with halothane 5% + 1 l/min O_2 mixture. When the rats are anaesthetised [motionless except for breathing, and painless (negative) tail-pinch reflex], they are taken from the induction chamber and put on the operating table with a 40°C thermostatically controlled heating pad and anaesthetic apparatus connected to a facial mask. On both donor and recipient, anaesthesia is maintained at 2% halothane, 0.2 l/min O_2 and 0.4 l/min N_2O mix during the amputation phase. After completion of the amputation on recipient, halothane is tapered to 1.5% and maintained at this level until skin closure. At commencement of skin closure, halothane is tapered to 1% and completely withdrawn after completing the transplantation.

If both hind limbs are used from the one donor, anaesthesia after the first limb amputation is maintained at 1.0–1.2% halothane, 0.2 l/min O_2 and 0.4 l/min N_2O mix until completion of the first transplantation on the recipient. Then the amputation of the second hind limb is carried out as described above.

Monitoring throughout anaesthesia is done using the tail-pinch reflex, respiratory rate, heart/pulse rate and tissue color. Anaesthesia is performed using a basic anaesthetic machine (Boyle Apparatus, Medishield, Sydney, Australia) (Fig. 7).

Donor Termination

After limb harvesting, the donor is terminated with 1 ml intracardial injection of Lethabarb (Lethabarb, 325 mg/ml pentobarbitone sodium) while under general anaesthesia.

Early Postoperative Management

After awakening from the anaesthesia, the animals are put in a clean, dry, warmed box on a

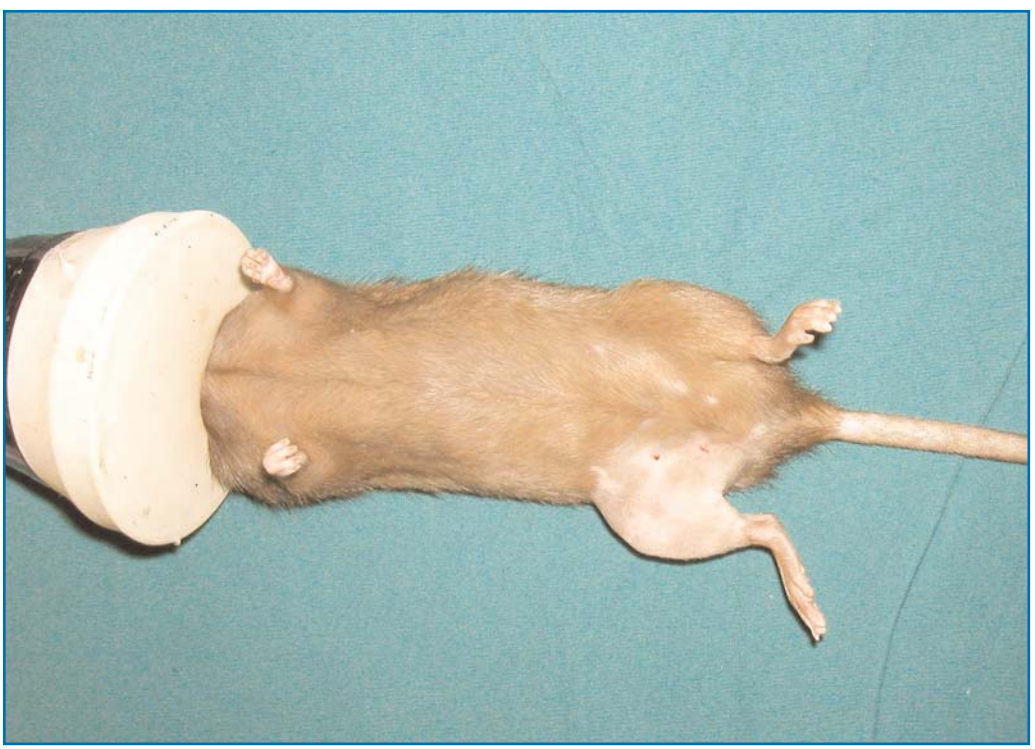

Fig. 7. Anaesthetised animal

folded towel in a quiet postoperative room (30–32°C) away from strong light. Immediately postrecovery, they are housed with a companion and allowed free access to food and water. Body weight bearing on the transplanted limb is not prevented after the operation. On the third day, they are transferred to the air-conditioned postoperative room kept at 20–24°C.

All transplanted limbs develop slight or moderate oedema within 24 h of the operation. This swelling slowly but steadily decreases, and usually completely subsides in 2–4 days. Aseptic conditions during surgery and antibiotic prophylaxis are the reasons wound infection occurs only exceptionally. Analgesia is performed by subcutaneous injection of Temgesic 0.1 ml/100 g twice a day for 3 postoperative days. Additive (self-administered) analgesia is carried out using analgesic jelly (4 ml jelly/animal, 0.125 ml Temgesic/1 ml jelly).

Per oral drugs, if needed, are administered by gavage with a 16-gauge rodent gavage catheter. When necessary, 2 ml of water is given by gastric lavage 3 times a day. No collar or other special device is used to prevent self-mutilation. If autophagy occurs, the wound is sutured under general anaesthesia, a single dose of antibiotics is administered (Clavulox 0.1 ml/100 g s.c.) and the limb is immersed in quinine solution (2.5 g quinine hydrochloride + 25 ml 0.5% chlorohexidine in 70% ethanol + 50 ml of distilled H_2O) twice a day to prevent continuing self-mutilation.

Nerve Regeneration and Bone-Healing Assessment

Femoral and sciatic nerve function is evaluated. Motor function of the femoral nerve is evaluated by observing walking and vertical climbing (thigh extensor muscle function) in comparison with the nontransplanted hind limb. The rat sciatic nerve is a combined sensory, motor and sympathetic nerve [6, 7]. Sensory function restoration is evaluated by the pinch test to determine skin-pain reaction. Pinch of the skin with fine surgical forceps on the dorsal (*n. saphenus* between 1–2 metatarsus and *n. peroneus* between 3–5 metatarsus) and plantar (*n. tibialis*) areas of the transplanted limb is carried out on the quiet animal, and a positive reaction is withdrawal of the limb, vocalisation or struggling in conjunction with the pinch. No walking track analysis (motor function) is done [6, 8]. Results are obtained by clinical observation of the rat walking and climbing in a quiet room for 3 min. Position and movement of toes, foot, knee joint and whole limb are assessed. Bone healing is evaluated by weekly gentle palpation to assess bone angulation. Normal healing is defined as less then 15° angulation.

Functional Recovery of the Transplanted Limb

Sensory nerve function is restored between weeks 4–6 postoperatively. Sensitivity of the plantar area of the foot is usually achieved in all rats; however, about 10% of animals exhibit insufficient sensitivity of the dorsal region. The less successful rate in sensory recovery of the dorsal aspect is possibly due to the small diameter of the saphenous nerve and sensory portion of the peroneal nerve where insufficient reinnervation is more likely to occur.

Thigh extensor function is preserved by surgical technique and is very good from the first postoperative day. Rats have a normal resting and walking position of the thigh, with optimal range of movement of the femur. Animals are able to stand on both the extended hind limbs and climb vertically (Figs. 8, 9).

Function of muscles innervated by the sciatic nerve is restored from weeks 6–8. About 80% of animals reveal excellent function of the transplanted limb, with normal gait on the plantar surface of the foot. In about 15%, motor nerve recovery is less favourable, and the animals walk either on the medial surface of the foot or on curled toes due to insufficient dorsal flexion. Recovery of the sciatic nerve can be, of course, unsatisfactory in some cases (about 5%). Such animals walk on three limbs pulling the transplanted limb behind.

In our experience, in about 50% of transplanted limbs, the toes remained adducted and

Fig. 8. Vertical climbing

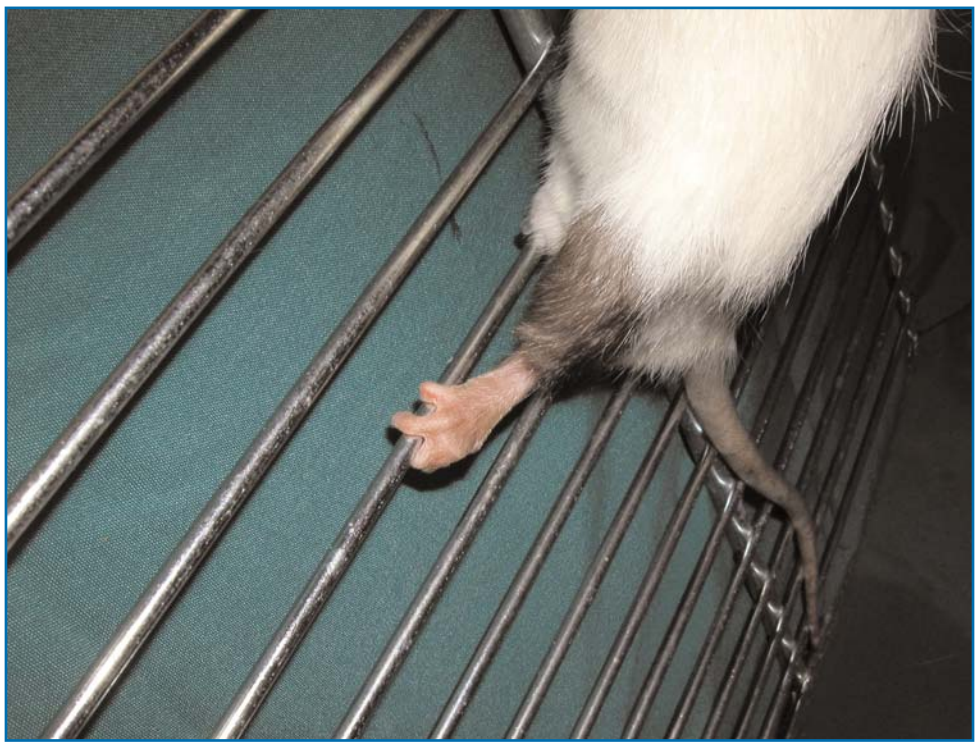

Fig. 9. Gripping by transplanted foot

partially or completely flexed during walking and resting. However, on inducement of a stress reaction (made by a quick and unexpected movement of the hand-held rat), most of these animals were able to involuntary extend and spread their toes (Fig. 10).

The femoral bone usually heals without complication. Angulation is less that 15° in more than 95% of animals (evaluated by posttermination inspection of the bone) (Fig. 11).

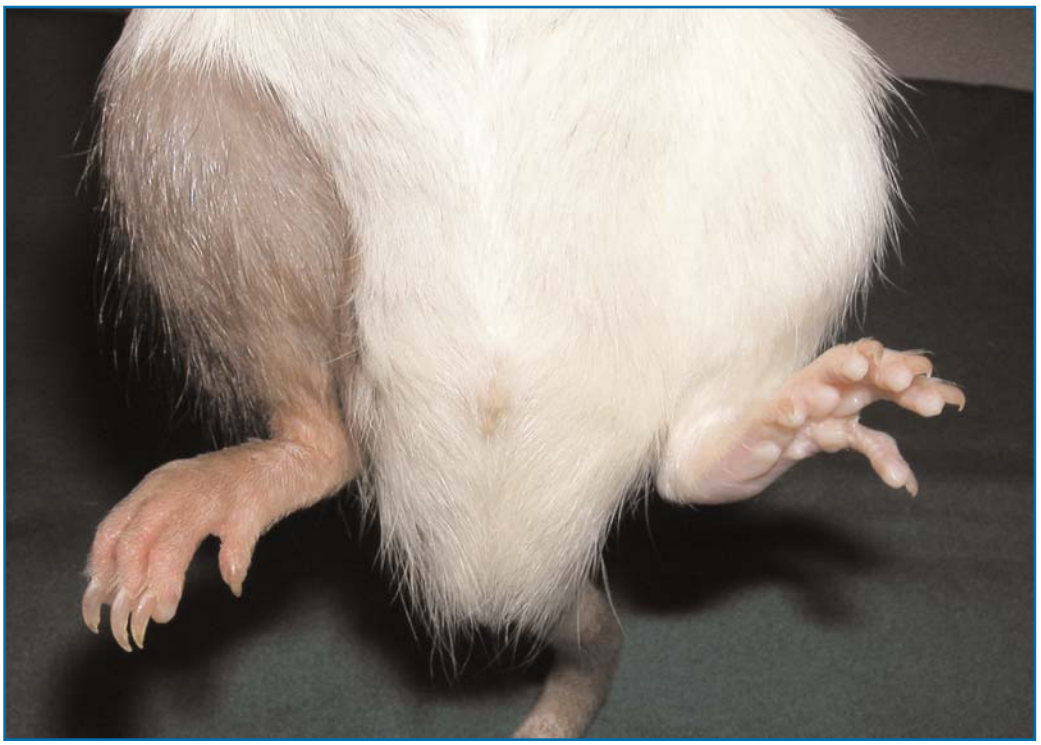

Fig. 10. Extension of the fingers

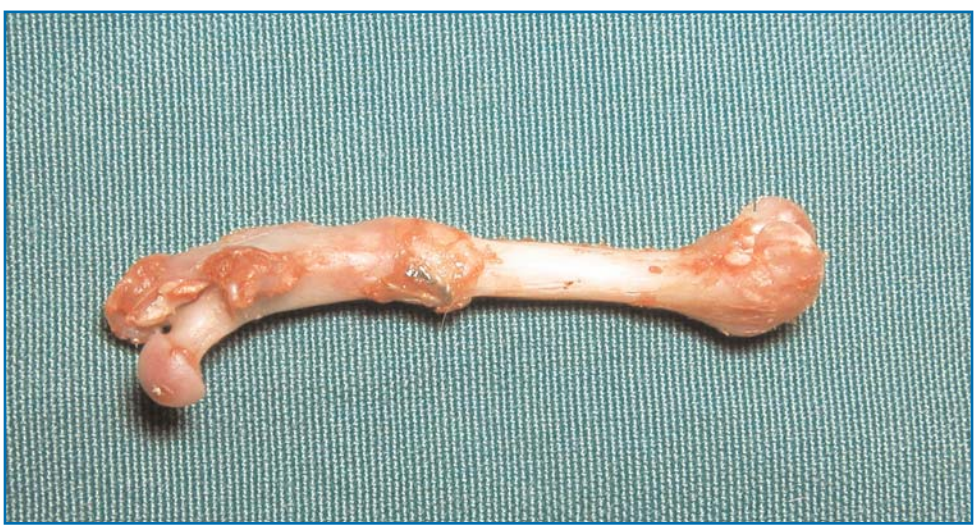

Fig. 11. Bone healing

Most Common Intraoperative Complications

Femur fracturing during tying of the bone suture wire can happen in the case of rough surgical technique. Shortening of the afflicted femoral bone stub and a new careful osteosynthesis can solve this complication.

The recipient's sciatic nerve can be ripped out when the nerve stump is caught by the bone drill during limb amputation. Usually, it is not possible to carry out reconstruction, even with a nerve graft, because a nerve is pulled out in line with the backbone. Such animals must be terminated or can remain in the protocol without neurorrhaphy.

Early vein thrombosis can occur. This is addressed by rapidly cutting off the anastomosis, evacuating the thrombus, washing out the distal and proximal vein stump with 0.9% sodium chloride solution and performing a new anastomosis. This procedure, when done quickly, is successful, and the limb survives without other complications. However, repeated venous thrombosis generally is irreversible, and because of limb venostasis, the animal must be sacrificed.

Acute arterial thrombosis can also occur. From our experience, the aforementioned method used in the vein thrombosis commonly is not successful. Limb ischaemia requires termination of the animal.

Review of the Literature

Femoral-Bone Osteosynthesis

Stable osteosynthesis of the femoral bone is a very important component for the well-being of the animals after hind-limb transplantation. Optimal bone healing promotes healing of the other tissues, especially muscles and nerves [6]. Severe bone angulation results in uncomfortable, difficult and painful walking. It is impractical to perform a reliable external splint of a rat thigh, and it would also be very stressful for the animal. Stability must be achieved with firm osteosyntesis. Various methods of femoral bone osteosynthesis have been used. Most often, a simple intramedullary rod – usually made from a stainless steel needle or a Kirschner wire – with firm suturing of muscles and tendons to support this osteosynthesis were performed [2, 3, 9–12]. Another reported method used two 4/0 stainless steel wire sutures placed perpendicular to each other [13, 14]. Yeh et al. described femoral osteosynthesis consisting of a combination of the intramedullary pin made from a stainless steel needle and methyl methacrylate cement. This method provides stable osteosynthesis; however, it requires a 7- to 10-min delay while the cement polymerises [6].

Combination of an intramedullary pin made from a stainless steel needle and one surgical-wire interosseal suture is quick, reliable and stable. Bone healing is prompt without pathological angulation and allows weight bearing from the first postoperative day.

Muscle Suturing

Muscle suturing must be precise, as failure leads to insufficient functional outcome and discomfort. However, it is not necessary to perform suturing of all muscles separately. Rejoining of the functional muscle groups is sufficient and provides a satisfactory result. Nonabsorbable or absorbable 6-0 to 4-0 single or mattress stitches can be used [2, 3, 6, 9–14]. Adequate tying of stitches is very important, as improper tying results in insufficient suture or muscle ischaemia and necrosis.

Vessel Anastomosis

An essential condition for successful limb transplantation is exact reconnection of the artery and vein. The technique of anastomosis can vary, but single stitches are mostly recommended. Ackland and Trachtenberg [15] reported a 13% narrowing of the vessel in the anastomosis site in 1 h and in 2 days after conventional end-to-end anastomosis. This narrowing increased to 16% on the fifth and 19% on the tenth day post-

operatively. In the third postoperative week, however, the narrowing disappeared. Use of continuous suture leads to even more narrowing of the anastomosis, and this is unacceptable in vessels with such a small diameter. An interesting method of widening the microanastomosis of the carotic arteries in rats was described previously [16]. This technique uses a single z-plasty, and the external diameter of the artery increased by 0.2 mm following the procedure. Mean vessel diameter was 0.87 mm, and a widening of 0.2 mm represents an increase of 23%. No failure or adverse effect was described, but the differences in patency rates between z-plasty and conventional end-to-end anastomosis were reported as not significant. Nevertheless, the average time of single anastomosis was 30.2 min, with 16 stitches required. This seems excessive in the case of femoral vessels in the rat, as conventional anastomosis delivers satisfactory results in less time.

The optimal number of single stitches is very important. Dense suturing with large bites causes inappropriate vessel narrowing. On the contrary, a large gap between stitches carries the risk of excessive bleeding, and small bites can cause vessel-wall tearing. No fat or other tissue envelope is used to decrease bleeding from the anastomotic site [13], and leaking stops after a few seconds of gently compressing the anastomosis with a wet gauze swab. Due to the vein's properties, suturing is technically more difficult, but in our experience, thrombosis of the anastomosis is more readily salvageable than in arteries. In their study, Shapiro and Cerra [2] reported that it is imperative to preserve the femoral profunda vein. We perform ligature of this vein in all rats and have not experienced any complications. Suturing both the femoral artery and vein using the conventional microsurgical single-stitch technique takes between 20–40 min.

Nerve Anastomosis

For human hand transplantation, nerve recovery is of paramount importance, as it deliveries functional recovery of the allotransplant and hence improvement in quality of life. Several previous reports have focused on developing a functional rat hind-limb transplant model [6, 17, 18]. For evaluation of nerve regeneration, walking track analysis, tibial, peroneal and sciatic functional indexes were developed [19, 20]. However, although indexes are important when quantitative data are needed, they are not very reliable and quite often do not correlate with clinical results of sensory or motor recovery. For qualitative nerve regeneration evaluation in a rat hind-limb transplant model, clinical examination, pinch test and observation of the rat walking is the most useful [6].

Shapiro in his pioneer work performed epineural sutures of *n. femoralis* and *n. ischiadicus* [2] and was followed by a many other researchers [3, 11, 13, 14]. Some reports show the technique with only the sciatic nerve suture [9, 10]. Amputation and reimplantation 5 mm below the knee with only the saphenous nerve suture was also carried out [21]. Yeh et al. obtained excellent functional and sensory restoration in their rat hind-limb transplantation model. They performed suturing of all three branches of the sciatic nerve separately. The saphenous nerve was not coapted and served as a control. However, procedure duration was between 4.5 and 6.5 h, with warm ischaemia from 75 to 90 min [6].

Dissection of the femoral nerve proximally from the branching into the muscular branches and *n. saphenus* retains short nerve stumps, and neurorrhaphy can be difficult and time consuming. From our experience, excellent functional recovery of thigh extensors can be achieved by transsection of *n. saphenus* and dissection of the thigh muscles 1 cm distally from the branching of the femoral nerve. This technique preserves long functional muscle stumps that are able to restore good function of the thigh extensor after transplantation without the need for dissection and suturing of the femoral nerve. Also, other groups of thigh muscles (medial, gluteal and posterior) remain functional after this distal dissection.

The rat sciatic nerve is a mixed-motor, sensory and sympathetic nerve and contains as many as 27,000 axons [6]. Functional restoration

requires precise fascicle adaptation because nerves posses target-specific regeneration [5]. The nerve itself includes three big branches – tibial, sural and peroneal – that can be easily recognised. Suturing these branches separately guarantees the best functional outcome. However, using simple epiperineurial suturing of the sciatic nerve en block, sensory and motor function recovery is very satisfactory.

Skin Closure

Optimal skin closure in our experience is by using running 4-0 nylon sutures. This suture is reliable and is readily removed without anaesthetising the animal. When surgery is performed under aseptic conditions with antibiotic prophylaxis and rats are postoperatively housed in a clean environment, no wound covering is necessary.

Autophagy

To prevent autophagy of the allograft, most often a plastic or metal collar is used [2, 6]. Ashur et al. reported a special plastic shielding case attached to the leg [21]. Cutting the incisors was also reported [22]. We consider these methods very uncomfortable and stressful for the animals. A key issue seems to be the proper choice of the recipient strain. Lewis rats are convenient because of their quiet and peaceful constitution, which results in easy handling and less self-mutilation in comparison with the other strains [6]. Adequate postoperative analgesia and quick sensory recovery of the allotransplant is also important. We do not use a protective device to prevent autophagy. Although in some rats autophagy occurs repeatedly, usually it is not extensive, and suturing, antibiotics and the use of the quinine solution is sufficient to rescue the limb allograft.

Anaesthesia

For postoperative recovery, a safe and abstemious anaesthesia is of equal importance to gentle and quick surgery. In rat anaesthesia, two principal methods are reported. The first is to use an intraperitoneal application of the anaesthetic, usually sodium pentobarbital [2, 6, 23] or a combination of intramuscular or subcutaneous first dose and maintenance by intraperitoneal application of the ketamine, xylazine or pentobarbital [3, 21, 24]. This method is reliable; however, it has been noted that the initial injection is difficult with a stressed animal, as is tailoring the dose to the animal response.

The second method is inhaling anaesthesia. This method carries several advantages. No premedication is needed, and the annoying and stressful injection is avoided. Also, it is not necessary to manipulate anaesthetic solutions during the operation. Sleep is fluent and steady without periods of insufficient or excessive anaesthesia, and this technique is very flexible and easily controlled according to the situation.

Conclusion

Hind-limb transplantation in the rat is a complex procedure involving reconnection of bone, muscles, nerves, vessels and skin. The rat hind-limb allograft process is the most popular animal model for human hand transplantation. This underlies the importance of having a fast, simple surgical procedure (with short anaesthesia) that provides good recovery for animals and consequently robust statistics. We believe that the described technique not only will help experimental microsurgeons to find an optimal method of hind-limb transplantation in rats but also that uniformity in surgical technique and postoperative management may lead to higher transparency and better comparability of results.

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Section 2-b

Indefinite Survival and Functional Recovery of Limb Allografts in Rodents

Takako Kanatani, Marco Lanzetta, G. Alex Bishop

Introduction

Over the past decade, the focus of experimental limb transplantation has been to obtain functional recovery and indefinite survival, as reported in previous review articles [1–10]. These factors are of paramount importance in non-life-saving procedures, such as hand transplantation, where quality of life is the main indication for reconstruction. There is an additional ethical issue in clinical composite tissue transplantation where continuous and extensive use of powerful immunosuppressive drugs required to obtain long-term limb allograft survival might have serious and possibly fatal consequences. It is necessary to balance the opposing requirements for prolonged graft survival with the need to minimise immunosuppressive drug treatment. One approach to this problem is to establish an animal model of limb transplant rejection to develop potential treatment strategies that can reduce or eliminate the requirement for lifelong immunosuppression. Here we describe our current approach to obtaining indefinite survival after tapering and withdrawal of immunosuppressive drug therapy to establish a rigorous system to test the effectiveness of future therapeutic developments. In addition we discuss our approach to measuring functional recovery of the transplanted hind limb in a high-rejector rat strain combination.

Indefinite Survival

Limb transplantation was performed across the strong histocompatibility barriers of Brown Norway (BN) to Lewis (LEW) (n=23) or BN to Fischer (F344) (n=8) rats with a standardised surgical technique, as previously described [11]. In the first two postoperative weeks, we administered the triple-drug therapy consisting of FK506, mycophenolate mofetil (MMF) and prednisone at dosages that closely approximate those used for the treatment for human hand transplant patients (Table 1). No animals showed any evidence of rejection episodes or morbidity due to immunosuppression during this time. Subsequently, MMF and prednisone were tapered and stopped at week 7 and, during this tapering period, there was no evidence of rejection. After week 7, FK506 was tapered to a maintenance dosage of 0.8 mg/kg per day and continued at this level until complete cessation at week 24 (Table 1). During tapering of FK506, no rejection episodes were shown in F344 recipient rats; however, rejection was common in LEW recipients, which reflected the high-responder status of the LEW strain. Episodes of rejection in LEW rats were treated with salvage therapy tailored to the severity of rejection. Rejection severity was scored according to a system formulated by Zdichavsky [12], and rejection was treated according to a protocol described in Table 2. Irreversible rejection episodes resulted in euthanasia of the transplant recipient.

Table 1. Regime for tapering of immunosuppressive drug administration

Time weeks (days)	Immunosuppression regimen FK506 (mg/kg per day)	MMF (mg/kg per day)	Prednisone (mg/kg per day)
1–2 (0–14)	2.0	15	0.5
3 (15–21)	2.0	12	0.4
4 (22–28)	2.0	9.0	0.3
5 (29–35)	2.0	6.0	0.2
6 (36–42)	2.0	3.0	0.1
7 (43–49)	2.0	0.0	0.0
8 (50–56)	1.6		
9 (57–63)	1.2		
10–24 (64–168)	0.8		

MMF, mycophenolate mofetil

Table 2. Regime of salvage therapy

	FK506 (mg/kg per day)	MMF (mg/kg per day)	Prednisone (mg/kg per day)
Grade[a] 1, 2	2 till reversed	15 till reversed	0.5 till reversed
Grade[a] 3, 4	10/3 days and 2 till reversed	30/3 days and 15 till reversed	0.5 till reversed

[a]Grade 0, no rejection; Grade 1, pink or slightly red; Grade 2, red; Grade 3, pink red or purple; Grade 4, blue purple, hair loss. From [12], used with permission

Overall, 17 of 23 (74%) LEW recipients of hind-limb allografts given this treatment and all F344 recipients survived under the treatment of low-maintenance dosage of FK506 until week 24. After withdrawal of FK506 at week 24, 6 of 23 LEW (26%) rats and 4 of 8 F344 (50%) rats survived until the 1-year end point without rejection (Fig. 1). Freedom from rejection in these animals was confirmed by histological examination of skin, muscle, artery and bone of the hind-limb transplant at the 1-year end-point of the experiment (Fig. 2). In addition, these long-term survivors accepted skin grafts from the donor (BN) strain and rejected third-party skin grafts from Sprague-Dawley donors at 9 months postoperatively, which confirmed donor-specific tolerance (Fig. 3c).

At the 1-year end-point, a low level of donor cell chimerism was found by flow cytometric analysis in the bone marrow (BM) of transplanted and contralateral femoral bones in long-term-surviving LEW recipients [13] (Table 3). Donor cells were found in the BM of the transplanted limb, including the donor and recipient segments of the femur, as well as in the contralateral, nontransplanted limb. Cellularity of the BM in the transplanted limbs was much lower, possibly due to the presence of an intramedullary rod that was used to fix the transplanted section of the femur to the recipient femur. Analysis of the extent of donor-cell chimerism showed that there were significantly more donor leukocytes in the contralateral BM of the tolerant recipients versus control (normal LEW blood) by Fisher's test (p=0.01). There was, however, no difference between the control and donor BM (p=0.09), recipient BM (p=0.17), and spleen (p=0.95). In addition, there was no significant difference between the control and donor T-cell component of the contralateral BM (p=0.89) and of the spleen (p=0.99). Bonfer-

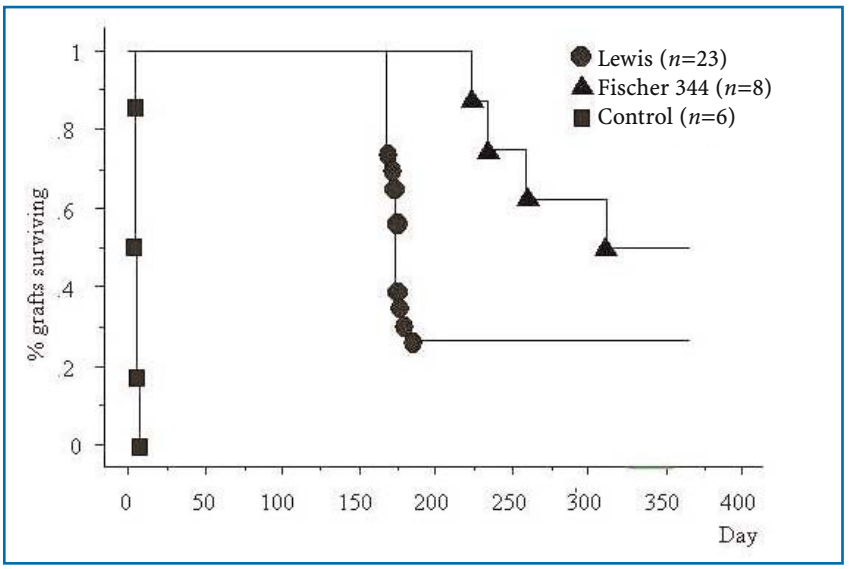

Fig. 1. Control group of 6 animals [Lewis (LEW): n=3, Fischer (F344): n=3) did not receive immunosuppressive treatment and showed rejection at an average of 7.4 days postoperatively. Six of 23 LEW (26%) and 4 of 8 F344 (50%) recipients achieved indefinite survival at the 1-year end-point. The remaining rats showed rejection at an average of 176 days after operation (7.8 days post withdrawal) in LEW recipient rats and at an average of 255.5 days after operation (87.5 days post withdrawal) in F344 recipient rats

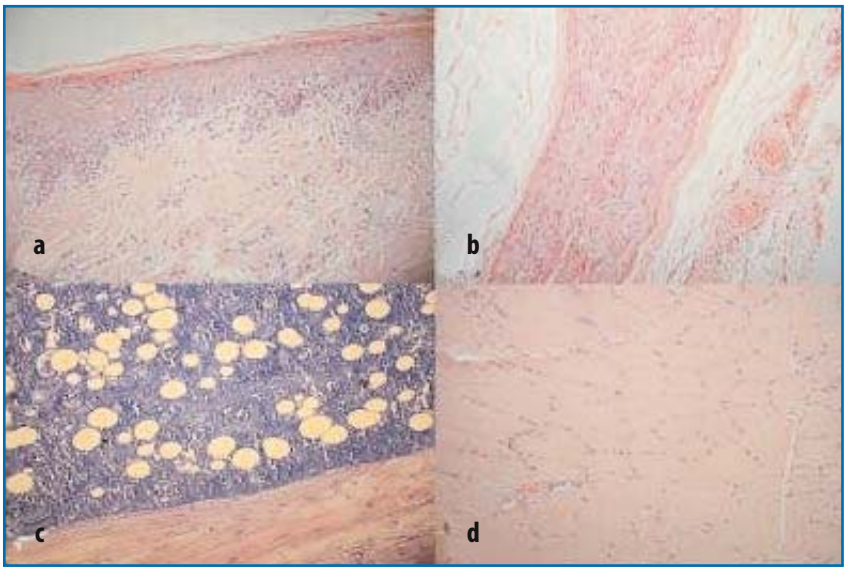

Fig. 2. Transplanted limb and donor skin graft at 1-year endpoint showing healthy appearance (**a**). Histological examination of skin from donor limb graft showed normal structure and little evidence of infiltrate by haematoxylin and eosin (H&E) staining (**b**), as did the donor skin graft (**c, d**)

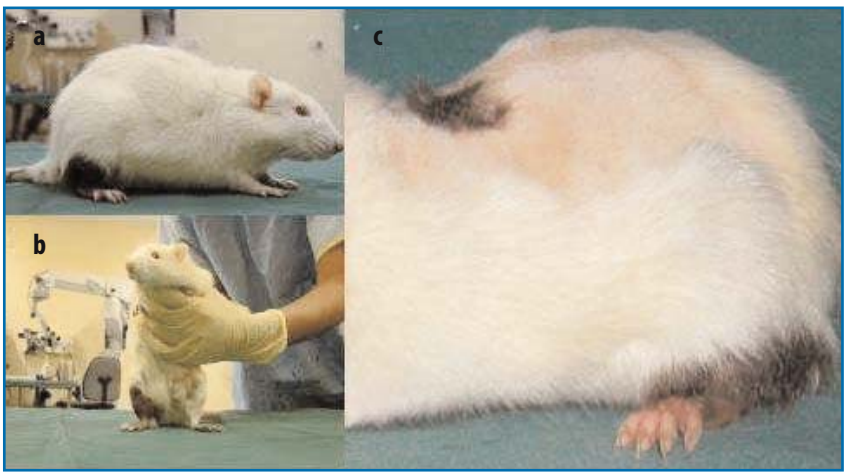

Fig. 3. Excellent results of functional limb allograft (**a**, **b**) and acceptance of donor skin graft (**c**)

Table 3. Flow cytometry analysis of donor cell percentage in donor and Lewis (LEW) recipient tissues

Case	Control[a]	Recipient BM[b]	Donor BM[c]	Contralateral BM[d]		Spleen		Peripheral blood	Thymus
				T cells	Non T cells	T cells	Non T cells		
1	0.00[e]	0.40	1.06	0.00	0.40	0.03	0.01	n.d.	n.d.
2	0.01	0.39	0.12	n.d.	n.d.	0.01	0.00	n.d.	n.d.
3	0.08	0.17	0.03	0.02	0.46	0.02	0.08	n.d.	n.d.
4	0.01	1.59	1.81	0.01	1.74	0.02	0.08	0.28	0.06
5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03	0.08	0.04
6	n.d.	0.03	2.8	0.33	2.49	0.03	0.07	0.12	0.05

BM bone marrow, *n.d.* not analysed
[a]Control was calculated from OX27 staining of normal Lewis blood collected freshly for each experiment
[b]BM obtained from the recipient half of the femur of the transplanted limb
[c] BM obtained from the donor half of the femur of the transplanted limb
[d] BM obtained from the femur of the recipient hind limb on the contralateral side to the graft
[e] Percentage of donor cells as a proportion of total cells. All live cells were gated for analysis

roni analysis of the analysis of variance (ANOVA) results did not show any group to be significantly different due to the large number of groups. No donor cells were detected by flow cytometry in recipient spleen, peripheral blood or thymus. Immunohistochemical staining of tissue sections confirmed flow cytometry results for the spleen and thymus and did not reveal cells of donor origin, identified by OX27, in recipient spleen, thymus or mesenteric lymph nodes by an indirect immunoperoxidase method [14].

Many studies of low-responder animal models in the past decade have demonstrated indefinite limb allograft survival. To our knowledge, however, Siemionow et al. [15] reported the first successful indefinite survival across the strong histocompatibility barrier (BN to LEW) using combination therapy of cyclosporine A (CsA) and anti-αβ T-cell-receptor monoclonal antibody (αβ-TCR). They demonstrated indefinite survival (>350 days) after withdrawal of the immunosuppression components. They reported that a short (7-day) course of αβ-TCR and CsA treatment without donor or recipient preconditioning was sufficient to promote long-term acceptance; however, the dosage of 250 μg of αβ-TCR was high compared with the dosage of OKT3, an antibody that also targets T cells and is used in clinical transplantation. Also, as OKT3 has significant side-effects, in humans it would be useful to examine the toxicity and side-effects in the animal model. Also, the αβ-TCR antibody used is specific for rats and cannot be used in humans, so it is necessary to produce and test an antibody that behaves similarly before it can be used in clinical trial.

Our group has taken a different approach to promoting limb allograft tolerance in rat models whereby we used a rigorous model of allograft rejection where the standard triple-therapy immunosuppressive drug regimen for human hand transplantation is used but with gradual tapering. One or more episodes of rejection can be expected in LEW rats under tapering conditions; however, if treated promptly, they can be reversed. There are a number of possibilities that could account for the effect of drug tapering. One is that during tapering, the gradual reduction of drug concentration allows a limited graft-versus-host (GVH) reaction mediated by donor T cells that ultimately leads to a chimeric state [16]. Another is that potentially alloreactive recipient T cells that are suppressed during high-dose drug therapy early after transplantation become activated during drug tapering and are then killed or inactivated by salvage therapy. In this case, an underlying assumption is that recipient T cells need to become at least partially activated before they can be killed or inactivated by high-dose immunosuppressive drug therapy, a concept that has some experimental support [13, 17].

Limb transplantation leads to the transfer of large numbers of donor BM cells in association with transplantation of the long bones of the leg. This has led to investigation of the extent of donor-specific mixed allogeneic chimerism as a marker for limb transplant tolerance. Initially, there are high levels of donor-cell chimerism identified in the recipient lymphoid tissues, which peak on days 2 to 4 after limb transplantation then decline [18]. The presence of chimerism does not always indicate tolerance, especially when there are few donor cells present; however, there is often a correlation between detectable chimerism and graft acceptance [19]. In the results reported here, there was a low level of chimerism in the BM of grafted limbs and contralateral limbs. However, we were unable to locate any donor cells in the spleen, MLN (mesenteric lymph nodes)and thymus. In all tolerant animals examined, this chimerism did not exceed 2.8%. Additionally, we were unable to detect significant numbers of T cells of donor origin, making the possibility that tolerance is due to a limited GVH reaction mediated by donor T lymphocytes unlikely. Our findings parallel those in human hand transplantation where patients with stable grafts treated with the same combination of drugs described here did not show detectable chimerism [20].

We believe our animal model of drug administration compares favourably with previous studies and allows for speculation that this approach can be tested in humans. There is little evidence that donor-specific chimerism contributed to tolerance in this model, as there were few donor T cells that could lead to peripheral tolerance by a limited GVH reaction. Also, there were no detectable donor cells in the thymus that could result in central tolerance although the possibility remains that donor MHC (major histocompatibility complex) peptides might promote thymic deletion.

Functional Recovery

During treatment, all animals showed a loss of weight evident at 2 weeks postoperatively followed by a plateau at 4 weeks. At around 8 weeks, they started to gain weight and continued steadily beyond their preoperative weights until the 1-year end-point. There was no evidence of morbidity due to immunosuppression and no sign of pneumonia, infection, tumors, splenomegaly or of a GVH reaction, including weight loss, recipient skin reddening or diarrhoea. Animals showed the appearance of protective sensation in all areas of the transplanted limb by week 7, as measured by pinprick stimulation. The positive grasping test, which measured the ability to grasp a wire grid by the transplanted limb, was displayed by week 10. Of the 6 LEW and 4 F344 long-term survivors, 1 LEW and 2 F344 rats showed a remarkable, complete functional recovery with full mobility of the transplanted limb. As we described previously [9], they could bear their full weight on the grafted limb (Fig. 3a, b), climb on a wire grid and generally perform functional tasks with the same ability as a normal nontransplanted animal. Gait analysis showed comparable print marks for both feet in terms of spread and print length and width, which was comparable with that obtained from healthy subjects. Of the remaining long-term survivors, 4 of 6 LEW and 2 of 4 F344 rats showed an almost complete recovery except for some degree of toe flexion contracture that limited their movements, which were otherwise normal.

Objective and accurate evaluation of sensory and motor function is a difficult problem in laboratory animals and must be assessed by several parameters, such as recovery of sensation, nerve regeneration by histomorphometric or electrophysiological studies, muscle strength, toe spread, weight bearing and gait [7]. Despite the limitation inherent in animal models, functional recovery of the transplanted limb with adequate immunosuppression therapy seems to be comparable with that seen in unoperated animals. Furthermore, the phenomenon that FK506 enhances neuroregeneration [21–23] would be an important consideration in experimental composite tissue allografts.

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Section 2-c

Induction of High-Level Chimerism in Composite Tissue Transplants

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Introduction

Although the prolonged survival of experimental composite tissue allografts (CTAs) is achievable using immunosuppressive drugs, long-term immunosuppression of CTAs is not acceptable in the clinical setting because of serious side-effects. The development of a model for reliable CTA tolerance induction across a major histocompatibility complex (MHC) mismatch and without the need for long-term immunosuppression is highly desirable.

Chimerism is the term used to describe cell traffic from the graft to the recipient and is frequently detected following successful organ transplantation in animal models. Starzl et al. [1] were the first to demonstrate that following successful liver transplantation, small numbers (less than 1%) of donor dendritic cells migrate into the recipient's lymphoid organs, including thymus, bone marrow, spleen and lymph nodes. They referred to this phenomenon as *microchimerism*. It is still controversial as to whether microchimerism can induce immunotolerance or whether it is merely a result of successful organ transplantation [2].

At present, the establishment of a high level (>10%) of chimerism may be the most stable strategy for donor-specific tolerance. This is called *macrochimerism* [2]. Pretransplant bone marrow transfusion (BMT) is a useful tool for induction of a state with mixed chimerism and immunotolerance. BMT-induced macrochimerism is an effective inducer of donor-specific tolerance to a variety of allografts, such as skin, heart, lung and pancreatic islets in a rodent model. Conventional experimental protocols for inducing macrochimerism involve a sequential course of pretransplant recipient conditioning, donor bone marrow transfusion, control of chimerism level by flow cytometry or molecular techniques followed by donor allograft transplantation. The delay period required between induction of chimerism and transplantation might not be as important in select cases of living, solid-organ transplantation. However, this delay is not possible in the case in CTA where, for example, a hand allograft is always procured from a cadaveric donor, and preconditioning of the recipient is therefore impossible.

Since whole-limb allograft contains bone marrow, it represents a vascularised bone marrow transplant where the bone marrow is surgically grafted with its microenvironment intact and where the donor bone marrow cells and stroma are able to function immediately upon transfer. This composite/vascularised bone marrow transplant model is likely to be a better source for bone marrow reconstitution than transplantation of cellular bone marrow cells. Our previous study in a rat limb allograft model showed that donor-origin cells migrated into the recipient's lymphoid tissues, but the ratio of donor and recipient cells remained unexpectedly low, resulting in microchimerism [3–5]. To raise the level of chimerism following limb transplants, novel methods of stimulating donor-cell migration into the recipient body are

required. Our issue concerns preconditioning of the recipient in order to raise the level of chimerism. Foster et al. and other studies used total body irradiation (TBI) to suppress recipient bone marrow cells [6, 7]. In the present study, we used the common chemotherapeutic drug cyclophosphamide (CYP) (Shionogi Pharmaceuticals, Japan) to achieve this. To our knowledge, the rodent limb allograft model has not been used to evaluate the toxicity and dose requirements of CYP monotherapy to induce stable bone marrow chimerism across the MHC barrier.

In this chapter, we introduce a new protocol for induction of a high level of chimerism following rat whole-limb allotransplantation. CYP was used to precondition recipient bone marrow, granulocyte colony-stimulation factor (G-CSF) (Chugai Pharma, Shionogi Pharmaceutical, Osaka, Japan) was administered for 4 days after limb allografting to activate donor-cell migration while temporal FK506 (Astellas Pharmaceuticals, Osaka, Japan) was used to immunosuppress the recipient.

Limb Transplantation to Preconditioned Recipient Rats

Animal Genetics

Hemizygous LacZ transgenic rats of Dark Agouti rat background were used as donors. These rats were provided by Jichi Medical University (Tochigi, Japan), and their generation has been described previously by Takahashi et al. [8, 9]. Adult male Lewis (LEW) rats (genetic expression, $RT1^{l}$) were used as recipients.

Transplantation Procedure

The rat hind-limb replantation model previously described by our group was used [10]. Briefly, the donor right hind limb, including bone, muscles, femoral vessels, sciatic nerve and skin, was amputated at the mid-femoral level. The skin distal to the knee joint was preserved to monitor circulation and skin rejection. The recipient's ipsilateral hind limb was amputated in a similar manner. Femoral osteosynthesis was performed using an 18-gauge needle as an intermedullary rod. The femoral vessels were anastomosed microsurgically with 10-0 nylon. The duration of ischaemia averaged 30 min. All rats were housed in cages and allowed normal cage activity without restriction. Special splints were used for all the recipients in order to prevent automutilation.

Experimental Design

Seventy-one animals were divided into 7 groups. Recipients in group I (n=6) were allograft controls. Recipients in group II (n=5) were given CYP at a dose of 150 mg/kg 2 days prior to transplantation and FK506 therapy at a dose of 1 mg/kg per day by intramuscular injection for 28 days after transplantation. Group III (n=12) were given CYP 2 days before transplantation and G-CSF for 4 days after transplantation at a dose of 25 μg/kg per day. Group IV recipients (n=12) were given G-CSF for 4 days after transplantation followed FK506 therapy for 28 days after transplant. Rats in group V (n=5) were administered CYP at a dose of 100 mg/kg, G-CSF in the same manner as group III and FK506 therapy for 28 days with the same dosage as group II. Rats in group VI (n=20) were administered CYP at 150 mg/kg, G-CSF and FK506 therapy for 28 days. Group VII (n=8) were given CYP at 200 mg/kg, G-CSF and FK506. No recipients were sacrificed during the course of the experiment, and the observation period was for up to 300 days after transplant (Table 1).

Evaluation Methods

Clinical Evaluation

General condition, survival and weight of the recipients and operated limbs were checked daily. Peripheral blood leukocyte counts and body weight were measured in some groups IV and VI recipients at various time points. Radiographs of the operated limbs were obtained at biweekly intervals

Table 1. Experimental designs and results (n=71)

Group	n	CYP* (mg/kg)	G-CSF**	FK506 ***	Death within 2 wks (%)	Limb survival >1 yr	Onset of Skin Rejection (days)	GVHD	Onset of GVHD (days)
I	6	-	-	-	0	0	3,3,4,5,5,5 (4.2)	0	-
II	5	150	-	+	5 (100)	0	-	0	-
III	15	150	+	-	10(67)	0	3,3,3,3,4,5,7,7,9,10 (5.4)	5	4,4,9,14,14
IV	12	-	+	+	0	0	47,52,53,56,57,67,67,68,70,71,72,88 (64)	0	-
V	5	100	+	+	0	0	85,87,91,92,95 (90)	0	-
VI	20	150	+	+	5(25)	1	90,90,99,100,100,101,135,138 (107)	7	92,95,98,124,142,164,201
VII	8	200	+	+	3 (38)	2	137,165,171 (158)	3	117,171,191

All transplantations were from LacZ Tg rats to LEW. * *CYP*, Cyclophosphamide was administered on two days before transplantation. ** *G-CSF*, Granulocyte-colony simulating factor was administered for four days from transplantation at a dose of 25μg/kg/day. *** FK506 was given at a dose of 1 mg per kilogram per day by intramuscular injection for 28 days after transplantation

after surgery to evaluate presence of bony union between the graft and recipient femurs. Skin rejection of the grafted limb was diagnosed by the finding of reddish discoloration, as reported previously [10]. Survival times for recipients and limb grafts were compared according to the Kaplan-Meier method.

Histology

Rejection of transplanted limbs was evaluated by examining each component tissue, including bone, marrow, muscle and skin. Bone samples were fixed in a 4% formaldehyde solution and decalcified in 10% EDTA solution, embedded in paraffin, sectioned at 5-μm thickness and stained with hematoxylin and eosin (H&E) for routine light microscopy.

Assessment of Graft-Versus-Host Disease

Animals with graft-versus-host disease (GVHD) were evaluated clinically and histopathologically. Clinical signs of GVHD onset included nonreversible weight loss, diarrhea, unkempt appearance, hair loss and rash on the paws, snout and ears. At the time of necropsy, sections of skin, liver and small intestine were stained with H&E and examined for the presence of lymphoid infiltration, subepidermal cleft formation and epidermal necrosis.

Detection of the LacZ Transgenic Gene by Semiquantitative Polymerase Chain Reaction

The LacZ gene (Amersham Pharmacia Biotech, Piscataway, NJ, USA) in grafted tissues was analysed semiquantitatively using polymerase chain reaction (PCR). The relative amount of GFP gene was compared with a known autosomal control gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The ratio of PCR product obtained from the LacZ-specific primer to that from the GAPDH-specific primer was used to determine the relative amount of LacZ-containing cells in the samples. The PCR reaction mixture contained 0.5 mg of genomic DNA, 25 pmol of LacZ-specific primers, PCR beads

(Ready-To-Go, Amersham Pharmacia Biotech, USA) and sterile distilled water in a final volume of 22 µl. PCR was carried out in a programmable thermal cycler (Intermountain Scientific Corporation, UT, USA) for 35 cycles of denaturation (94∞C for 15 s), annealing (55∞C for 15 s) and extension (72∞C for 15 by electrophoresis on a 2% agarose gel in parallel with a 50 base pair ladder of standard markers (Boehringer Mannheim, USA). Gels were stained with ethidium bromide and exposed to ultraviolet (UV) light to visualise the PCR product.

Specificity and sensitivity of PCR was evaluated using serial dilutions of LacZ-positive and LEW (LacZ-negative) DNA as templates. DNA from LacZ and LEW animals was mixed in ratios varying from 1:0 to 1:10,000. Each DNA mixture underwent PCR using the same conditions. After 35 PCR cycles, the LacZ band was stronger than GAPDH at a 1:1 DNA ratio, identical at 1:10, lower at 1:100 and present only faintly at 1:1,000 dilution. No LacZ band was detected at a 1:10,000 ratio.

Long-Term Acceptance of Allogeneic Limb Allografts

Clinical Observations

1. Recipient survival: The animals in group I (allograft control) and group IV (treated with FK506) showed no serious symptoms, and all except one survived more than 300 days. Body weight was lost acutely (-43 g) at 8 days and recovered after 28 days to pretransplantation weight. Group II (CYP/FK506) suffered severe weight loss, and all animals died within 2 weeks posttransplantation. Group III (CYP/G-CSF) also showed severe weight loss, and 10 recipients (67%) died within 2 weeks posttransplant. Group V (CYP100/G-CSF/FK506) showed weight loss, but all survived after transplantation. Group VI (CYP150/G-CSF/FK506) showed severe weight loss, and 5 recipients (25%) died within 2 weeks posttransplantation. Three group VII (CYP200/G-CSF/FK506) animals (38%) died within 2 weeks posttransplantation; however, of the remaining 5 animals, 3 survived more than 100 days and 2 more than 300 days.
2. White blood cell (WBC) count: Four groups IV and VI animals were analysed for WBC count. In group VI, the leukocyte count dropped to <1,000/mm^3 at 1 week posttransplantation, and leucopenia less than 5,000/mm^3 was continuously observed up to 3 months posttransplantation. In group IV, leucopenia was not observed up to 10 weeks posttransplantation.
3. Radiography: In groups VI and VII, long-term recipient survivors, solid bony unions between the femur junctions were confirmed by 4 weeks posttransplantation. In group I, no recipient achieved bony union.
4. Onset of skin rejection: Limb allografts in group I showed skin rejection on average at 4.2 (range, 3–5) days posttransplantation and were acutely mummified thereafter. In group III, onset of skin rejection varied from 47 to 88 (mean 64) days. Skin rejection was confirmed in 10 group IV animals, and the mean onset time was 5.4 days. In group V, mean onset was 90 (range, 85–95) days. In group VI recipients, skin rejection was observed in 8, and the mean onset was 107 (range, 90–138) days. One recipient (5%) showed no skin rejection after more than 1 year. In group VII, three limb allografts showed skin rejection, and the mean onset was 158 (range, 137–171) days. Two limb allografts (25%) showed no skin rejection after more than 1 year (Fig. 1). Onset time for skin rejection was significantly prolonged with CYP/G-CSF therapy prior to FK506 administration.

Histological Study

In group I, all components in the limb allograft were rejected simultaneously after onset of skin rejection. In groups VI and VII, even if the skin component of the limb allograft was rejected, other components such as muscle, bone and small vessels showed no evidence of rejection over a long period. Bone marrow cells in the grafted limb were few, showing aplastic, marrow whereas the recipient showed hypoplastic marrow.

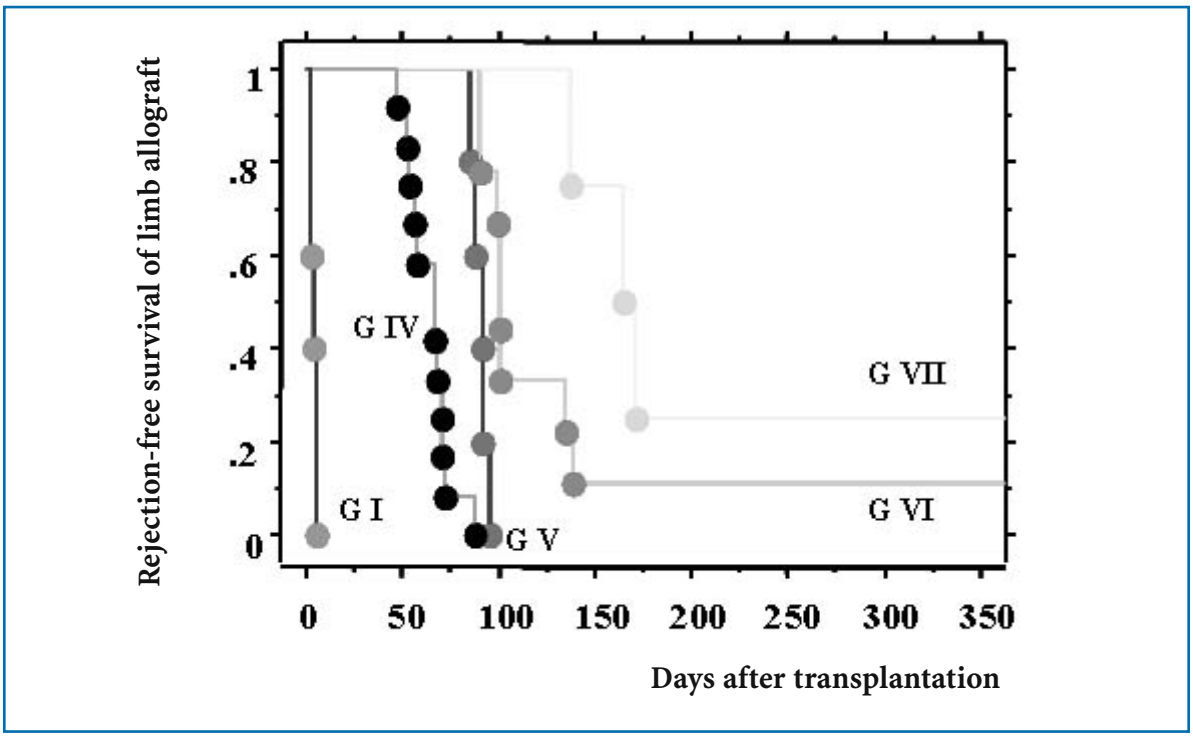

Fig. 1. Onset of skin rejection. Limb allografts in group I showed skin rejection on average at 4.2 days. In group IV, mean onset was 64 (47–88) days. In group V, mean onset was 90 (85–95) days. In group VI, skin rejection was observed in 8 recipients, and mean onset was 107 (90–138) days; one recipient (5%) showed no skin rejection after more than 1 year. In group VII, three showed skin rejection, and mean onset was 158 (137–171) days; two (25%) showed no skin rejection after more than 1 year. Onset time for skin rejection was significantly prolonged with cyclophosphamide/granulocyte colony-stimulating factor (CYP/G-CSF) therapy prior to FK506 administration

Occurrence of GVHD

No recipients in groups I, II, IV and V showed clinical evidence of GVHD. Five recipients in group III (33%) experienced acute lethal GVHD with dermatitis of the ear and paw and mean onset time of 9 days after transplant (Fig. 2a). Severe infiltration of the inflammatory cells was observed histopathologically in skin specimens of skin, small intestine and lung. All animals died within 1 week after the onset of GVHD. In group VI, occurrence of chronic GVHD was confirmed in 7 recipients, with a mean onset time of 131 (range 92–201) days. These rats showed severe hair loss but no dermatitis. Five showed rejection-free limb allografts, but two showed signs of skin rejection. Weight loss was not as severe, and all rats survived for 1–3 months after onset of GVHD. Histopathology showed mild infiltration of inflammatory cells in the skin (Fig. 2c), small intestine (Fig. 2d) and lung tissues. Three recipients in group VII showed clinical signs of chronic GVHD (Fig. 3a), and the mean onset for this was 160 (range 117–191). Two recipients survived more than 1 year without rejection of the grafted limbs. At the final examination of 14 months posttransplantation, histopathology showed no inflammatory cell infiltration in the skin (Fig. 3c), liver or small intestine (Fig. 3d).

Detection of Donor-Derived LacZ Genes in Recipient Bone Marrow Using PCR

Bone marrow chimerism was confirmed using the LacZ-specific PCR technique. In group III, 2 recipients showed a high level of chimerism (10%) (Fig. 2b), 2 showed moderate levels (1%) and 3 showed none. In the 3 recipients showing GVHD, 2 revealed 10% chimerism and the other 1% chimerism. The level of bone marrow chimerism correlated with the occurrence of

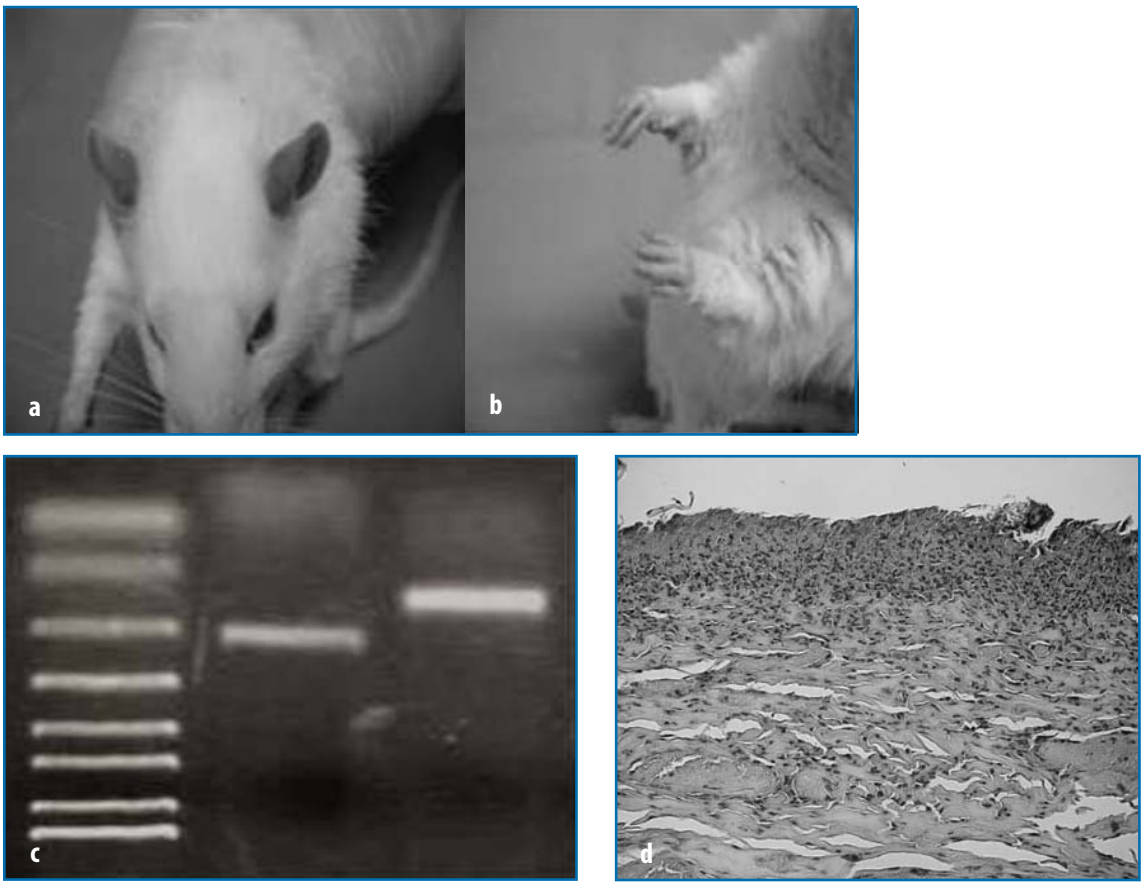

Fig. 2a-d. a Five recipients (25%) in group III [cyclophosphamide/granulocyte colony-stimulating factor (CYP/G-CSF)] experienced acute lethal graft-versus-host disease (GVHD) with dermatitis of the ear and paw and a mean onset of 9 days posttransplantation. **b** Bone marrow chimerism detected by polymerase chain reaction (PCR). *Lane 2*: LacZ band showing high level of chimerism (10%). *Lane 3*: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of the same sample. **c, d** Histopathology of acute GVHD. Severe infiltration of inflammatory cells was observed in skin specimens (**c**) and small intestine (**d**)

acute GVHD. Bone marrow chimerism was not found in groups IV and V recipients with rejected limbs. In group VI, 3 recipients showed a high level of chimerism (10% level) whereas the 7 recipients with rejected limbs showed none. A high level (10%) of bone marrow chimerism was found in the 3 group VII recipients with chronic GVHD (Table 2; Fig. 3b).

Pretransplant Bone Marrow Transfusion for Composite Tissue Allografts

The ultimate goal of organ transplantation is to establish a nontoxic, easily applied regimen for induction of donor-specific tolerance so that grafts can be accepted across MHC barriers without the requirement for chronic immuno-

Table 2. Occurrence of GVHD and chimerism

Group	n	CYP (mg/kg)	FK506 (days)	Limb rejection	GVHD	Chimerism (level)
III		150	-		3 (acute)	2(10%) + 2(1%)
IV	5	-	28	5	none	none
V	5	100	28	5	none	none
VI	10	150	28	7	3 (chronic)	3 (10%)
VII	3	200	28	none	3 (chronic)	3 (10%)

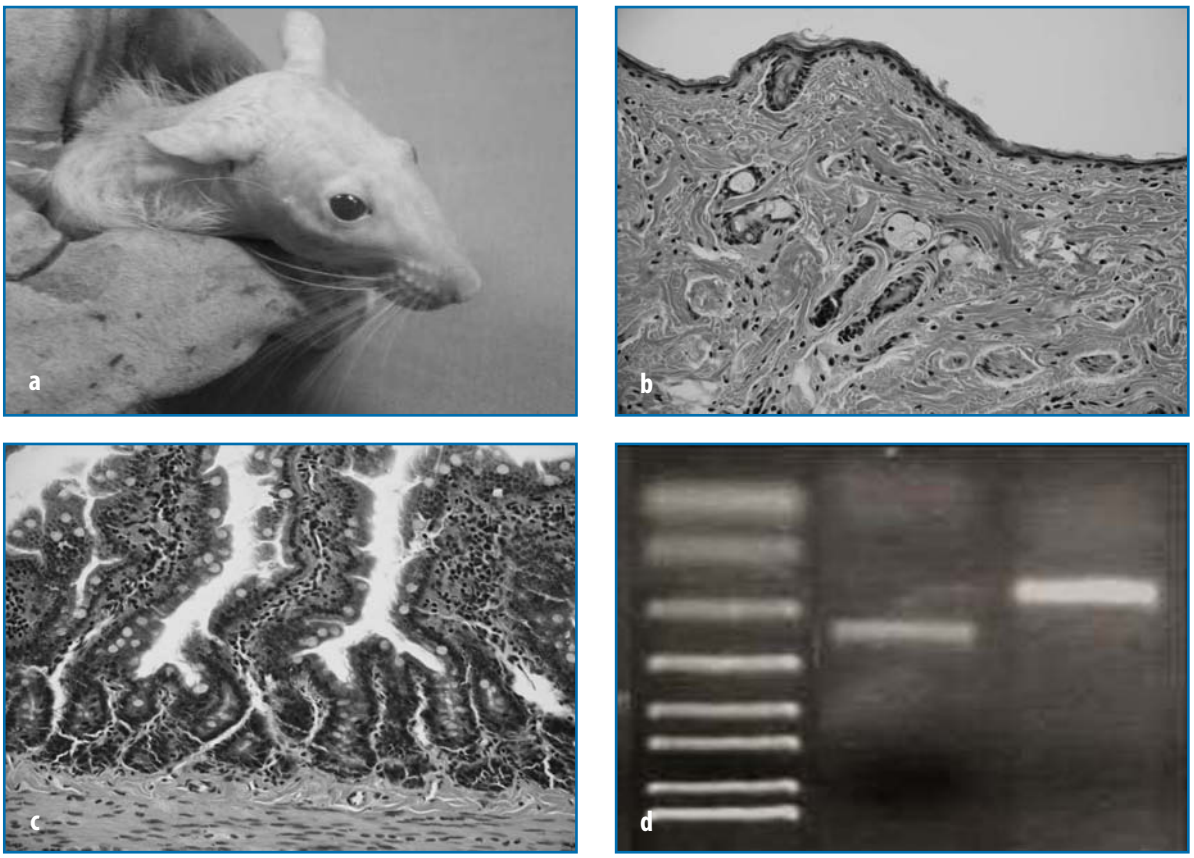

Fig. 3a-d. a Group VII recipient [cyclophosphamide/granulocyte colony-stimulating factor (CYP/G-CSF)/FK506] showing clinical signs of chronic graft-versus-host disease (GVHD) with severe hair loss. **b** Bone marrow chimerism detected by polymerase chain reaction (PCR). *Lane 2*: LacZ band showing high level of chimerism (10%). *Lane 3*: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of the same sample. **c, d** Histopathology showed no inflammatory cell infiltration in the skin (**c**) and small intestine (**d**)

suppressive therapy. Macrochimerism induced by BMT is one of the strategies used to induce a state of immunotolerance. BMT patients who subsequently require a kidney transplant become tolerant to the transplanted kidney and do not require long-term immunosuppression if the kidney is transplanted from the same donor. Therefore, surgeons have attempted to establish macrochimerism by BMT after pretreatment of the recipient lymphoid system. Colson et al. first achieved reliable mixed allogeneic chimerism by transplanting a mixture of T-cell-depleted bone marrow cells into pretreated recipients in a rat cardiac allograft model [11]. Chimerism ranging between 12% and 93% was achieved in 91% of cases across several strongly antigenic, MHC-disparate strain combinations.

Hewitt et al. first applied BMT for limb allotransplant [12]. Their study showed that low-level (18 ± 3%) mixed lymphocyte chimerism was associated with induction of tolerance after limb transplantation but that a high-level (60 ± 14%) of chimerism was associated with development of GVHD. Foster et al. developed reliable tolerance across a strong histocompatibility barrier by inducing a state of mixed chimerism with BMT in a rat limb transplant model [6, 7]. There were no signs of rejection of the limb allografts for more than 100 days in the 6 out of 10 animals with a donor chimerism level greater than 60%. All 3 animals with chimerism less than 20% rejected the limb transplant. Interestingly, Esumi et al. demonstrated successful allogeneic rat limb allotransplantation using combined pretreatments of fludarabine injection, low-dose irradiation, and BMT directly into the bone marrow cavity [13]. These limb allografts survived more than 1 year without signs of rejection. The conclusion from these studies was that development of high-level mixed chimerism allowed long-

term survival of a limb allotransplant similar to that of other visceral organ allotransplants.

Raising the Level of Chimerism Following CTAs Using Pretreatment with CYP, G-CSF and FK506

Recent studies have demonstrated donor-cell migration following limb transplant. Ajiki et al. developed a green fluorescent protein transgenic rat for marking the donor cells and showed that the ratio of donor cells in recipient bone marrow was about 1% at 48 weeks posttransplantation [14]. Our previous study demonstrated similar results, and the level of bone marrow chimerism was 1% at 24 weeks and 10% at 48 weeks [3]. Mathes et al., using a miniature swine model and flow cytometry, found no evidence of donor-cell engraftment in a recipient animal [15]. Similarly, Granger et al. reported that donor-cell microchimerism in clinical hand transplant patients was barely detectable in some early posttransplantation specimens but was undetectable thereafter [16]. These results indicated the level of chimerism following limb allotransplantation was unexpectedly low, resulting in microchimerism.

To our knowledge, there have so far been no studies attempting to raise the level of chimerism following limb allograft/vascularised bone marrow transplant. The present study is the first to demonstrate the induction of fully allogeneic bone marrow chimerism in whole-limb allografting using a simple pretreatment protocol involving CYP, G-CSF and FK506 combination therapy. Destruction of recipient bone marrow cells by CYP is advantageous in that it creates a new space for the survival of donor-marrow cells. While TBI-induced chimerism and tolerance is stable, toxicity for recipients of the preconditioning regimen appears too severe to allow clinical application of this strategy. Considerable efforts have therefore been undertaken to reduce the requirement for irradiation. Chemotherapeutic drugs have been evaluated as possible substitutes for TBI, and in the present study, we focused on CYP.

Tomita et al. reported induction of skin allograft tolerance by using CYP and BMT in a murine model [17]. The same group, Zhang et al., also reported induction of heart allograft tolerance in the same experimental model [18]. Several mechanisms for the efficacy of CYP in inducing allograft acceptance have been considered, with perhaps the most important being the specific effect of CYP on proliferating T cells. Mature T or B cells reactive against alloantigen cause clonal expansion after the transplant of allogeneic cells, and CYP may selectively destroy these allo-stimulated mature reactive T cells. Proliferating cells are especially sensitive to CYP, and thus the clones are selectively destroyed with this agent [19, 20].

Dosage and Timing of CYP administration and G-CSF

Dose and timing of CYP injection appear to be critical for limb graft acceptance. Establishment of stable macrochimerism and allogeneic limb graft survival are likely to depend on the CYP dose. Okayama et al. evaluated the dosage of CYP required to induce macrochimerism prior to BMT [21]. They used a single dose of CYP ranging from 50 to 200 mg/kg and found that pretreatment with 200 mg/kg induced macrochimerism but caused lethal GVHD. A 150 mg/kg dose of CYP appears to be optimal for induction of tolerance without GVHD whereas lower dosages cannot induce high enough levels of chimerism for graft acceptance. Iwai et al. performed a similar study with mouse skin allografts and found that pretreatment with a single dose of 200 mg/kg CYP induced a significantly higher degree of chimerism compared with a 100 mg/kg dose [20]. In our study, 100 mg/kg of CYP were not enough to induce stable bone marrow chimerism. While recipients treated with 150 mg/kg of CYP showed prolonged survival of limb allografts and high levels of chimerism, only 1 of 20 recipients (5%) showed long-term graft acceptance. Two of 8 recipients (25%) treated with 200 mg/kg of CYP showed long-term acceptance of limb allografts; however, toxicity was more severe than with 150 mg/kg treatment. Hence, the current findings demonstrate that CYP has dose-dependent effects on survival

of limb allografts and on induction of chimerism.

Mayumi et al. reported that CYP injection on day 2 after the infusion of donor-derived bone marrow and splenocytes induced the acceptance of allogeneic skin grafts in a mouse model whereas CYP injection before BMT failed to induce skin graft acceptance [19]. In contrast, Okayama et al. studied the timing of CYP administration in a rat heart transplant model and found that injection 1 day before BMT was the most effective for induction of allogeneic macrochimerism. We cannot assess which protocol is superior; however, CYP injection followed by the BMT protocol worked in the present limb allograft study.

Okabe et al. investigated the effect of G-CSF on mice pretreated with CYP [22]. An acute CYP-induced drop in neutrophil count was successfully reversed by G-CSF administration at a dose of 25 μg/kg per day for 4 days. To our knowledge, there have so far been no studies that have attempted to raise the level of chimerism following limb allograft or vascularised bone marrow transplant. In the present study, we used G-CSF at 25 μg/kg per day for 4 days to stimulate donor-cell migration into the recipient and thus demonstrate that the level of bone marrow chimerism could be raised by G-CSF therapy. From the histopathological results of bone marrow, the majority of donor-marrow cells migrated into recipient marrow space and lymphoid tissues, resulting in aplastic marrow.

Occurrence of GVHD

Although increased chimerism and significantly prolonged limb allograft survival could be induced with CYP and G-CSF treatments, recipients frequently showed chronic nonlethal GVHD. Other recent studies have also demonstrated GVHD following limb allografting. Ramsamooj et al. transplanted limb allografts from Lewis to Lewis x Brown Norway F1 rats and reported that 7 of 19 recipients (38%) with rejection-free limb allografts showed lethal acute or chronic GVHD [23]. To prevent GVHD in chimeric hosts, Gorantla et al. transplanted irradiated limb allografts into BMT pretreated recipients [24]. All limb allografts survived up to 5 months without clinical signs of GVHD. The same group, Prabhume et al., then transplanted irradiated limb allografts and BMT simultaneously in order to raise the level of chimerism and prevent GVHD [25]. Following 28 days immunosuppression with FK506 and mycophenolate mofetil (MMF), limb allografts survived without rejection and without GVHD in the recipient.

To prevent GVHD after limb allografting, it may be necessary to regulate the chimeric cell [24]. Lethal GVHD after BMT has not been experienced until now because T cells were depleted *in vitro* and the bone marrow cell count was adjusted to 1×10^7–1×10^8. Following limb allograft or vascularised bone marrow allograft, all donor-cell types, including haematopoietic stem cells and stromal cells, can migrate into the recipient. We have no data on the total number of migrating bone marrow cells into the limb graft. CYP/G-CSF/FK506 combination therapy resulted in prolonged survival of limb allografts, but more studies aimed at controlling chronic GVHD are necessary.

Conclusion

Limb allografting could function as a vascularised carrier for bone marrow transplantation and contribute to a high level of chimerism in the recipient. Pretransplant CYP followed by G-CSF and FK506 treatment significantly prolonged survival of limb allografts but frequently caused chronic nonlethal GVHD in recipients. Pretreatment with CYP had dose-dependent effects, and a dose of 200 mg/kg appeared to significantly prolong limb-graft survival.

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Section 2-d

Simultaneous Vascularised Bone Marrow Transplantation to Promote Acceptance of Limb Allografts

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Introduction

Rejection is prevented in clinical hand transplantation using immunosuppressive drugs. However, as these are non-life-saving transplants, it would be ideal to achieve a level of chimerism and/or tolerance sufficient to withdraw the antirejection drugs without compromising functional recovery and sensibility return. One perceived means to obtaining improved survival of transplanted tissues is administration of bone marrow (BM) cells, which has been used in both clinical practice and experimental investigation [1, 2]. There are several approaches to using these cells. One is to use donor bone marrow cells in conjunction with conventional or modified immunosuppression to promote survival. This has been used in the clinic where it has met with limited success and in experimental transplantation where the outcome has generally been improved. Another approach is to use donor BM in conjunction with recipient preconditioning to create a stable mixed haematopoietic chimera. This has led to donor-specific tolerance in conjunction with the presence of donor cells in the thymus by a process involving central tolerance.

However, donor BM cells are usually administered as a single-cell suspension i.v., and their supply to the recipient is obviously limited to this. We hypothesise that vascularised donor bone graft containing BM cells, a vascularised bone marrow (VBM) allograft, may provide a continuous supply of donor-derived progenitor cells to the recipient and therefore may work better than isolated BM cells in inducing a useful level of chimerism and possibly tolerance. It has been shown that VBM can modestly prolong survival of skin grafts from the VBM donor strain when transplanted together [3]. The mechanism of prolongation of graft survival by VBM is not known but could involve central deletion of alloreactive T cells in the thymus, similar to mixed allogeneic chimerism [4].

An additional practical advantage of VBM allograft in hand transplantation would be that achieving a critical level of chimerism would serve as an indicator to begin tapering immunosuppressive drugs without risk of prompting an immediate rejection. In other words, drugs could be tapered only when reaching a level of chimerism deemed sufficient to avoid acute rejection.

Experimental Study

In a recent study, we examined whether a simultaneous transplantation of a limb allograft and VBM led to improved limb allograft survival compared with a more conventional administration of isolated donor BM cells. We also examined the pattern of donor chimerism in the recipients.

Brown Norway (BN) rats were used as donors and Lewis rats as recipients for orthotopic hindlimb transplantations and simultaneous vascularised BM transplantations or BM cell i.v. infu-

sions. There were three experimental groups, which consisted of: hind-limb transplantation alone (HLTX); hind-limb transplantation plus injection of recipient with BM harvested from crushed tibia and femur bones of BN rats (HLTX+BM) in which BM were flushed out by a syringe and injected i.v. through the femoral vein; hind-limb transplantation plus grafting of BN-strain VBM to the recipient (HLTX+VBM). The surgical procedure of hind-limb transplantation followed the one previously described [5, 6]. In the group receiving HLTX+VBM, we used a method for VBM involving an osteomyocutaneous free thigh flap that included a groin skin flap on the same general vascular pedicle as the femoral bone [7, 8]. The donor femur and skin were transplanted to an inguinal area adjacent to the HLTX. We used a modification of the published method because the VBM skin flap must have long enough vascular pedicles to carry out end-to-side anastomoses. This required the groin flap to be harvested with the superficial epigastric vessels. After reperfusion, the bone transplant was fixed by two stitches to the front muscle group to prevent malrotation. Finally, the skin wound was closed by interrupted 4-0 sutures. The skin groin flap allowed monitoring of VBM transplant survival.

Animals were sacrificed on days 1, 7, 30, 90 and 120 to assess chimerism. Donor chimerism was evaluated in spleen, blood, donor and recipient BM and thymus by two-colour flow cytometry. Rejection was assessed by our previously described visual grading system [9] as grade 0, no rejection; grade 1, pink or slightly red; grade 2, red; grade 3, pink red or purple; grade 4, blue purple, hair loss. All transplanted animals received immunosuppressive therapy of FK-506 2.5 mg/kg per day; mycophenolate mofetil (MMF) 20 mg/kg per day and prednisone (Pred) 0.5 mg/kg per day from the first postoperative day. After day 27, Pred and MMF were both tapered by 20% of the initial dose per week and stopped at day 56. FK-506 was tapered by 20% of the initial dose per week after day 49 and then stopped on day 70 postoperatively. Salvage therapy was used in all groups at the time of the first apparent signs of rejection. Survival at 3 months after transplantation was significantly better in the HLTX+VBM group (p=0.03 compared with HLTX), and there was no significant difference in the HLTX+BM compared with the HLTX group (p=0.22). At the completion of the experiment at 4 months, the HLTX+VBM group showed prolonged survival compared with the HLTX and HLTX+BM groups although this was not quite statistically significant (p=0.056). Analysis of donor-cell chimerism showed large numbers of donor cells surviving after hind-limb transplantation. This was most marked in the VBM graft. Thirty-four percent of cells were positive compared with 0.13% stained by the control, isotype-matched antibody. The number of donor cells diminished with time, and their distribution changed. In the early stages after transplantation (<30 days) most donor cells were found in the transplanted BM and particularly in the VBM of those animals that received a VBM transplant. In the VBM, there were 47.6±28% of donor cells while in the transplanted hind-limb BM, there were 17.8±32% of donor cells in animals that received a hind-limb transplant alone. Despite this increased level of chimerism in the VBM, there was no statistically significant difference between groups due to the large variability in levels of chimerism within groups. At the later stages after transplantation (>90 days), the pattern of chimerism had changed, and most donor cells in the marrow of the transplanted hind limb and the transplanted VBM graft had been replaced by recipient cells so that less than 2% of cells in the donor BM were of donor origin. At this later stage, the majority of donor cells were found in the recipient spleen and peripheral blood. There were no statistically significant differences in the extent of chimerism between treatment groups by analysis of variance (ANOVA) although there was a tendency for more donor cells in the VBM recipients in most compartments tested.

Discussion

Providing a potential source of donor BM that might increase the level of donor chimerism should improve the outcome of limb transplan-

tation by inducing the form of central tolerance that accompanies induction of mixed haematopoietic chimeras or through induction of microchimerism or by whatever mechanism donor BM promotes organ allograft acceptance. The approach we took to increasing the level of donor chimerism was to transplant a vascularised BM graft from the donor of the hind limb transplant, and this provided a continuing source of donor cells. This led to a slightly improved survival compared with untreated animals or those receiving an injection of BM cells. On the contrary, injection of BM cells did not result in significantly prolonged survival compared with HLTX alone. Many studies have shown that donor BM can promote acceptance of transplants in a number of animal models, and some studies have shown improvement of the outcome of clinical transplantation resulting from perioperative donor BM infusion [10].

The inability to promote acceptance in our model using donor BM might have been due to differences compared with published studies in dose, timing and number of injections used or to the fact that the HLTX model in Lewis strain recipients is a very rigorous one in which only treatments that have a marked immunomodulatory effect will prolong survival. Another reason for the lack of effect of BM cells is that conventional immunosuppressive drug therapy with calcineurin inhibitors or corticosteroids can inhibit donor leucocyte-dependent graft acceptance. The mechanism by which the VBM graft might have prolonged survival could have been by providing greater numbers of donor leucocytes in the induction phase of the allograft response, the time when most studies find that injected BM cells or leucocytes are most effective. Our examination of the levels of chimerism in recipients found a high percentage of donor cells in the VBM graft itself early after transplantation; however, the levels of chimerism in the tissues of VBM recipients did not show a significantly higher level compared with BM-treated or -untreated animals. This was the case in all tissues examined at all times after transplantation.

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Section 2-e

Experimental Approaches to Composite Tissue Allograft Transplants

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Introduction

Composite tissue allotransplantation has been recently introduced as a potential clinical treatment for complex reconstructive procedures, include traumatic injuries, cancer ablative surgeries, or extensive tissue loss secondary to burns. Composite tissue allografts (CTAs) consist of heterogeneous tissues derived from ectoderm and mesoderm, including skin, fat, muscle, nerves, lymph nodes, bone, cartilage, ligaments, and bone marrow, with different antigenicity. Thus, composite tissue structure is considered to be more immunogenic than solid organ transplants. While cartilage, ligaments, and fat present low antigenicity, bone, muscles, nerves, and vessels present moderate antigenicity, and skin is the component that develops the most severe rejection because of the abundance of dendritic cells within the epidermis and dermis. To study the mechanisms of CTA acceptance and rejection, different experimental models, strategies and different immunosuppressive protocols have used [1, 2].

Composite Tissue Allograft Transplantation Studies Using Immunosuppressive Protocols

In 1971, before the introduction of the cyclosporin A (CsA) to the CTA transplantation area, Lance et al. developed a canine hind-limb allotransplantation model and undertook painstaking comparison of immunomodulatory regimens that included combinations of azathioprine, hydrocortisone, and antilymphocyte serum. The researchers reported in excess of 300 days' graft survival rate in one animal. For the first time in the literature, this study revealed that immunologic barriers in CTA could be overcome and that long-term successful CTA transplantation was possible [3, 4]. In 1978, developments in the technical expertise of microvascular repair of rat hind limbs revealed alloantigenic systems which established the rat hind limb as the prototype model in CTA transplantation in [5–7]. In 1979, Doi published a study of rat limb allotransplantation across known histocompatibility barriers utilizing three nonspecific immunosuppressive agents (azathioprine, 6-mercaptopurine, and prednisolone) in different doses and combinations [8]. In this study, graft survival was shown to be extended in the treatment groups, especially in those treated with a combination of azathioprine and prednisolone. However, the most significant feature of this study was that 100% of the treated rats died from side-effects from the immunosuppressive regimen. For this reason, graft survival in these groups could not be ascertained because of early animal death (9–24 days posttransplant); histologic evidence of rejection was not apparent. All untreated allografts rejected between 10 and 15 days, with histologic confirmation. The researcher concluded that immunosuppressive therapy would require dramatic improvement

before CTA could be realized in clinical application [4].

The first report of CTA using CsA appeared in 1982. Black et al. [9] performed rat hind-limb transplantation in inbred strains of rats, with Lewis (LEW) ($RT1^1$) rats serving as recipients and Lewis Brown Norway (LBN) ($RT1^{1+n}$) F1 animals serving as donors. They reported extension of rat hind-limb survival from 18±5 days in untreated grafts to 101±13 days in animals receiving a 20-day perioperative course of 25 mg/kg per day dosage of CsA. This marked a significant breakthrough in CTA, making CsA the mainstay of immunomodulatory therapy in subsequent investigations for many years [4].

Most experimental studies on CTAs have been performed on rodents by using a hind-limb transplantation model. Monotherapies using calcineurin inhibitors [CsA, tacrolimus (FK-506)] are known to prolong allograft survival only if they are given in high doses and throughout the recipient's life. Only a few studies reported survival longer than 1 year by using CsA on major histocompatibility complex (MHC)-mismatched animals [10, 11]. The combined use of CsA with prednisone reduced the amount of CsA dose and prolonged graft survival up to 210 days, but again, infection and rejection rates were high [12]. We found that CsA combined with topical fluocinolone acetonide to prevent skin rejection extended allograft survival and allowed for the use of a reduced dose of CsA to achieve long-term survival [13]. The use of FK-506 as a single-dose (10 mg/kg) protocol on the day of transplant followed by single weekly injections (3 mg/kg) produced complete graft survival over 200 days [14]. FK-506 was also found to be more potent than CsA in a study using 114 hind-limb transplants across an MHC mismatch [15]. It is clearly understood that combination therapies are more successful than monotherapy protocols. Low doses of CsA with mycophenolate mofetil (MMF) showed long-term survival over 231 days in 89% of recipients, with a return of full sensory and partial motor function [16]. Combining FK-506 and 15-deoxyspergualin showed a 120-day rejection-free survival when both drugs were given for 30 days after transplantation [17]. Experimental rodent data reveal that effective immunosuppression and graft survival in CTAs can be achieved by using chronic administration of combination therapies, with substantial morbidity and mortality.

Experimental data on CTAs from large animals are limited. Ustuner and colleagues employed the swine as a large animal model for CTA research. Transplantation of a radial forelimb osteomyocutaneous flap between outbred swine using the combined treatment of CsA, MMF, and prednisone was reported. In this model, the graft consisted of a segment of the forelimb that included a portion of radius, the flexor carpi radialis, and overlying skin. The vascular supply was through the brachial artery and cephalic veins, and a segment of median nerve was included in to the CTA model [18]. In this study, three of eight allografts were found to be rejection free at 90 days after transplantation. Antirejection effect and systemic side-effects of combined FK-506 and MMF were assessed in a radial forelimb osteomyocutaneous flap model in outbred pigs. Five of nine animals survived for 90 days without any signs of rejection. It was found that this combination provided superior antirejection effect when compared with CsA/MMF regimen but showed more toxicity [19].

Lee et al. recently reported an inbred swine model of heterotopic partial limb allotransplantation [20]. The graft consisted of donor tibia, fibula, knee joint, distal femur, and associated musculature on a femoral arteriovenous pedicle; no skin was included. The vessels were anastomosed in an end-to-side fashion to recipient vessels and the graft inserted into a subcutaneous pocket on the recipient's abdomen. This is the only large animal model of CTA with a genetically defined histocompatibility barrier enabling the study of specific transplantation barriers between the donor and the recipient. CsA at 10mg/kg per day was administered intravenously to the recipient pig for 12 days, and the dose was adjusted based on serum levels. In this study, allografts from MHC-mismatched donors treated with CsA showed signs of rejection in less than 6 weeks, but in similarly matched donors, 178- to 280-day allograft survival was accomplished. Allografts in similarly matched group were harvested between 178 and 280 days after transplant. All grafts demonstrated patent vessels, bleeding from marrow cavities, and

viable bone and soft tissues on microscopic examination at harvest time. This study shows that tolerance to vascularized skeletal tissue allografts could be induced in MHC-matched miniature swine with minor antigen differences following a 12-day course of CsA [4].

Although primates are the best choice due to their close similarity to human immunophysiology, there are few studies to date concerning CTAs in primates. It was shown that hand transplantation in nonhuman primates is technically feasible and that functional results are promising. Daniel et al. reported four hand transplants and seven neurovascular free-flap allograft transplants in baboons [21]. High doses of CsA and steroids as immunosuppressive therapy were used. As a result, three of four hand allografts rejected, and only one survived up to 304 days. However, four neurovascular free flaps survived more than 4 months. Stark et al. used CsA (20 mg/kg) and prednisolone (1.5 mg/kg) in the hand transplantation model in baboons and reported long-term survival in one transplant (296 days); the others were rejected within 15 days [22].

In a partial hand transplantation study by Stevens et al. on rhesus monkeys, it was reported that the 21–33 survival days achieved by CsA/prednisolone treatments were increased up to 79–179 days by the addition of monoclonal antibodies (specific for CD3+, CD4+, CD8+, and MHC class II DR-positive cells) and blood transfusion [23]. Unfortunately, all recipients were lost due to sepsis or lymphoid tumor. In this study, the first signs of sensory recovery and motor reinnervation were detected at days 41 and 28, respectively.

Gold et al., in a study on cynomolgus monkeys using mandibular allograft as a CTA model, showed that mandibular allograft transplantation was technically possible, and recipients survived up to 65 days on CsA monotherapy [24].

Antilymphocyte Serum

Antilymphocyte serum (ALS) is a nonspecific lymphocyte-depleting agent. Massive depletion of T cells by ALS has been reported to induce tolerance [25]. Combinations of ALS with conventional immunosuppressants, such as CsA and tacrolimus, have been studied in different transplantation models [26]. Allograft recipients treated with a short protocol of antilymphocyte serum combined with the infusion of donor-specific bone marrow-derived cells showed induction of donor-specific tolerance in skin allografts in mice [27].

In our studies using a hind-limb allograft transplant model (between LBN and LEW rats), rejection-free survival was achieved over 420 days by using ALS and CsA therapy for 21 days [28]. Long-term survivors revealed 35–42% of donor-specific chimerism in peripheral blood. The unresponsiveness to donor specific antigens was confirmed by skin grafting in vivo. Mixed lymphocyte reaction revealed suppressed response against donor-type antigens and increased response to third-party antigens. The same protocol of combined antilymphocyte serum and CsA therapy for 21days was used in fully mismatched hind-limb allograft transplants [between Brown Norway (BN) and LEW rats] showed a survival rate of 51 days. Donor-derived chimerism peaked to 17% at day 35 and fell to 0% at the time of rejection [29].

Anti-T-Cell Receptor Monoclonal Antibodies

T-cell recognition of foreign MHC molecules plays a crucial role in the initiation of allograft rejection. T lymphocytes are classified as αβ or γλ, depending on the type of disulfide-linked heterodimeric glycoprotein receptor. Early studies targeting the T-cell receptor (TCR) by using a specific depleting mAb against its αβ subregion have been reported on an experimental cardiac allograft model [30]. The combined use of αβTCR mAb and CsA protocol successfully depleted αβTCR cells and created a therapeutic window of immunological incompetence, resulting in induction of donor-specific tolerance across the MHC barrier without recipient conditioning. A total of 120 hind-limb transplantations were performed in semiallogeneic models across MHC barriers between LBN and LEW rats. Allograft controls without treatment had graft rejection within 5–7 days after transplantation. Transplant recipients receiving monothera-

py with either CsA or αβTCR showed prolongation of limb survival up to 21 and 13 days, respectively. All transplants under the combined αβTCR/CsA protocol for 35 days survived more than 750 days. The combined use of αβTCR antibody and CsA therapy successfully depleted T-cell population (by more than 95%) and created a therapeutic window of αβT-cell mediated immunological silence between days 21 and 35. At day 35, immunosuppressive therapy was discontinued, and T-cell levels returned to 84% of pretransplant rate at day 64. After cessation of immunosuppression, tolerance was confirmed by acceptance of donor skin grafts 100 days after transplantation [31, 32].

Experimental Models of CTA Transplants

During the almost 20 years of our research in the field of CTA transplants, we have designed and developed different CTA models testing different immunosuppressive protocols of tolerance induction. These models include: hind-limb transplants, composite vascularized skin (VS) and femoral bone transplants, bone marrow transplants (BMT), VS allograft transplants, vascularized bone marrow transplants (VBMT), bilateral vascularized femoral BMT, composite hemiface/calvarium transplants, rat maxilla allotransplants, composite osteomusculocutaneous hemiface/mandible/tongue-flap transplants, combined semimembranosus muscle and epigastric skin flap transplants, cremaster muscle CTA transplants, vascularized laryngeal allograft transplants, full face/scalp transplants, and hemiface transplants.

Hind-limb Transplant Models

We have performed hind-limb transplantation techniques in over 1,000 hind-limb transplants across MHC barriers between fully allogeneic BN and semi-allogeneic LBN donors and LEW recipients. Different immunosuppressive protocols of CsA monotherapy, combined CsA with topical fluocinolone acetonide, FK-506 monotherapy, combined CsA/ αβTCR mAb and CsA/ALS were used for different dose and time regiments. Flow cytometry was used to assess the efficacy of immunosuppressive protocols and donor-specific chimerism. Clinical tolerance and immunocompetence were confirmed by skin grafting in vivo and by mixed lymphocyte reaction in vitro. Combined protocols of CsA/ αβTCR mAb and CsA/ALS resulted in long-term survival and donor-specific tolerance in the hind-limb allografts [13, 28, 29, 33–35]

We also evaluated the role of host thymus in tolerance induction in CTA across the MHC barrier during a 7-day αβT-cell-receptor (TCR)/CsA protocol. In this study, we evaluated the role of host thymus in tolerance induction in CTA across MHC barrier during a 7-day αβT-cell-receptor (TCR)/CsA protocol. Isograft transplants survived indefinitely. For thymectomized rats, the median survival time (MST) of limb allograft in nontreated recipients was 7 days; monotherapy with αβTCR extended MST to 16 days, and CsA therapy extended it to 30 days. Using the αβTCR/CsA protocol, the MST of allografts was 51 days. For euthymic rats, the MST of limb allograft in nontreated recipients was 7 days; monotherapy with αβTCR or CsA extended MST to 13 or 22 days, respectively. Treatment with αβTCR/CsA resulted in indefinite allografts survival (MST=370 days). Mixed leukocyte reaction (MLR) and skin grafting confirmed donor-specific tolerance in euthymic recipients. Flow cytometry showed stable chimerism in the euthymic rats and transient chimerism in thymectomized limb recipients. Immunoperoxidase staining revealed the persistence of donor-derived cells in the lymphoid tissues of euthymic recipients. We found that the presence of thymus was imperative for induction of donor-specific tolerance in rat hind-limb CTAs using a αβTCR/CsA protocol [36].

Composite Vascularized Skin and Femoral Bone Transplant Models

To test the importance of vascularized bone marrow in tolerance induction, we designed a new model of combined vascularized groin skin

and bone marrow transplantation. Transplants were performed between LEW rats. Combined groin-skin and femoral-bone flaps were transplanted based on the femoral vessels. All flaps survived over 100 days posttransplant. Histologic examination of the femoral bone revealed active hematopoiesis with viable compact and cancellous bone components at day 100 posttransplant. These models can be applied directly to tolerance induction study across the MHC barrier, where bone will serve the same as delivery of donor stem and progenitor cells and the skin component will serve as a monitor of graft rejection.

In this model, we describe the anatomical and technical feasibility of a new VBMT model in which the rat femur is transplanted with the groin cutaneous skin flap. In this model, vascularized bone marrow is transplanted within its own space, along with its natural microenvironment, which facilitates cell engraftment and does not need recipient conditioning to create space for such an engraftment.

Mean times for the composite skin/bone graft harvesting ranged from 10 to 20 min and for isograft transplantation about 45 min; mean ischemia time was around 45 min. Successful flap transplantation was accomplished in all ten animals. All animals tolerated the operation well and returned to their normal activities the day after transplantation. Body weight was stable, and no signs of infection were noticed. Mild to moderate hematoma formation was observed under the skin flap in four animals; this resolved spontaneously within 8–12 days. Clinically, all flaps were pink and pliable during the entire observation period. New hair growth was observed within 20–25 days posttransplant. Histological results showed normal (grade 0) skin histology and a viable compact and cancellous bone in the entire femur, except for the femoral head. Active hematopoiesis of the transplanted bone marrow was noted. Radiological evaluation with barium sulfate showed that the main arterial branches supplying the femur were well preserved within the flap [37].

Based on this study, we introduced different experimental models of modified VS/bone marrow (VSBM) transplantation techniques for tolerance induction, monitoring, and maintenance studies. In this skin/bone transplantation model, the technical feasibility of concurrent or consecutive transplantation of the combination of bilateral VS, vascularized bone marrow, or VSBM transplants was investigated.

Isograft transplantations were performed between genetically identical LEW RT11 rats. Five different experimental designs in five groups of five animals each were studied: group 1: bilateral VS transplantation; group 2: bilateral VS/bone transplantation; group 3: VS transplantation on one side and VS/bone transplantation on the contralateral side; group 4: vascularized bone transplantation on one side and VS/bone transplantation on the contralateral side; group 5: vascularized bone transplantation on one side and VS transplantation on the contralateral side. All skin flaps remained pink and pliable and grew new hair. The viability of the compact bone, bone marrow, and skin at 100 days posttransplant was confirmed by histologic evaluation, and bone marrow revealed active hematopoiesis.

The bilateral skin/bone transplantation model may serve as an experimental tool to study new strategies in tolerance induction by altering the amount of immunogenic load in the form of skin transplant and bone marrow delivery in the vascularized form, allowing for expedited engraftment of stem and progenitor cells [38].

Bone Marrow Transplantation Models

To extend VS allograft survival, we cotransplanted crude bone marrow without marrow processing or recipient conditioning. Skin graft transplants were performed between semiallogeneic LBN donors and LEW recipients under CsA or αβTCR mAb alone, or combined CsA and αβTCR mAb for 35 days. Monotherapies combined with crude BMT extended survival of skin allografts up to 21 days under CsA and up to 10 days under the αβTCR mAb protocol. The use of combined CsA and αβTCR mAb therapy with crude BMT extended skin allograft survival up to 65 days in this VS allograft model.

We have also investigated the effect of different routes and dosages of donor-derived bone marrow cell transplantation on donor-specific tolerance induction and chimerism across the MHC barrier under a short, 7-day protocol of CsA monotherapy and combined CsA/ αβTCR therapy. Intraosseous and intravenous bone marrow cells were transplanted between BN donors and LEW recipients. Flow cytometry assessed immunodepletion and donor-specific chimerism. All animals survived without graft-versus-host (GVH) disease. Intraosseous transplantation of donor-specific bone marrow cell was 75% more efficient in induction of donor-specific chimerism compared with intravenous transplantation, and the level was 50% higher in animals that received 70?106 bone marrow cells (9.9%) when compared with animals that received 35?106 bone marrow cells (4.9%). These studies confirmed tolerogenic properties of donor BMT directly into the bone, representing natural microenvironment for bone marrow seeding and repopulation [39, 40].

Vascularized Skin Allograft Transplant Model

We used a vascularized groin skin allograft transplant model to evaluate the potential for tolerance induction in this highly immunogenic tissue graft. Vascularized groin skin transplants based on the femoral vessels were performed between fully allogeneic ACI (RT1a) donors and LEW recipients. Animals received either αβTCR mAb, CsA, or FK-506 therapy or combined different immunosuppressive protocols αβTCR mAb/CsA and αβTCR mAb/FK-506 therapy given only for 7 days to test potential for chimerism induction and to extend graft survival. The combined αβTCR mAb/CsA and αβTCR mAb/FK-506 protocols were effective in inducing and maintaining chimerism and substantially extended the survival of the VS allograft transplants across the MHC barrier [41] (Figs. 1, 2).

Vascularized Bone Marrow Transplantation Models

VBMT as a part of CTA transplantation forms a solid basis for chimerism. Tolerogenic- and chimerism-inducing effects of bone marrow cells are known. Vascularized bone as a component of CTA provides the stromal hematopoietic microenvironment as well as pluripotent progenitor cell source and seems to be more efficient and essential for posttransplant lym-

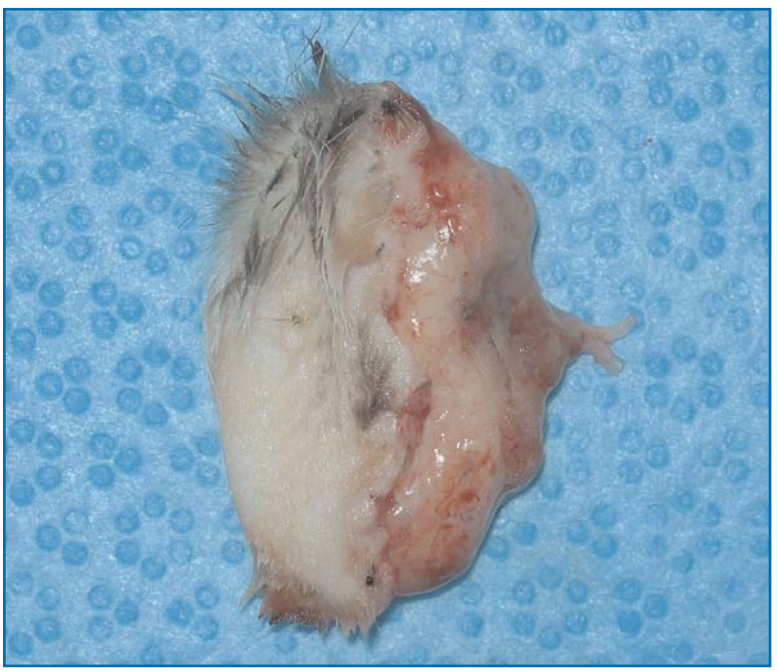

Fig. 1. Appearance of the harvested vascularized skin allograft from fully allogeneic ACI (RT1a) donors

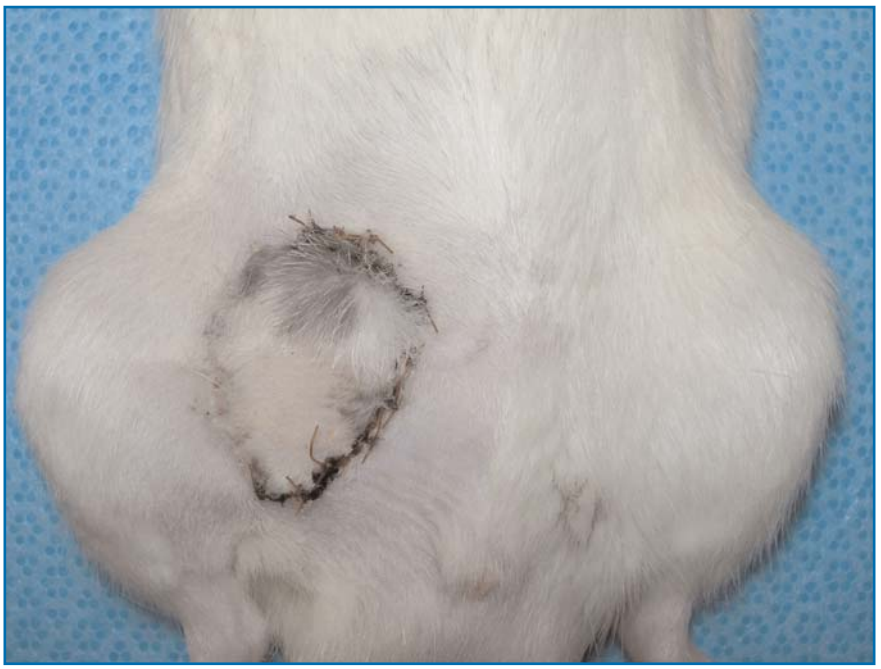

Fig. 2. Vascularized groin skin transplants based on the femoral vessels were performed between fully allogeneic ACI (RT1a) donors and Lewis (RT11) recipients

phopoiesis when compared with cellular bone marrow transplanted intravenously in the form of cellular suspension.

Different models of VBMTs have been introduced. Limb transplantation was the first vascularized bone marrow model used for this purpose. Hind-limb allografts are vascularized carriers of bone marrow, serving as constant sources of donor hematopoietic stem cells capable of inducing donor-specific tolerance and chimerism [42]. It has been suggested that vascularized bone marrow in the form of the hind-limb graft could provide repopulation of bone marrow cells in rats receiving total-body irradiation [43]. This rapid engraftment and repopulation of bone marrow cells were probably related to the preservation of bone marrow stromal cells in the form of vascularized bone marrow, a component of the limb allograft. Hind-limb transplants were performed between LBN donors and LEW recipients to test the effect of 21-, 7-, and 5-day protocols of combined αβT-cell-receptor monoclonal antibody (αβTCR mAb)/CsA treatment on tolerance induction [33]. All transplants under combined αβTCR/CsA therapy survived over 350 days. Clinical tolerance and immunocompetence were confirmed by skin grafting in vivo and MLR in vitro. All recipients at day 100 posttransplant uniformly accepted skin allografts from donor (LBN) and the recipient (LEW) but rejected third-party (ACI) grafts. We confirmed that the 5-day protocol was long enough to maintain immunological unresponsiveness of a new repertoire of recipient T cells, which led to the engraftment of donor hematopoietic stem cells. This was confirmed by donor-specific chimerism of 10–12 % for double-positive CD4 cells and 6–9% for double-positive CD8 T-cell subpopulations on day 120 posttransplant in all combined treatment groups.

The hind-limb transplant model has the disadvantages of containing different types of tissues, such as skin, ligaments, and muscles with their variable degree of antigenic properties. It is also associated with a high ratio of recipient morbidity and mortality. To get rid of the antigenic properties of other tissues, isolated VBMT models were developed. These include femur, sternum, and tibia as a source bone marrow cells [44–46].

We introduced a new model of combined vascularized groin skin and femoral BMT based on femoral vessels [37] (Fig. 3). Our research on VBMT by using rat epigastric free flap alone or in combination with vascularized femur allografts under treatment of αβTCR/CsA for 7 days

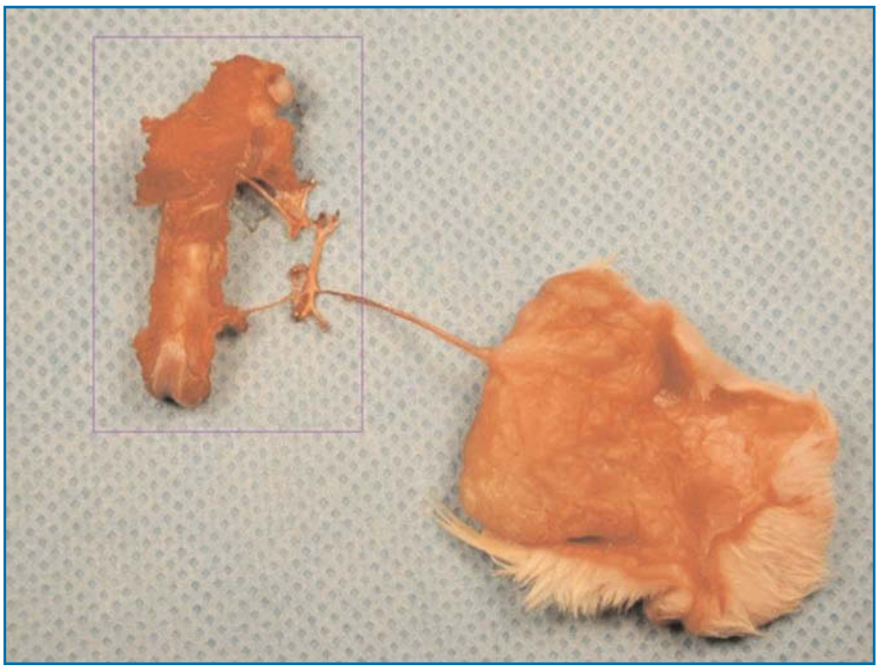

Fig. 3. Anatomy of harvested composite vascularized skin/bone graft

revealed that when the epigastric free flap was used alone, the survival rate of the flap was 25 days. When skin flap transplantation was combined with vascularized femoral allografts, survival over 60 days was achieved, with no signs of rejection or GVH [47] (Fig. 4).

We investigated the effect of different routes of bone marrow cell transplantation on donor-specific tolerance induction across the MHC barrier under our established 7-day protocol of αβTCR/CsA therapy in a fully MHC-mismatched rat model. Our observation indicated that donor-specific chimerism at day 63 was 75% higher in intraosseous (9.9%) when compared with intravenous (3.4%) transplant groups receiving 70×10^6 of BMT. Following intraosseous BMT under the αβTCR/CsA protocol, multilineage chimerism was 50% higher in the group receiving 70×10^6 bone marrow cells (9.9%) compared with 75×10^6 bone marrow cell delivery (4.9%) [48].

Tai et al. [49] described the extraperitoneal model with placement of the bone into a subcutaneous pocket created in the anterior abdominal wall of the recipients. They passed a single-loop stitch around the neck of the femur bone to the rectus muscle to prevent graft migration of the graft.

In our ongoing studies regarding tolerance induction after vascularized femoral bone transplantation, vascularized grafts were harvested in a manner similar to that described by Suzuki et al. [44]. Briefly, after wide exploration of the right inguinal region and the leg, the superficial epigastric and saphenous vessels were ligated and divided. The popliteal space was explored, and muscular branches and femoral vessel were ligated and transected distally. Both the lateral femoral circumflex and superficial circumflex iliac arteries were preserved, as they are the nutrient and periosteal arteries to the femoral bone. The femur bone was disarticulated both proximally and distally. Femoral vessels were then dissected proximally, and the flap was harvested. In the recipient, vascular anastomoses were performed to femoral vessels in an end-to-end fashion. Flap insetting was completed in the inguinal region of the recipient. Unlike Tai et al., [49] we placed the vascularized bone graft directly into the inguinal region of the recipient without creating any additional subcutaneous pocket or applying a suture for bone fixation. This decreased the operative time and the possibility of complications caused by the creation of a subcutaneous pocket, such as seroma or hematoma. After approximation of the inguinal

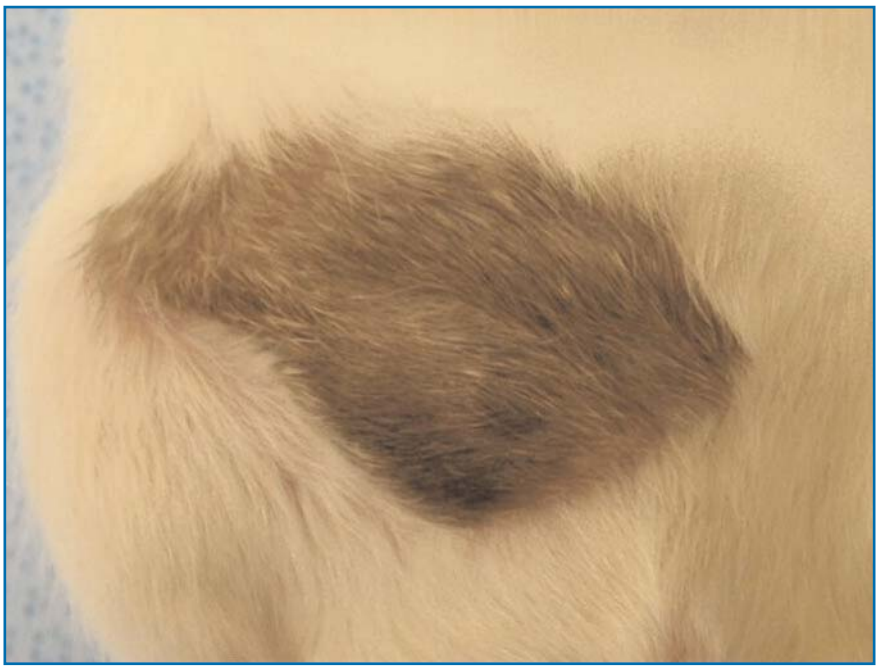

Fig. 4. Composite vascularized skin/bone graft at day 60 posttransplant revealed complete survival

fat pad over the flap with three interrupted absorbable sutures, the skin was closed using 4-0 chromic catgut sutures. More than 50 transplants were performed, and the patency of the anastomosis was confirmed in all animals. Like Tai et al., [49] we also found that ligation of the femoral vessels had no critical effect on the vascularity of the recipient's hind limb. Placement of the vascularized bone graft in the inguinal region did not interfere with the recipients' mobility [50].

Bilateral Vascularized Femoral Bone Marrow Transplant Model

Encouraged by the tolerogenic properties of VBMT, we introduced a new model of VBMT – the bilateral vascularized femoral bone (BVFB) isograft and allograft transplant based on abdominal aorta and inferior vena cava. Transplants were performed between LEW rats. In the donor, both femoral bones were harvested based on the abdominal aorta and inferior vena cava. In the recipient, the harvested isograft transplants were transferred into the abdominal cavity. The vascular pedicles of transplants were patent, and the bones were viable during the follow-up period of 63 days posttransplant. We confirmed the feasibility of BVFB transplantation based on abdominal aorta and inferior vena cava.

As a continuation of the previous study, we tested the efficacy of the BVFB model in induction of chimerism across the MHC barrier under the combined CsA/ αβTCR mAb protocol given for 7 days. Transplants were performed between BN donors and LEW recipients. At day 21, peak level of donor-specific chimerism of 24.2 % was confirmed in peripheral blood of BVFB recipients. At day 63, the level of chimerism declined to 1.5% and was maintained at this level thereafter. Histological examination revealed viable bone marrow cells up to 35 days posttransplant. The BVFB transplant model can be used for tolerance induction protocols [51, 52].

Composite Hemiface/Calvarium Transplantation Model in the Rat

We introduced a new composite hemiface/calvarium transplantation model in the rat. The purpose of this composite tissue model was to extend application of the face/scalp transplantation model in the rat by incorporation of the vas-

cularized calvarial bone, based on the same vascular pedicle, as a new treatment option for extensive craniomaxillofacial deformities with large bone defects. Seven composite hemiface/calvarium transplantations were performed across the MHC barrier between LBN and LEW rats. Seven donor and seven recipient rats were used in this study. Hemicalvarial bone and face grafts were dissected on the same pedicle of the common carotid artery and jugular vein and were transplanted to the deepithelized donor faces. All rats received tapered and continuous doses of CsA monotherapy. Evaluation methods included flap angiographies, daily inspection, computed tomography (CT) scan, and bone histology. Flap angiography demonstrated the vascular supply of the bone. The average survival time was 154 days. No signs of rejection and no flap loss were noted at 220 days posttransplantation. Bone histology at days 7, 30, 63, and 100 posttransplantation revealed viable bone at all time points, and CT scans taken at days 14, 30, and 100 revealed normal bones without resorption. For extensive face deformities involving large bone and soft tissue defects, this new osteomusculocutaneous hemiface/calvarium flap model may serve to create new reconstructive options for coverage during one surgical procedure [53].

Maxilla Allotransplantation Model

We developed a rat model to test the effects of vascularized maxilla allotransplantation on composite maxillary substructures. Allograft maxilla transplantations were performed across the MHC barrier between ten LBN and ten LEW recipient rats under CsA monotherapy. Grafts were dissected along Le-Fort II osteotomy lines based on the common carotid artery and external jugular vein and transplanted to the anterior abdominal wall via microvascular anastomosis. Allografts were examined by tomography, flow cytometry, angiography, and histology. llograft survived up to 105 days without signs of rejection. A high level of donor-specific chimerism for T-cell and B-cell lineages was maintained. The incisors continued to grow; teeth buds, bone, cartilage, and mucosa remained intact. Moderate inflammation of the nasal, oral mucosa, and keratinous metaplasia were noted histologically. We created a maxilla allotransplantation model that allows the study of immunologic responses and demonstrates potential clinical applications based on growth properties of the allograft. n the long-term surviving allograft recipients, over 105 days, there were no indications of flap loss, partial necrosis, or rejection. The incisors grew over 10 mm during the follow-up.

Flow cytometry analysis of donor-specific chimerism in the peripheral blood was performed at day 105 posttransplant and revealed 12.5% of CD4FITC/RT1n-Cy7 and 5.3% of CD8PE/RT1n-Cy7 T-cell subpopulations. Analysis of the B-cell population revealed 4.7% of CD45RAPE/RT1n-Cy7 donor-derived cells in the peripheral blood of the maxilla recipient. Histologic evaluation revealed intact and remarkable structures, including teeth, teeth buds, teeth pulp, bone, cartilage, oral mucosa, nasal mucosa, and soft-palate musculature. No histopathological signs suggesting allograft rejection were noted for any component of the graft, including teeth, mucosa, bone, muscle, cartilage, nerve, or vascular tissue [54].

Composite Osteomusculocutaneous Hemiface/Mandible/Tongue-Flap Model

We introduced a new model of composite osteomusculocutaneous hemiface/mandible/tongue allograft transplant. The purpose of this new flap model was to extend application of the face/scalp transplantation model in the rat by incorporation of the vascularized mandible, masseter, and tongue, based on the same vascular pedicle, as a new reconstructive option for extensive head and neck deformities with large soft- and bone-tissue defects.

A total of 12 composite osteomusculocutaneous hemiface/mandible/tongue transplantations were performed in two experimental groups. Group 1 isotransplantation between LEW rats served as control without treatment (n=6). Group 2 (n=6) composite hemiface/mandible/tongue transplants were performed

across the MHC barrier between LBN donors and LEW recipients. Hemimandibular bone, masseter muscle, tongue, and hemifacial flaps were dissected on the same pedicle of the external carotid artery and jugular vein and were transplanted to the donor inguinal region. All allogeneic transplant recipients received 16 mg/kg per day CsA monotherapy tapered to 2 mg/kg per day and maintained at this level thereafter. All animals were monitored for signs of rejection, such as erythema, edema, hair loss, and desquamation. Flap angiography and CT scan evaluated allograft viability. Flow cytometry assessed donor-specific chimerism for MHC class I RT1n antigen. Hematoxylin and eosin (H&E) staining revealed bone histology and tested inflammatory response and grade of allograft rejection.

Isograft controls survived indefinitely. Six hemiface/mandible/tongue allotransplants survived up to 100 days (under observation at time of this writing). Flap angiography demonstrated intact vascular supply to the bone. No signs of rejection and no flap loss were noted. CT scan and bone histology confirmed viability of bone components of the composite allografts. Viability of the tongue was confirmed by pink color, bleeding after puncture, and histology. H&E staining determined the presence of viable bone marrow cells within the transplanted mandible. Donor-specific chimerism at day 100 posttransplant was evaluated by the presence of donor T cells (2.7% CD4/RT1n, 1.2% CD8/RT1n) and B cells (11.5% CD45RA/RT1n).

Long-term allograft acceptance was accompanied by donor-specific chimerism supported by VBMT of the mandibular component. This model may serve as a new reconstructive option performed in one surgical procedure for coverage of extensive head and neck deformities involving large bone and soft-tissue defects [55, 56].

Combined Semimembranosus Muscle and Epigastric Skin Flap Model

We developed a new model of combined semimembranosus muscle and epigastric skin free flap based on a single pedicle consisting of the muscular branch of the semimembranosus muscle and superficial epigastric vessels in continuity with femoral vessels.

Eight combined semimembranosus muscle and epigastric autogenous skin flaps based on the muscular branches and superficial epigastric vessels in continuity with femoral vessels were transferred to the neck (four flaps) and contralateral groin (four flaps) recipient sites. All animals survived after surgery, and all flaps were viable as checked by direct observation of color and temperature. Ligation of the femoral vessels had no significant effect on the vascularity of hind limbs both on the side from which the flap was harvested and on the contralateral side where the flap was anastomosed to the femoral vessels in the end-to-end fashion. All flaps, including muscle and skin components, were viable at postoperative day 7. Vascular patency of the pedicles was confirmed under an operating microscope. The success rate for the flap transfer was 100%.

This model of combined muscle and skin flap has several advantages. It is reliable, versatile, and easy to dissect, with a long vascular pedicle and adequate vessel diameter for anastomoses. It can be used for different applications, including microcirculatory, pharmacologic, physiological, biochemical, and immunologic studies [57].

Cremaster Muscle Composite Tissue Allograft Transplantation Model

A new mouse CTA transplantation model was developed to study microcirculatory changes during acute allograft rejection and ischemia/reperfusion (I/R) injury. Donor cremaster muscle allografts were prepared as tube flaps, harvested on the common iliac vessels, transplanted to the neck region of the recipient, and anastomosed to the recipient's ipsilateral carotid artery and external jugular vein using the standard end-to-end microsurgical technique. In group 1 (n=6), the hemodynamics of cremasteric muscle microcirculation was measured in C57BL/6N mice without transplantation for baseline data. In group 2 (n=6), isograft transplantations were performed between

C57BL/6N mice. In group 3 (n=5), allograft transplantations were performed across a high histocompatibility barrier between C3H and C57BL/6N mice. Following transplantation, cremaster muscle tube flaps were prepared for standard microcirculatory measurements of functional capillary perfusion, diameter, and red blood cell (RBC) velocities of first-, second-, and third-order arterioles and venules, and numbers of rolling, adhering, and transmigrating leukocytes and lymphocytes. Hemodynamic parameters of microcirculation did not differ significantly between the three groups. However, the number of rolling, adhering, and transmigrating polymorphonuclear leukocytes and lymphocytes was significantly increased in the allograft group (p<0.001) as early as 2 h following transplantation.

Cremaster muscle transplantation in mice is a reliable and reproducible model, with a 95% immediate success rate. The model offers the unique possibility of studying leukocyte–endothelial interaction during acute allograft rejection and I/R injury in the mouse [58].

Vascularized Laryngeal Allograft Transplantation Model

In 1992, Strome et al. developed a vascularized laryngeal allograft transplantation model to reexamine the potential for laryngeal transplantation, and this model contributed to the first successful human larynx transplant in 1998. In the first model introduced in 1992, the allografts were sited in tandem with the intact recipient larynges and were not innervated. A total of 16 animals were studied, and 14 rats had a 64% arterial patency at intervals of 1–14 days. Over 1,500 rat transplants later, numerous modifications have improved the applicability of this model to the CTA transplantation field [59, 60].

We applied the αβTCR mAb protocol along with tacrolimus to the existing rat model of laryngeal transplantation as a tolerance-inducing strategy. Larynges were transplanted from LBN donors to LEW recipients. Recipients received 7 days of treatment with tacrolimus and mouse anti-rat αβTCR mAbs. Histology, MLR, skin grafting, and flow cytometry assessed functional tolerance, efficacy of immunodepletion, and donor-specific chimerism. All ten recipients survived until sacrifice at 100 days. Histology suggested functional allograft tolerance. In this rat laryngeal-transplantation model, functional tolerance was induced under the combined tacrolimus and αβTCR protocol [61].

New Applications of CTA Transplants

Facial transplantation is the new approach in CTA transplantation to treat patients whose facial disfigurement cannot be addressed by conventional methods of reconstructive surgery.

Face Transplantation Models

In preparation for facial allograft transplantation in humans, we have developed full-face and hemiface skin transplant models to test different immunosuppressive protocols and tolerance induction across semi- and full allogeneic histocompatibility barriers [62–66].

Full-Face/Scalp Transplant Model

We have confirmed the feasibility of total facial/scalp allograft transplantation across MHC barriers in the rodent model for the first time. Transplants were performed between semi-allogeneic LBN donors and LEW recipients. In donors, based on the bilateral common carotid arteries and external jugular veins, the entire facial skin and scalp flap, including both ears, were harvested. In the recipient, a facial/scalp defect was created by excising facial skin, scalp, and external ears. Facial nerves and muscles and the perioral and the periorbital regions were preserved to avoid functional deficits, which could interfere with animal feeding, breathing, and eye closure. Both common carotid arteries were used to vascularize the full facial/scalp flap. Arterial anastomoses were performed to the

common carotid arteries (end to side) or external carotid arteries (end to end) of the recipients. Venous anastomoses were performed to the external jugular and anterior facial veins (end to end). Postoperatively, the recipient animal received CsA monotherapy of 16 mg/kg per day tapered to 2 mg/kg per day over 4 weeks and maintained at this level during the follow-up period [62, 63].

Modifications of Full-Face/Scalp Transplant Model

The full-face/scalp transplant model is technically challenging and takes over 6 h to perform. To improve the survival of facial/scalp allograft recipients, two different modifications of arterial anastomoses in recipients were introduced. The unilateral common carotid artery of the recipient was used to vascularize the full transplanted facial/scalp flap.

In the first modification, following arterial anastomosis between the left common carotid artery of the donor face flap and the left common carotid artery of the recipient (end to side), the right common carotid artery of the flap was anastomosed to the left common carotid artery of the flap using the end-to-side technique. In the second modification, following the arterial anastomosis between the left common carotid artery of the donor face flap and the left common carotid artery of the recipient (end to side), the right common carotid artery of the flap was anastomosed to the long stump of the internal carotid artery on the left side of the face flap in the end-to-end manner.

These arterial modifications have significantly reduced the complications associated with the bilateral common carotid arteries anastomoses and subsequently the post-operatave mortality of the animals. Full facial/scalp allograft transplants were performed between fully allogeneic ACI donors and Lewis recipients. The same tapered dose CsA monotherapy immunosuppressive protocol was used and over 180 days of facial/scalp allograft transplant survival was achieved [64].

Hemiface Transplant Model

To further shorten surgery and brain ischemia, time we introduced the hemifacial allograft transplant model, which is technically less challenging compared with the full-face/scalp model. This model was used to test induction of operational tolerance across MHC barriers. Hemifacial allograft transplants were performed between semiallogeneic LBN and fully allogeneic ACI donors and LEW recipients. Composite hemifacial/scalp flaps including the external ear and scalp and based on the common carotid artery and external jugular vein were harvested from the donors. In the recipient, the hemifacial/scalp skin, including the external ear, was excised. The arterial and venous anastomoses were performed to the common carotid artery (end to side) and to the external jugular vein (end to end), respectively. The same CsA monotherapy immunosuppressive protocol was used, and 400 days' survival was achieved for semiallogeneic transplants and 330 days in the fully MHC mismatched hemifacial transplant recipients [65, 66] (Figs. 5, 6).

Conclusion

We present the experimental applications of CTA transplantation. Functional and aesthetic outcome following application of conventional reconstructive procedures or prosthetic materials is not satisfactory, especially in patients with severe deformities and disabilities. Since the first successful hand transplantation in France in1998, CTA transplantation has gained a great deal of interest in the field of plastic surgery. So far, more than 50 CTA transplants have been reported. It is obvious that CTA transplantation will improve patients' life quality, but this might be at the expense of decreasing the life expectancy of these patients. Currently, the main obstacle for CTA transplantation is the use of life-long immunosuppression therapy because of its well-known side-effects, such serious infections, organ toxicities, and malignancies. In addition,

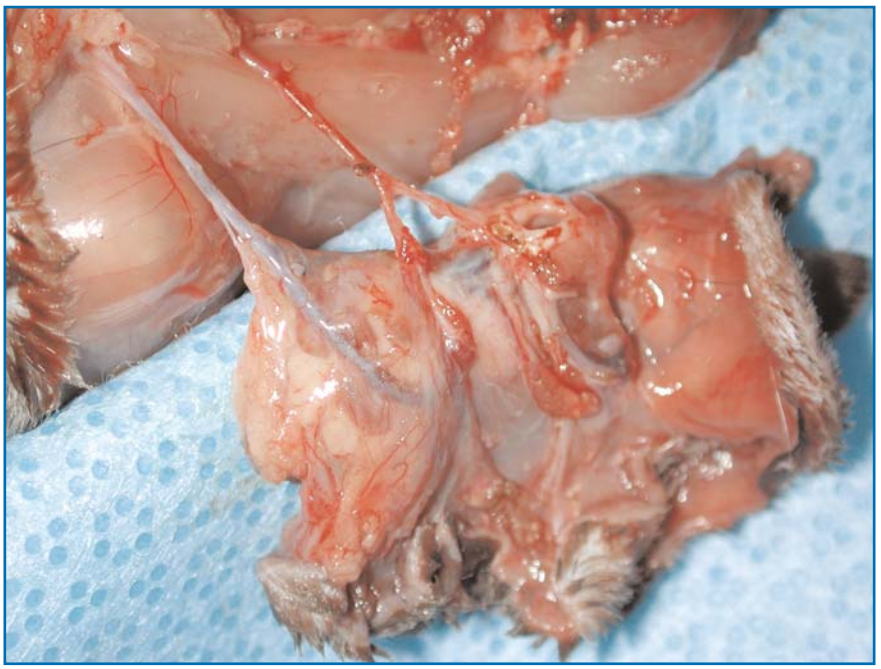

Fig. 5. Hemiface transplantation: composite hemifacial/scalp flaps, including the external ear and scalp, based on the common carotid artery and external jugular vein, were harvested from the donors

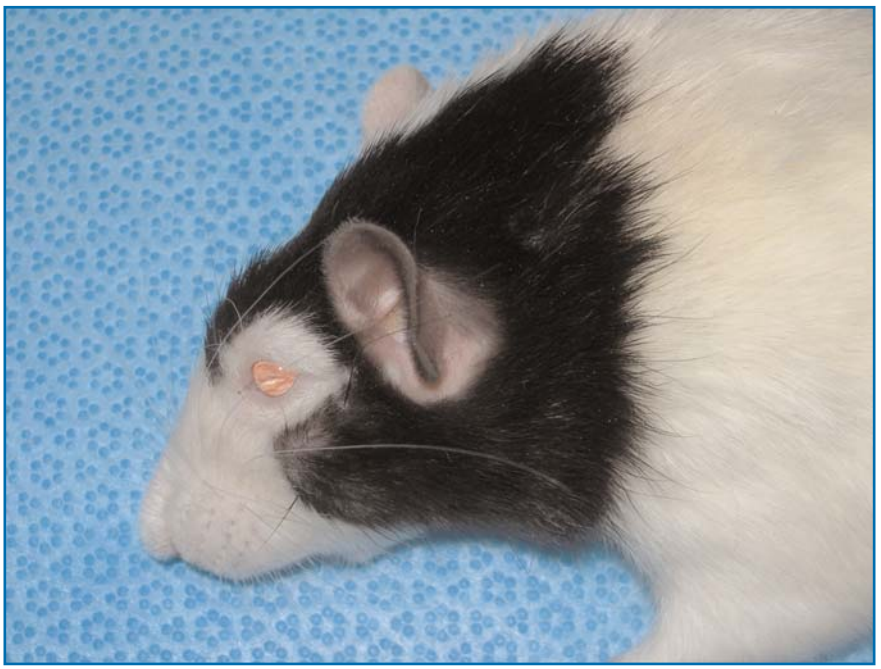

Fig. 6. Hemifacial allograft transplants were performed between semiallogeneic Lewis Brown Norway (RT11+n) donors and Lewis (RT11) recipients. Late postoperative view (day 150) with no signs of rejection under low-maintenance dose of cyclosporin A

ethical, social, and psychological issues are raised when discussing face transplantation. The long-term results of the recently performed partial face transplantations will be critical in order to judge the future applications of partial or total face transplantation.

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Section 2-f

A Review of Current Strategies to Achieve Tolerance in Animal Models

Dragos Zamfirescu, Ioan Lascar

Introduction

The current objective of transplantation is to prolong allograft survival indefinitely without the complications associated with chronic immunosuppression (infection, malignancy, adverse metabolic effects, drug toxicity) and without the development of chronic rejection. However, the ultimate goal of all transplant surgeons, the "Holy Grail" of transplantation, is to achieve indefinite clinical allograft acceptance without the need for long-term immunosuppression while the immune response to all other antigens remains intact – donor-specific tolerance [1, 2]. Over the last 50 years, various strategies have been tried to induce transplantation tolerance. Many of these have been validated in rodent models but have shown less degrees of success after extension to large animals, nonhuman primates and humans.

The promising field of composite tissue allografts (CTAs) offers tremendous potential for reconstruction of composite tissue defects, but the balance between the risks of lifelong immunosuppression *versus* quality-of-life improvement benefits is more difficult to justify than for the life-saving transplants. Therefore, a safe protocol for tolerance induction to CTAs that would eliminate the need for chronic immunosuppression and may also prevent the onset of chronic rejection could significantly expand the indications for composite tissue transplantation and could revolutionise the field of reconstructive surgery.

A CTA has two characteristics that make it different from solid-organ allografts: it is made up of tissues that are highly antigenic and capable of differential tissue rejection, and it may contain bone marrow (BM), which has the potential to modify the recipient's immune response. A vascularised CTA is composed of a large spectrum of ectodermal tissues: epidermis and epidermal derivatives (nails, hair, sweat and sebaceous glands and exocrine glands); and nerves and mesodermal tissues: dermis, subcutaneous tissue, muscles, fascia, bones, articular cartilage, tendons, other supportive and connective tissues, vessels, and haematopoietic tissues or cells from BM, blood or lymph nodes. Each tissue has differing antigenicity, and therefore, CTAs elicit nonsynchronised immune responses of differing intensity among their tissue components. The differing immunogenicity of different organs and tissues and the classical organs antigenicity hierarchy are a well-described phenomenon: *skin* > BM > small bowel > lung > pancreas > islets > kidney > liver > bone > cornea. Combining the result of many modern studies [3, 4], we summarised the tissues hierarchy of the antigenicity in CTAs: *skin* (epidermis > dermis) > subcutaneous tissue/BM > vascular endothelium (vein > artery)/muscle/periosteum > nerve > bone (osteocytes) > tendons/cartilage (chondrocytes).

Skin is often an important component of a CTA and is clearly the most immunogenic tissue. The high degree of antigenicity of skin was the

major obstacle to earlier applications of CTAs. The difficulty in achieving tolerance after transplantation of skin allografts, even in tolerogenic models capable of inducing tolerance to organ allografts, is another well-known phenomena, named split tolerance [5].

Unexpectedly, the antigenic character of CTAs (i.e., rodent-limb allografts or even human hand transplants) is lower than any of its single-tissue component antigenicity [3], and the dosage of immunosuppression for experimental and clinical cases is almost similar with kidney transplant [6, 7]. Therefore, the current hierarchy of transplant antigenicity seams to be: free skin allograft > vascularised skin allograft > BM > small bowel > lung > heart > pancreas > limb (CTA) ≥ kidney > liver.

The presence of BM contained within some CTAs implies that they could be considered as vascularised bone marrow transplants (VBMT). This unique condition leads to the possibility that a CTA could achieve immediate marrow engraftment and repopulation in the recipient BM without the need for cellular bone marrow transplant (BMT). The transplanted CTA brings with it functional BM within its microenvironment, which serve as a constant source of donor haematopoietic stem cell delivery capable of inducing donor-specific tolerance and chimerism [8]. Actually, a composite VBMT has been recognised as a better source for BM reconstitution than transplantation of BM cells (BMC) [9–14]. A rat hind limb CTA acts as a VBMT and repopulates the host, with donor chimerism levels between 2–10%, but this level of mixed chimerism does not allow tolerance induction, developing clinical signs of rejection once immunosuppressants is withdrawn.

A VBMT prolonged the survival of a skin graft from the same donor [15, 16]. Similarly, the skin allografts in rats that simultaneously received limb allografts (containing BMC) survived much longer than those in recipients that received vascularised skin or muscle (not containing BMC), and the vascularised skin allografts survived longer than nonvascularised skin. Skin allografts in recipients that received limb allografts irradiated 7 days before transplantation were rejected earlier than those in recipients that received nonirradiated limb allografts. Talmor et al. [13] reported that BM-derived chimerism was established in nonirradiated, Cyclosporine-treated rats that received vascularised limbs, and chimerism was abolished if the grafted limb was irradiated. In conclusion, this prolongation of skin survival might be due to the tolerogenic activity of some BMC from the limb or some APCs from vascularised skin [17].

The tolerogenic activity of limb allografts is superior to that of BMC. VBMT can provide a continuous supply of donor-derived progenitor cells with a significant amplification of donor chimeric cells. Contrary, the achievement of chimerism by transfusion of donor-derived BMC is very low [18, 19]. The persistence of donor-derived leukocytes, which are capable of proliferating, trafficking, and interacting within the recipient tissues, has been proposed as an important mechanism that facilitates the induction of tolerance.

Strategies to Achieve Tolerance

Successful strategies to induce transplantation tolerance copy classical mechanisms of self-tolerance: deletion (the removal of T cells specific for a given antigen), anergy (the functional inactivation of responding T cells) and suppression (the inhibition of T-cell function by another cell).

Strategies Based on T-Cell Depletion

Since allograft rejection is mainly a T-lymphocyte-mediated process, the depletion of recipient alloreactive CD8+ and CD4+ T cells around the time of transplantation creates a transitory immunodeficiency in the recipient, compromising the recipient's capacity to reject the transplant. The goal of this strategy is to prevent immune engagement during the period of lymphocyte activation. Delaying the initial encounter between the allograft and the immune system to a more quiescent posttransplant peri-

od may result in a shift of the immune response towards one of indifference rather than rejection and incorporate several peripheral mechanisms of anergy into the maintenance of tolerance. This is mainly a peripheral effect involving circulating lymphocytes and those T cells present in secondary lymphoid organs.

Lymphoid depletion can be induced through radiation, pharmaceutical agents and antibodies. An early, nonspecific method was total lymphoid irradiation (TLI), which induces profound lymphocyte depletion but has additional effects, such as a Th2 cytokine shift and induction of suppressive cell populations. However, because the potential complications of TLI (especially infectious complications and secondary malignancies) are not acceptable for routine transplantation, this approach has not been widely followed.

Another approach involves intensive T-cell depletion by various antilymphocytic monoclonal or polyclonal antibodies. The first antibody was polyclonal antithymocyte globulin (ATG). It was demonstrated that T-cell depletion with ATG is not enough for tolerance induction. Many strategies that result in depletion of leukocytes (antithymocyte globulin, anti-CD52) or T cells (anti-CD3 with or without immunotoxin, CD2, CD4 and CD8) have been investigated in small and large animal studies [20]. In small animals, the short-term depletion of T cells appears to be sufficient in some situations for tolerance to develop and be maintained in the long term.

More profound T-cell depletion in nonhuman primates was achieved with an anti-CD3 immunotoxin [21], but again, tolerance was not fully achieved by this approach. Data from primates using an anti-CD3 immunotoxin in combination with deoxyspergualin suggest that T-cell depletion can be used to induce tolerance to donor alloantigens. When leukocytes are depleted, maintenance of tolerance depends on graft transplantation within a window of depletion of donor-reactive cells in the thymus and the periphery when cells repopulate the periphery. Donor-reactive cells are deleted or eliminated as a result of the presence of the surviving graft. The effects of T-cell depletion can be enhanced by combining this approach with administration of donor antigen to try to achieve some level of chimerism [20].

A newer antibody, alemtuzumab (Campath-1H), a humanised anti-CD52 antibody, is a powerful depletional agent for both T and B lymphocytes but does not affect BM stem cells and appears to induce *prope, or almost* tolerance in kidney allograft recipients [22].

Similarly, daclizumab, an antibody to CD25, the alpha chain of the IL2 receptor, also appears to induce "partial" tolerance in transplant recipients [23].

There are only a few reports of tolerance induction in CTA using depletional T-cell strategies. Siemionow et al. [24, 25] and Ozer et al. [26] used antilymphocyte serum for induction of donor-specific tolerance in rat hind-limb allografts. Thomas et al. [27] demonstrated that the CD3-IT and DSG induction protocol have high success rates, minimal toxicity and no risk of GVH disease, and they concluded that the CD3-IT/DSG tolerance induction could be the best option for CTAs. Siemionow et al. induced tolerance under a 35-day protocol [25] and even under a short-term (7-day) protocol [28] using cyclosporine A (CsA) and a mouse monoclonal antibody against rat αβ-T-cell receptor (TCR) in fully major histocompatibility complex (MHC)-mismatched rat hind-limb allograft recipients. A high level of donor-specific chimerism was associated directly with unresponsiveness to alloantigens and tolerance induction and maintenance. The immunomodulating strategy of the combined αβ-TCR antibody and CsA therapy successfully depleted the combined αβ-TCR+ T-cell subpopulation (by >95%) and created a window of immunological unresponsiveness needed for engraftment of the donor-derived BM haematopoietic stem cells delivered with the limb allograft within the recipient's central and peripheral lymphoid organs, resulting in establishment of stable lymphoid chimerism, peripheral anergy and tolerance induction. To the best of our knowledge, these are the first really feasible protocols demonstrating the induction of tolerance across the MHC barrier in CTA transplants.

Strategies Based on Blockade of Costimulation

The blockade of costimulation prevents efficient antigen presentation and has been shown to induce T-cell anergy, peripheral T-cell deletion and regulatory mechanisms. Unfortunately, the durability of this state of anergy appears susceptible to reversal by immune activation occurring after the removal of the agent responsible for costimulation blockade.

Blocking CD28-B7 costimulation using CTLA-4-Ig prolongs allograft survival in rodent models and in some instances induces tolerance. The most usually used reagent for this purpose has been CTLA-4-Ig, which potentially blocks all CD28 and CTLA-4-B7 interactions. The use of CTLA-4-Ig has only moderately prolonged allograft survival. The combined use of anti-CD80 and anti-CD86 antibodies was more effective, but no tolerance was induced [29].

The use of anti-CD154 antibodies to block the CD154-CD40 costimulatory pathway has been more successful. Kidney graft rejection in monkeys can be prevented completely with antibodies to CD154 [30]. The mechanisms of CD154 blockade in vivo include CTLA-4-dependent anergy or regulation, T-cell apoptosis and induction of regulatory cells [31]. The introduction of anti-CD154 antibody treatment into clinical studies has been restricted due to the thromboembolic complications observed in human trials. However, ketorolac, a nonsteroidal anti-inflammatory drug, seems to prevent thrombocyte activation induced by anti-CD154 treatment.

The combination of CD40 and CD28 blockade together has been used in rodents and nonhuman primates and appeared to be more efficacious than the manipulation of either pathway alone [32]. Blockade of costimulatory pathways alone has not been sufficient to induce the long-term survival of highly antigenic tissues such as skin, but simultaneous blockade of both pathways leads to long-term survival of skin grafts in a rodent model.

To our knowledge, several studies are in progress to date with this technique in the area of CTA. It can be supposed that if the immune response to the highly antigenic skin component of CTA can be overcome, the possibility of long-term CTA survival should increase. There is also evidence that a continuing source of donor antigen is beneficial in promoting graft acceptance. Thus, large CTA grafts may, in fact, be more amenable to anti-CD154 therapy than isolated skin grafts. This mechanism may be equivalent to antigen enrichment with donor-specific transfusion (DST). DST when combined with costimulation blockade may lead to long-term graft survival by providing more opportunity for alloreactive T cells to encounter antigen while being deprived of costimulation and thus undergo apoptosis. Studies of Elster et al. [33] using anti-CD154 both with and without DST in a nonhuman primate skin allograft model have been encouraging, and they postulated that anti-CD154 offers the most suitable therapy for treatment of CTA transplants in primates and humans. However, the thromboembolic side-effects must be resolved. Graft survival approaching 1 year has been achieved for full-thickness nonhuman primate skin allografts. It would thus appear that anti-CD154 can overcome the differential rejection of skin and suppress immunity to skin-specific antigens [34].

Iwasaki et al. [35] reported that a single administration of CTLA-4-Ig significantly prolonged limb allograft but failed to induce tolerance. They supposed that high doses of CTLA-4-Ig were required for induction of transplantation tolerance.

Strategies Based on Signaling Blockade

The specific tolerance by early evasion of antigen-presenting cells-lymphocyte interactions with T-helper-2 cytokine deviation (STEALTH) protocol [36] combined CD3 immunotoxin with deoxyspergualin (DSG), a substance known to interfere with nuclear factor (NF)-B signaling, a transcription factor involved in the signaling of many cytokine receptors. It led to long-term, rejection-free, probably based on a regulatory mechanism. This protocol was characterised by 3 interrelated elements: profound T-cell depletion,

arrest of dendritic-cell (DC) maturation in peripheral lymph nodes and a striking switch of cytokine expression towards an immunoregulatory pattern, with sustained production of IL-10.

Another substance that interferes with cytokine signaling is sirolimus (rapamycin). Theoretically, sirolimus is an attractive candidate to include in protocols for tolerance induction since it preserves signal 1 mediated via T-cell receptor (which is blocked by calcineurin inhibitors) but inhibits either costimulation (signal 2) or cytokine activation (signal 3). Rapamycin was included in protocols involving intensive T-cell depletion with immunotoxin and with anti-CD52 antibody instead of cyclosporine with encouraging results [1].

Donor Antigen Infusion

If signal 2/3 is blocked, signal 1 may be insufficient, and only a small amount of donor antigen may reach regional lymph nodes via passenger leukocytes, and even a smaller amount is likely to be found in the thymus. Therefore, the effect on T cells may be neutral, and as soon as the blocking treatment is stopped, rejection occurs. Consequently, intensifying signal 1 by additional donor antigen infusion (donor whole blood, lymphocyte, BM infusion, *facilitating* cells, DCs, embryonic stem cells, spleen cells or other stromal cells) in combination with T-cell depletion and either costimulation or signaling blockade has been an attractive concept for tolerance induction [37]. Donor-derived cells have a dual role: in some conditions, they promote immunogenicity, but in other microenvironments, they induce tolerance. These cells stimulate intense (IL-2 and γ-IFN-associated) activation-induced apoptosis of donor-responsive recipient lymphocytes and sustain a multilineage chimerism via engraftment and/or survival of donor haematopoietic stem cells. In addition, recent reports indicated that donor antigen infusion may generate regulatory T cells [38].

There are data suggesting the portal route, intra-bone-marrow injection or isolated limb perfusion are more efficient than the intravenous route for achieving engraftment of donor haematopoietic stem cells and increase the likelihood of tolerance [37].

Several successful CTA strategies to induce tolerance have been reported in animals. Early studies to induce immunological acceptance in CTAs reviewed by Kann et al. [39] reported the induction of immunologic tolerance in a canine hind-limb CTA model in newborn puppies involving subtotal blood exchange from an adult donor. Poole et al. [40] used immunologic enhancement with recipient-derived antidonor antiserum to induce tolerance in rats before limb transplantation. Black et al. [41] demonstrated that the use of preoperative whole-blood administration as a means of introducing donor antigen and inducing transplant tolerance offered no significant benefit in rat hind-limb allotransplantation.

Strategies Based on Haematopoietic Chimerism Induction

The concept of immunologic ablation (cytoreduction) with haematopoietic reconstitution was a consequence of the original demonstration by Billingham who infused replicating donor haematopoietic cells into the naturally immunologically incompetent neonate to induce tolerance. This led to adult chimerism and donor-specific allograft tolerance [37].

In chimerism, two genetically different cell populations coexist in the same organism. Two types of chimerism have been described: microchimerism and macrochimerism. At a state of microchimerism, the level of donor cell (passenger leukocytes or DCs but not donor-specific haematopoietic stem cells) is less than 1% in the recipient's peripheral blood and less than 2% in the recipient's BM and detectable only with highly sensitive methods, such as polymerase chain reaction. The interaction between passenger leukocytes from the transplanted allograft and the recipient's own leukocytes may lead to induction of donor-specific tolerance [42]. There is no need to condition the recipient before allotransplantation. It is debated whether

microchimerism is responsible for tolerance or is a side effect of tolerance. Rejection of organ allografts in the presence of microchimerism and long-term allograft survival in the absence of microchimerism has been reported. Macrochimerism results after BMT. Once donor pluripotent stem cells are engrafted into recipient lymphoid organs and thymus, the levels of donor-specific cells in peripheral blood are higher compared with levels observed in microchimerism, allowing the detection of macrochimerism by flow cytometry analysis, and may lead to either full or mixed allogeneic chimerisms. In fully allogeneic chimerism, BM cells in the recipient are donor derived without presence of recipient cells. This can be obtained by a complete myeloablation of the recipient before allogeneic BMT. In mixed allogeneic chimerism, both recipient and donor BM cells coexist, which results from an incomplete myeloablation of the recipient before BMT or when a fully myeloablated recipient receives a mixture of syngeneic and allogeneic BMT. Mixed allogeneic chimerism demonstrates superior immunocompetence compared with fully allogeneic chimerism [42].

The strategies for chimerism induction require some type of recipient conditioning (irradiation and/or cytoreductive chemotherapy) combined with T-cell-depleting antibodies and/or conventional pharmacologic immunosuppression. The intent is to create a "clean space" for the donor haematopoietic cells to engraft and survive in the recipient-vacated BM interstices. This is followed by infusion of donor BM alone or a combination infusion of donor and recipient BM. Once the immune compartment has begun to reconstitute itself, tolerance is primarily induced and maintained by central deletion of potential donor-reactive T cells although peripheral mechanisms are also likely to contribute to the process. This occurs when the immature T cell encounters donor antigen expressed on donor-derived APCs that reside in the thymus. Tolerance associated with mixed chimerism is strictly dependent on the persistence of donor cells. Tolerance achieved by haematopoietic chimerism has been widely recognised as the ideal and most robust form of T-cell tolerance since it is systemic and most donor-reactive clones are eliminated from the repertoire [1]. There seems to be general agreement that when there is established macrochimerism of donor BM cells, solid-organ and other grafts will be accepted. Unfortunately, toxicity and risk of graft versus host disease (GVHD) of such regimens have limited their use in larger species and man. Total body irradiation (TBI) is the most toxic form of ablation.

Recently, less toxic non-radiation-based protocols have been developed using nonlethal cytoreduction (sublethal or fractionated TBI, thymic irradiation) with or without additional immunosuppressive therapy (polyclonal, multiple monoclonal antibodies, Campath H antibody, immunotoxin or chemical immunosuppression such as sirolimus). Haematopoietic-cell reconstitution could be made using whole BMC (T-cell depleted), *in vitro* cultured and cytokine-expanded BM with or without autologous recipient marrow (depending on the degree of cytoreduction used) or peripheral stem cells. These nonmyeloablative techniques lead to a mixed chimerism and low incidence of GVHD [43]. There seems to be a direct relationship between BM dose, he level of chimerism achieved, and tolerogenic efficacy, so high cell doses should be used. Donor-type T cells and T-cell chimerism are not required in non-radiation-based protocols, thus permitting use of a large infusion of T-cell-depleted BMC without concern for occurrence of GVHD. The mechanisms involved in induction and maintenance of this tolerance-included induction of host and donor-specific immunoregulatory (suppressor) cells, as well as clonal deletion and anergy [44]. No evidence for regulation was found in protocols leading to high levels of chimerism.

Reliability and robustness of tolerance induced through mixed chimerism makes it one of the most promising approaches for CTAs [39, 45–48]. Th team of Hewitt [49–51] used rat hind-limb transplantation to study the role of VBMT in inducing chimerism and tolerance or GVHD. They demonstrated that the development of low-level, stable, mixed-lymphocyte chimerism after limb allografting is associated with alloimmune tolerance induction while unstable, high-level

lymphocyte chimerism is associated with the development of GVHD.

Lee et al. [55] have been successful in inducing long-term tolerance to vascularised musculoskeletal allografts in major histocompatibility-complex-matched minor antigen mismatched pigs after only a 12-day postoperative course of cyclosporine A. The same team have also used intrathymic injection of donor-derived BM cells along with the short-term use of an antilymphocyte serum to prolong survival of skin allografts in rats [56]. Butler et al. have demonstrated prolonged survival of allogeneic skeletal tissue transplants without immunosuppression after pretransplant, neonatal injection of donor-derived BM cells in a rat hind-limb allograft model [57]. Foster et al. [58, 59] described the first reliable model demonstrating rejection-free CTA survival and immune tolerance induction in an adult rat hind-limb allograft model without long-term immunosuppression across a strongly antigenic MHC mismatch. This was performed by the induction of a state of stable mixed chimerism. They prepared mixed chimeras by injecting a mixture of T-cell-depleted syngeneic and allogeneic rat BM into recipients that were conditioned with 500–700 cGy of irradiation, antilymphocyte serum and tacrolimus.

Active Suppression and Regulation-based Protocols

Active regulation and suppression of immune responses has been described as a mechanism for inducing and maintaining tolerance to donor antigens. The immune response mediated by activation of peripheral T-cells by foreign antigen could be downregulated by the emergence of T suppressor (TS) and regulatory cells (TR). There might be a fragile balance between immunity and tolerance that may depend on size of the effector populations or prevalence of inflammatory or inhibitory cytokines that may affect the "immunogenic" or "tolerogenic" phenotype of APCs. Decrease or increase in frequency of effector cells versus alloantigen-specific TS/TR cells changes the balance in favour of rejection or tolerance, respectively. Tolerogenic host professional APC, such as DCs and nonprofessional APC such as endothelial cells (EC), create a privileged local microenvironment, eliciting the expansion of TS and TR cells. Eventually, this will become the dominant population of allopeptide-specific T cells in the peripheral circulation and lymph nodes [60].

To date, a distinct population of donor alloantigen-specific CD8+CD28– FOXP3+ T suppressor cells have been described [23]. These are MHC class-I-restricted cells and suppress antigen-specific CD4+ TH cell responses, inhibiting their capacity to produce IL-2 and preventing upregulation of CD40 ligand (CD40L). After exposure to CD8+CD28– TS cells, APCs lost the capacity to stimulate T-cell alloreactivity, inducing instead anergy in allospecific TH1 cells. Similar to CD8+CD28– TS, the anergic CD4+ CD25+ FOXP3+ T regulatory cells were shown to act directly on APC, inducing upregulation of inhibitory receptors ILT3 and ILT4. This modulated dendritic cell is then able to preferentially present antigen to induce further cohorts of CD4+CD25+ regulatory T cells that mimic the process of infectious tolerance. These data demonstrate that tolerogenic DCs are crucial to the generation of antigen-specific CD8+ TS and CD4+ TR. The bidirectional interaction between regulatory cells and APCs perpetuates a cascade of events that downregulate T-cell alloreactivity [60].

Experimental models in rodents have demonstrated that it is now possible to reprogram the immune system towards a state of antigen-specific donor tolerance that ultimately depends on the development of TS/TR cells. This can be achieved either by modulating the responding T cells, often by the use of monoclonal antibodies that block full T-cell activation or by modifying APCs. The effective deletion of alloreactive T helper and cytotoxic cells in conjunction with the expansion of antigen-specific suppressor and regulatory T cells creates a milieu in which the graft is well tolerated under an "umbrella" of low-dosage immunosuppression [23].

There are some ongoing studies looking for evidence of tolerance-inducing cells, which may act by means of suppression or regulation of alloreactive T cells in CTAs.

Final Considerations

To date, the translation of successfully tolerogenic therapies from rodents to nonhuman primates and humans has failed because adult humans have more complex and redundant immune systems than animals. One explanation for this may be heterologous immunity induced by a variety of pathogens, thus accumulating memory T cells that crossreact with an additional inflammatory stimulus and overcome any tolerance therapy – a situation essentially different from rodents raised under pathogen-free conditions. Heterologous immunity might best be overcome by a deletional tolerance approach since any type of regulation or anergy could potentially be overcome by an infectious disease. Therefore, clinical tolerance strategies that induce donor-specific hyporesponsiveness and rejection-free (acute and chronic) survival with minimal nontoxic dose of maintenance immunosuppression may be a logical compromise, ensuring guard against any intercurrent infection or other hazards.

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Section 2-g

The History of Experimental Hand Transplantation in Primates

Rollin K. Daniel, Kevin A. Brenner

Introduction

The senior Author's (Rollin K. Daniel) interest in experimental hand transplantation evolved from the development of free tissue transfers. In 1971, microvascular surgery was struggling with development of sutures capable of sewing 1-mm blood vessels. Once this was solved, it became possible to replant fingers, but elective reconstructive procedures posed new ethical dilemmas. Attempts at replantation surgery were easily justified, but was it ethical to try free flaps when viable alternatives existed including cross-leg and tube flaps? Experimental studies in animals with similar cutaneous blood supply provided a rational scientific basis to begin clinical trials in very select cases. Once initial clinical success of free-flap transfers was achieved by Daniel and Taylor in 1973 [1], a broad range of tissue transfers began. Perhaps the most pertinent were the toe-to-hand transfers, as they represented composite tissue transfers with excellent restoration of function and sensation. Obviously, the thought of true tissue transplantation occurred to the pioneers of reconstructive microsurgery, but again, scientific and ethical issues arose. The scientific questions were numerous, but included the following: (1) most tissue transplants had been done in lower species of pure immunological strains, (2) assessment of sensation and function were mainly cursory and (3) extrapolation of data to clinical application could not be justified. The principle ethical issue was "quantity of life" versus "quality of life". Obviously, the major side effect of the immunosuppression regimes of the early 1980s could only be justified in cases where death was the only alternative. Quality-of-life transplantations would have to await advancements in immunotherapy, which would sharply reduced graft rejection.

Experiments devised in the reconstructive microsurgery laboratories at the Royal Victoria Hospital and McGill University, Canada were truly major breakthroughs, as they provided the scientific basis for all quality-of-life tissue transplants, including hand and facial transplants. Specifically, these were complete hand transplants remarkably similar to humans. The animals were of the highest primate species possible – baboons – and were nonhabituated and had a diverse DNA profile. There was no attempt at genetically matching donor and recipient. The surgical procedure had to be developed with decisions as to bony, tendon and neural repairs that were different than the usual hand replant. The postoperative immunosuppression regime and management of rejection crises was difficult, including twice-a-day medication every day of the year. Ultimately, long-term surviving animals were able to feed themselves with their transplanted hand thus validating gross muscle function. However, the central question was whether or not precise motor and sensory function would be regained: would the nerves reinnervate the end organs in the midst of immuno-

suppression designed to keep the host from recognising foreign tissues, i.e. would the host nerve recognised the correct foreign end organ and would reinnervation occur? The experimental model of hand transplantation that we selected was truly the most difficult imaginable, and if it worked, it would justify clinical trials once acceptable regimes of immunosuppression were developed.

In 1999 and again in 2000, Dubernard et al. reported on the first case of a human hand transplant [2, 3]. Since that time, the medical literature has been replete with information about technique, feasibility, disadvantages, ethics, successes and failures of human-extremity transplantation [4–18]. The advent of cyclosporin and other newer immunosuppressive medications made possible the survival of solid organ, and now composite tissue, transplants that were not possible only thirty years ago [19]. As with almost all other novel breakthroughs in surgery, composite hand transplant was initially worked out in multiple animal models. Mice, rats, dogs, and rabbits have all served as useful models for allogeneic experimentation [20–28]. However, early success in primate composite hand transplant is what paved the way for ultimate feasibility in humans.

Primates are an ancient and diverse eutherian group, with around 233 known living species. They dwell in arboreal habitats primarily and have adapted to this distinct habitat through time by developing several important traits by which we recognise and categorise them. Amongst these traits are: opposable hallucis and pollicis muscles (in the hand and foot, respectively), unfused and highly mobile radii and ulnae in the forelimb and tibia and fibula in the hind limb. Homo sapiens have evolved into land dwellers, but it is these common features that make primates an ideal model for forelimb transplantation studies [29].

Review of the Literature

As with all other areas of tissue transplantation, once the technical aspects of the operation are championed, the underlying essential question is: "Does it work"? Can a host axon grow into and functionally innervate a transplanted histoincompatible tissue under the guise of systemic immunosuppression [30]? Further, even if reinnervation occurs, will the end organ function work correctly? What stumbling blocks lay in the path to successful transplantation and immunosuppression, and how do we overcome them? These are the questions that have driven investigation of forelimb transplantation in primates.

Investigation into composite tissue transplantation began in 1984 when Egerszegi et al. described two experimental models in the primate to study the survival and reinnervation of transplanted tissue [31]. Specifically, the authors were investigating the degree of reinnervation and functional recovery possible in the presence of immunosuppression with cyclosporin A (CyA) in a primate species. The baboon Papio anubis was chosen for several reasons: (1) the hand is anatomically similar to that of humans, (2) the skin contains similar sensory receptors and nerve pathways to those of humans, (3) the neurovascular bundles are of sufficient size to allow microsurgical repair and neurophysiological recording, (4) neural function had been previously investigated by the same lab and (5) CyA had been previously used successfully in primates. The two models designed were a neurovascular soft tissue free flap for the index finger and an entire hand transplant through the distal forearm.

The same group performed a follow-up study in 1986 showing preliminary results of tissue transplants in primates demonstrating evidence of reinnervation. In the neurovascular free-flap (NVFF) transplant, the entire skin coverage of the second digit was performed using two median-derived digital nerves and small branches of the dorsal radial nerve. The complete hand transplant, designed to assess survival and function of multiple tissues, was performed in four animals in a manner similar to that of replantation of a distal forearm amputation. For the NVFF group, 3 of the 7 successfully performed flaps developed signs of early rejection. Some flaps survived to between 161 and 211 days. All of these flaps demonstrated some degree of rein-

nervation in nonscarred regions of the flap tissue. Low-threshold, well-defined receptive fields of both slowly and rapidly adapting receptor classes were observed in both glabrous and hairy skin. Two of the 4 complete hand transplants survived long term, to 188 and 304 days, respectively. The other two suffered loss to acute rejection episodes. In the surviving limbs, both slowly and rapidly adapting cutaneous mechanoreceptors were observed to have low threshold receptive fields in both hairy and glabrous skin. The average threshold for the cutaneous, rapidly adapting mechanoreceptors was higher than normal. Joint and muscle spindle afferents were also observed. Interestingly, the investigators were able to demonstrate that the thenar and intrinsic muscles had multiple motor units serving them. In addition, by comparing transplants with a rejection episode to those with little or no rejection, it was apparent that many of the abnormalities correlated with the degree of rejection such that axons serving tissue with minimal rejection had more normal response properties [32].

In 1987, Stark et al. reported their experience of hand transplantation in baboons [33]. In that model, eight baboons underwent complete composite microsurgical hand transplantation. The animals were tested for class 1 antigens of the major histocompatibility complex (MHC), and donor-recipient pairs were chosen that differed in at least one BabLA locus, the baboon equivalent to the human leukocyte antigen (HLA) class system. Immunosuppression was achieved with CyA and methylprednisolone. Six hands were lost at 2, 5, 7, 13, 13, and 15 days; 5 of these were due to hyperacute rejection. One animal suffered an anesthetic-related death. One hand survived to day 296 following two separate bouts of acute rejection that resulted in areas of skin desquamation. Both episodes were treated successfully with steroid augmentation and intravenous antibiotics. This hand demonstrated thumb function in week 21, followed by long-finger function in week 22. Further, radiographs showed continuous noncallous bony union, and neurophysiological study showed good motor and sensory function in the graft. Histological postmortem analysis of the one long-term survivor showed the following: chronic skin rejection with loss of normal dermal and epidermal architecture, hair follicle loss, diseased but patent radial artery, very moderate atrophy of the intrinsic musculature, and viable osteocytes in the donor bone. One interesting finding was a lymphoplasmacellular infiltrate in the recipients' autologous tissue, consistent with multiple areas of graft-versus-host disease (GVHD).

In 1988, Samulack et al. addressed the differences that exist in axonal form and function between rejected and nonrejected allograft tissues through electrophysiologic measurement and histologic documentation [32]. Two surgical models were designed and implemented in 12 baboons Papio c. anubis. In the first model, an index finger neurovascular free flap (TNVFF), the entire soft tissue coverage of the digit along with its neurovascular pedicle was removed and transplanted to the same site on another animal. A surgical control neurovascular free flap (CNVFF) involved the same dissection except that instead of transplanting the flap, it was inserted into its own donor bed. The hand transplant model involved composite replacement of all tissues up to a level 4 cm proximal to the wrist. Both the TNVFF and CNVFF had a short period of slight edema due to manipulation and ischemia. Their denervation led to Wallerian degeneration of the host axons and Schwann cell demyelination within the nerve fascicles, which led to atrophy of the peripheral sensory and motor structures. Histological analysis of cutaneous sensory mechanoreceptors showed that Meissner, Pacinian, and Ruffini complexes; Merkel cells, and hair follicles all underwent morphological changes after denervation. Even in the control CNVFF nerves, a percentage of the mechanoreceptors that became denervated never recovered. In the nonrejected TNVFF flaps, distribution of identifiable receptive fields was not very different from those of the CNVFF. However, in the rejected TNVFFs, the number of identifiable axons that reached the flap was significantly reduced. These differences were due to a decrease in target mechanoreceptor number as well as a disruption of the allografted Schwann-cell pathways due to rejection processes. Conduction velocities were reduced in both CNVFF and TNVFF flaps (compared with normals), and was likely due to the

process of nerve transection and repair. Further, conduction velocities in the TNVFF were decreased compared with CNVFF and was likely due to the foreign environment in which host axons exist. Rejection episodes result in mononuclear cell infiltrate into the nerve fascicles and Schwann-cell destruction, reducing TNVFF flap conduction velocities even further. The most disturbing finding in this study centered on the differences noted in the conduction velocities of axons to normal skin without immunosuppression and those with immunosuppression. Abnormal myelination in the immunosuppressed host axons may have been the result of a neurotoxic effect from CsA, possibly by altering vascular adrenergic neurotransmission.

In 1990, Stevens et al. addressed the immunological aspects of partial hand transplantation in the rhesus monkey, specifically focusing on reversal of rejection with monoclonal antibodies specific for CD3+, CD4+, CD8+ and MHC class 2 antigens, looking at what effect rejection had on allograft reinnervation [34]. Twelve partial hand transplantations were performed successfully; 6 survived short term (21–33 days), and 6 survived long term (79–179 days). Half the animals received third-party blood transfusions preoperatively, which appeared to have no significant effect on graft survival and timing of rejection episodes. Overall, rejection occurred in 10 animals. Five of these were treated with monoclonal antibodies (mAbs); in 2 of these 5, rejection was reversed. The other 5 received increasing methylprednisolone (diadreson faquaosum, DAF) doses; none of these rejection episodes were reversed. Sensory and motor function recovery occurred in all long-term graft survivors. Sensory recovery began at a mean of 41.8 days postoperatively. Episodes of rejection decreased the skin area in which the withdrawal reflex could be evoked, likely because the sensory receptors lay at the dermal–epidermal junction, the main target of rejection. Importantly, reversal of rejection enabled renewed reinnervation after 2 weeks, showing that host axons can grow in viable histoincompatible tissue and establish reinnervation of sensory receptors. Motor function was first detectable after a mean of 28 days. In the grafts in which rejection was reversed, episodes of rejection did not affect the latency and amplitude of motor action potential, suggesting that nerve and muscle fibers are less antigenic than dermis and epidermis.

In 1991, Stevens et al. addressed the relationship between immunologic aspects of allogeneic partial hand transplantation in the rhesus monkey and the complications encountered [35]. Twelve allogeneic transplantations of the first ray (thumb) along with a radial forearm flap were performed in unrelated donor–recipient combinations mismatched for rhesus major histocompatibility (RhLA). Immunosuppression was achieved with CyA and DiadresonFaquaosum (DAF). Rejection episodes were treated with either high-dose DAF or with mAb directed at rhesus CD3+, CD4+, CD8+, and MHC class 2 antigens. Ten of 12 successfully transplanted hands developed a rejection episode of their composite tissue allograft that was not reversible by increasing steroid doses. However, mAb therapy did reverse rejection in 2 of 5 monkeys that received it. In the grafts that survived longer than 70 days, anorexia and weight loss occurred. Deaths were related either to overwhelming opportunistic infections and sepsis or to lymphoid-tumor-related multiple-system organ failure. Importantly in this work, treatment of rejection episodes with mAb was significantly more effective than treatment with steroids.

In 1992, Hovius et al. addressed the technical aspects of allogeneic transplantation of the radial side of the hand in the rhesus monkey, with specific attention to the value of monitoring the microcirculation and functional recovery in a rhesus monkey model [36]. Twelve rhesus monkeys, Macaca mulatta, underwent allogeneic transplantation of the radial side of the hand. The animals were divided into 4 different treatment groups for the purpose of evaluating the effect of third-party blood transfusions and mAbs for the treatment of rejection. Immunosuppression was ensured with CyA and DAF. Half the animals received mismatched third-party blood transfusions preoperatively. In cases of rejection, half the animals received increasing doses of DAF while the other half received mAbs. Graft survival times were short in 6 cases (21–33 days) and long in the other 6 (79–179 days). Adminis-

tration of blood transfusions conferred no significant difference in the onset of allograft rejection, nor did it facilitate reversal of graft rejection. Ten of 12 hosts experienced some degree of histologically confirmed rejection. In all of them, skin was more rapidly rejected than nerve and muscle. Five animals received mAb therapy for rejection, which reversed rejection in only 2. Five animals received high-dose DAF for rejection, which failed to reverse rejection in all 5. MAb antirejection therapy prolonged survival significantly longer than an increase in steroids. Doppler flowmetry measurements and skin temperature readings offered no assistance at predicting graft rejection. Sensory and motor recovery occurred in all long-term survivors. In the monkeys with long-term graft survival, the first signs of sensory recovery occurred in the median nerve distribution after a mean of 42 days. The percentage of median nerve reinnervation increased over time; rejection episodes clearly decreased this area. In cases of reversal of rejection, renewed reinnervation was seen within 2 weeks. The first sign of motor recovery was seen at a mean of 43.8 days postoperatively. The quality and amplitude of conduction increased over time but was clearly decreased following rejection episodes.

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Section 2-h

Hand Transplantation in Monkeys: Technical Details and Immunological Aspects

Steven E.R. Hovius, H. Mischa Zuijdendorp, Jeroen J.P.D. Stevens

Introduction

In the late 1980s, increased interest could be perceived in reconstruction of acquired or congenital deformities with allogeneic composite tissue. This revival was attributed to the introduction of new immunosuppressive drugs in combination with the widespread possibilities of microsurgical reconstruction. As a consequence, composite tissue transplantation studies were performed not only on rodents and dogs but also on monkeys as preparation for future hand transplantation in humans. As far as the literature is concerned, three groups in the world were active at that time on this particular subject. The group of R.K. Daniel in Montreal, Canada and the group of G. Stark in Pittsburgh, Pennsylvania, USA, published in 1986 and 1987, respectively. Both groups worked on baboon [1, 2]. Furthermore, our group in Rotterdam, The Netherlands, worked on rhesus monkeys and published in 1991 and 1992 [3, 4].

This chapter is devoted to our experiences with composite tissue allograft (CTA) transplantation in monkeys and more specifically, the (partial) hand. Before going into detail, the prelude to these studies should be clarified. As this chapter concerns a certain time frame (till the early 1990s), it is important to realise that large-scale applied modern immunosuppression, with drugs such tacrolimus, is of later date and will hardly be discussed here.

First of all, the individual parts of composite tissue will be discussed separately to understand the role they play in transplantation procedures. The individual parts are skin, nerve, muscle, tendon and bone. It should be stressed again that only studies before the 1990s are considered, as later studies were not relevant concerning this chapter.

Skin transplants can survive indefinitely in rodents and humans. In rats, survival greater than 100 days could be obtained by administering a maintenance dosage of 15 mg/kg cyclosporine A (CyA) subcutaneously every fourth day following an initial 2-week course [5, 6]. At the time of the CTA transplantation studies in monkeys, publications on long-term skin allograft survival in humans mainly concerned burn treatment. Achauer et al. and Frame et al. described 4 patients with massive burns who received short-term CyA treatment after application of allograft skin. In two cases, the allografts survived only during treatment. In two patients, no evidence of rejection was seen up to two years after cessation of CyA, presumably due to the immunosuppressive effect of the thermal trauma or replacement of the allograft by autologous epithelial cells [7, 8].

In nerve transplants, indefinitive survival could be reached at that period with only initial immunosuppression. Long-term treatment was not necessary. Studies were performed in rats, primates and humans. Mackinnon et al. reported that nerve regeneration in rats receiving nerve allografts treated with 5 mg/kg CyA for 8 weeks was comparable to that seen across autografts

and in permanently immunosuppressed controls [9]. Fish et al. and Bain et al. compared regeneration across allografts in primates with and without long-term immunosuppressive treatment using CyA 25 mg/kg per day. Excellent regeneration was demonstrated in both group [10, 11]. A clinical case using CyA was reported by Mackinnon and Hudson. A 23-cm sciatic nerve defect was reconstructed using ten nerve allografts. At 26 months postoperatively, evidence of nerve regeneration was observed, and immunosuppression was discontinued. Nineteen months after cessation of immunosuppression, the patient regained functional sensibility of his foot [12].

Vascularised muscle allografts in rats using CyA in a dose of 10 mg/kg per day subcutaneously were published in 1991 by Tan et al. Survival rates of at least 70 days were obtained with this treatment scheme [13]. Only limited studies were available concerning muscle transplants. Vascularised and nonvascularised tendon allografts in the same time frame were not very antigenic and therefore did not need special attention [14].

Allogeneic vascularised bone grafts were performed in rats and rabbits in that period. Paskert et al. showed long-term survival of vascularised knee allografts in rats using CyA 10 mg/kg per day [15]. Siliski et al. achieved these results in rabbits immunosuppressed with CyA 15 mg/day [16]. Nonvascularised allogeneic bone grafts have been performed in humans without administration of immunosuppressive drugs. These procedures have a high complication rate and poor outcome [17].

In summary, isolated parts of composite tissues demonstrated different rates of antigenicity. Research at that time showed that skin was the most antigenic. However, Lee et al. compared the immune responses in rats 1 week after transplantation of a limb and the individual components separately [18]. Although skin was considered to be strongly antigenic, vascularised muscle graft elicited even greater cell-mediated responses, followed by bone, subcutaneous tissue and skin [18, 19]. The highest humoral responses were elicited by skin, subcutaneous muscle and bone allografts. Limb allografts generated lower responses, and vessel allografts were least antigenic. The relative low immune responses of limb allografts might be explained by decreased immune responsiveness, possibly due to the high antigen load [18].

When considering hand transplantation, research was further focused on CTA transplantation in animals in order to mimic the human situation as much as possible. CTA transplantation was mostly performed in rats and dogs. Monkey studies are of a later date. Two periods can be distinguished concerning immunosuppressive therapy in relation to CTA transplantation in this time frame: the periods before and after cyclosporine A.

Before the introduction of CyA, a few experimental studies were published concerning CTA transplantation. For instance, a limb transfer that survived for 14 days using parabiosis before 2 weeks of age in rats, as described by Schwind in 1962 [20], or the 2-month survival of a limb transplantation in a dog 9 months after complete exchange transfusion of the donor at the age of 9 days [21].

As soon as the combination of 6-mercaptopurine, azathioprine and prednisone was successful in kidney transplantations, it was also tried in CTA studies [22]. In 1966, Goldwyn et al. treated dog limb allografts with 6-mercaptopurine and azathioprine. Survival could be prolonged for a short time only, and even fatal drug-induced side-effects occurred [23]. With 6-mercaptopurine and prednisone, Doi et al. could only obtain a CTA survival rate in rats with a maximum of 24 days [24]. In 1971, Lance et al. reported long-term allograft survival (60, 200 and 300 days in three dogs, respectively) using unrelated beagles. The treatment scheme in these dogs consisted of a short-term, massive immunosuppressive regime followed by either or not splenectomy and thymectomy. Subse-quently, immune tolerance was induced from donor splenic cells or exchange transfusion [25].

Following the introduction of CyA, survival of vascularised allogeneic hind-limb transplantations in rats treated with CyA as immunosuppressive therapy was first reported by Furnas et al. in the early 1980s [24–28]. They transplanted hind limbs from hybrid Brown Norway

(BN)/Lewis rats to Lewis rats. Transplant survival increased dramatically following 20 days' administration of 25 mg/kg per day CyA. In one animal, allogeneic limb survival was even beyond 225 days. Under a continuous dose of moderate CyA, even indefinite CTA survival was obtained in rats by Fritz et al. in 1984 [29].

In our preliminary studies, we started to master the CTA vascularised hind-limb transplantation technique in rats by performing the operation as described by Fritz et al. [29]. Furthermore, we had to learn more about the process of rejection. In the first study, transplantation was performed in 20 rats using the inbred strain of BN rats (BN/Bi) as donor and the Wistar Albino Glaxo/Rijswijk (WAG/Rij) rat as acceptor. These rats were known to have a strong mismatch. The control group consisted of six WAG/Rij rats in which hind-limb replantations were performed. The purpose of the study was to find parameters to indicate the initial onset of rejection. Parameters used were clinical examination, laser Doppler flowmetry (LDF), blood gases, glucose, lactate and biopsies, always comparing the transplanted limb with the contralateral nonoperated hind limb. Parameters were measured at different time frames between day 2 and day 14 following transplantation. The onset of rejection was mostly clinically seen from the seventh postoperative day onwards by progressive epidermolysis, crust formation, exudation and leathery skin. The parameters blood gases, glucose and lactate were not useful in this study. Clinical, histological and LDF examination, however, were good parameters for rejection and correlated well [30].

The evident next step was to turn to higher species to come closer to the human situation. Interestingly, the concept of different groups doing similar research without knowing of the others' work frequently emerges at the same time in different parts of the world. Our grant proposals date back to the very early 1980s, initiated by Dr. A.R. Smith. The hand of most monkeys is closely related to the human hand regarding anatomy and functional use. At the primate centre (at TNO, Rijswijk, The Netherlands), most research was performed on rhesus monkeys. Therefore, these monkeys were used for our studies. Before this could take place, numerous protocols were admitted to various (animal) ethical committees: local, regional and even to a national level. Considerable resistance was encountered, especially when emotional arguments took over from rational ones. For example: "Should I receive the hand of my grandmother" and similar remarks. As soon as the protocols were approved, the feasibility of the model was tested. The use of the whole hand was not approved for functional and ethical reasons.

Materials

Therefore we designed a functional anatomical model of a partial hand encompassing all relevant tissues and suitable to use. Furthermore, extrapolation from obtained data to human hand transplantation had to be possible. Subsequently, the technical model comprised of the first ray enlarged with the radial forearm flap. In the flap, the median nerve, including the palmar cutaneous branch, motor branch of the ulnar nerve and superficial branch of the radial nerve, was included, as well as the thenar muscles, flexor pollicis longus and extensor pollicis longus tendons. The model was disarticulated at the carpometacarpal joint (Fig. 1).

In all, 15 fresh cadaver upper extremities of the rhesus monkey (*Macaca mulatta*) were dissected to define the model, with emphasis on all anatomical details aforementioned. By including sensory and motor nerves and thenar muscles, sensory and motor function could still be tested in the model following attachment at the acceptor site. Moreover, when using this model, basic function was still left in the remaining wrist and four fingers in the event of a donor monkey or in the recipient when transplantation failed. Technical feasibility of the research model was tested by performing four replantations of the first ray of the hand of the rhesus monkey with the radial forearm flap. The maximum follow-up time of these monkeys was 3 years [31]. The monkeys were born and raised at the primate center at TNO, Rijswijk. Their average weight was 7.5 kg and their age varied from 9 to 24

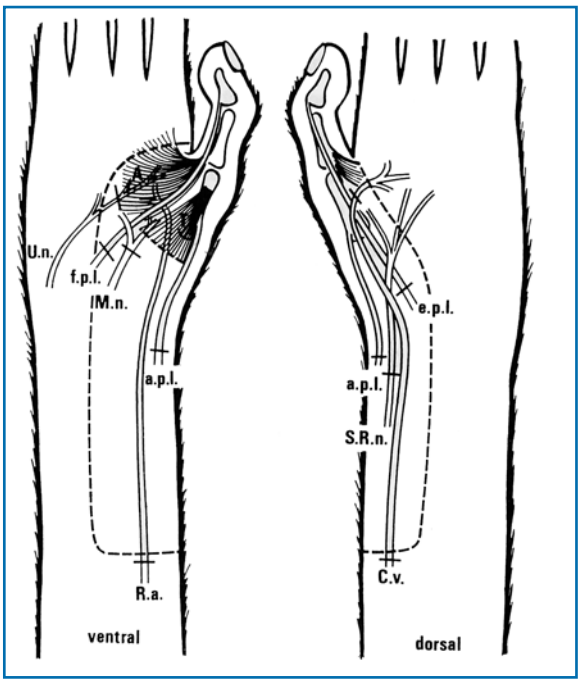

Fig. 1. The radial transplant model. The dotted line marks the radial hand unit. Ventral: *A*, adductor muscle; *T*, thenar muscle; *U.n.*, ulnar nerve; *M.n.*, median nerve; *f.p.l.*, flexor pollicis longus; *a.p.l.*, abductor pollicis longus; *r.a.*, radial artery. Dorsal: *e.p.l.*, extensor pollicis longus; *a.p.l.*, abductor pollicis longus; *s.r.n.*, superficial radial nerve; *c.v.*, cephalic vein. From [1], used with permission

years. Male to female ratio was two to one. The hands had not been injured previously; also, hand dominance was assessed if possible. The animals were selected on normal liver and kidney function.

Methods

Operation

The operations were performed under routine general anesthesia and axillary brachial plexus block. Administered medication consisted of Rheomacrodex intravenously during and directly after operation, acetylsalicylic acid on the day of operation and antibiotics intramuscularly for 5 days. Following dissection of the radial forearm flap (20–25 cm^2) with vessels, nerves and tendons, the thumb sustained a proximal metacarpal osteotomy in the donor monkey in case of an allograft. In transplantation experiments, the donor structures were taken longer than expected to prevent problems with matching at the acceptor site. Before the transplant was detached, it remained on its pedicle to be recirculated after tourniquet release. On another operating table, first the acceptor site was prepared taking into account the discrepancies in size with the donor monkey. Following separation of the transplant, it was attached by an intraosseous wire at the proximal metacarpal bone. The adductor pollicis was reinserted and the flexor and extensor as well as the abductor longus of the thumb were reconnected. Nerve ends of the motor branch of the ulnar nerve, the common trunk of the median nerve, the palmar cutaneous branch of the median nerve and the superficial radial nerve were all microsurgically sutured. Furthermore, the radial artery and the cephalic vein were sutured end to end under the microscope with 10/0 nylon. The skin was approximated. At the end of the operation, the thumb was monitored for microcirculation with LDF, and a pin-prick test was performed for bleeding of the pulp of the thumb.

The following aftercare and measurement methods were used for both replants and allogeneic transplant [3, 31, 32].

The arm was dressed, and a specially designed upper-arm thermoplastic splint was administered. This splint was secured with nuts and bolts, as monkeys are very strong and are able to tear almost anything apart [31].

Two times per week, the wound was inspected under light sedation and the microcirculation monitored with LDF.

Sensory Reinnervation

Sensory reinnervation was assessed by an electrical, bipolar, small-current stimulator, the sensiometer. The idea was to induce a withdrawal reflex following stimulation of designated lines on the skin along the transplant with currents ranging from 0.14 to 1.8 m. A working from distal to proximal. The contralateral side was used as control. The lines on the skin correlated with the areas of the different nerves. The number of sites with positive withdrawal reactions related

to the total number of measuring sites (range, 30–40, depending on size) provided the percentage of reinnervation.

Motor Recovery

Motor recovery was assessed by using electromyography (EMG) with surface electrodes in the same way as in the human situation. Latency and amplitude of compound motor action potentials (CMAP) of the thenar muscles in the operated side were compared weekly to the contralateral side. Latency of CMAP was measured from stimulus artifact to peak of negative deflection in milliseconds. Amplitude of CMAP was measured from peak of negative deflection to baseline in millivolts. Sensory and motor recovery were measured in the transplant cases from the first week postoperatively.

Function Tasks

Videos were taken from several replant and transplant monkeys.

Blood Parameters

Apart from classical parameters, such as leukocyte and lymphocyte count, monitoring was performed on through levels of CyA, levels of circulating monoclonal antibodies (MAbs), and relative numbers of Rhesus antigens.

Histology

Skin biopsies were taken from the transplant on a weekly basis. In the event of rejection, biopsies were taken two to three times a week. Regular HA staining was performed to grade rejection. Also, immunohistochemical studies were done to demonstrate expression of various Rhesus antigens. To examine nerve ingrowth and muscle reinnervation, the thenar muscle was removed from the operated and contralateral nonoperated side. Histochemical methods were applied to detect vital motor end plates. Immunohistochemical staining was used specifically for the presence of neurofilaments

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Statistical Analysis

Tests used according to requirements were: Fischer's exact test, Mann-Whitney *U* test, two-sample *t* test and log-rank test. Differences were considered significant if $p<0.05$.

Immunosuppressive Treatment Scheme

In our studies on allogeneic transplantation of the radial side of the hand in nonhuman primates, donor–recipient combinations were mismatched for rhesus major histocompatibility complex (MHC) class I and II antigens. The MHC antigens of rhesus monkeys are well defined and show close resemblance with the immunological status of humans. Immunosuppressive treatment consisted of high doses of CyA in combination with prednisone [Di-Adreson F-aquosum (DAF)]. CyA was administered by subcutaneous injections twice daily (25 mg/kg per day) to obtain whole blood through levels of 400–1,000 ng/ml. DAF was given in an initial high dosage (12 mg/kg per day) for the first 3 days postoperatively and then was tapered slowly until a maintenance dose (1 mg/kg per day) was reached 12 days later. Furthermore, preoperative blood transfusions were added to the research protocol for their known favourable effect on graft survival.

Studies on hand transplantation in baboons by the groups of Daniel and Stark showed that even high doses of CyA could not prevent rejection in the majority of cases. Reversal of rejection with an increase in prednisone was reported in two out of four and one out of eight hand transplantations, respectively [1, 2]. To improve the immunosuppressive regimen, rejection episodes were treated with a combination of seven MAbs specific for CD3, CD4, CD8 and MHC class II antigens [33]. Once daily, MAbs were administered as an intravenous bolus injection for a period of 10 days [3]. Using MAbs,

selective T-cell populations relevant for rejection can be manipulated without impairing the host's immune competence. MAbs were significantly more effective for reversing rejection of transplanted organs compared with conventional steroid treatment [34]. Stevens et al. reported that a combination of MAbs prolonged skin allograft survival times in rhesus monkeys significantly, from 8.3 (SD=0.7) to 19.3 (SD=1.8) days [34].

Results

Concerning the replants, one monkey died postoperatively due to cardiac arrhythmias, with at time of death a well vascularised replant [31]. This left three monkeys for follow-up. Splints were removed at days 45, 57 and 59, respectively, in the different monkeys. No signs of automutilation or ulcers were encountered after removal of the splint. With the sensiometer, the percentage of sensory reinnervation of the median and superficial radial nerve area was 96%. The unconnected nerve branch of the lateral antebrachial cutaneous nerve showed a positive withdrawal reflex in only 38% in its referral area. Motor recovery measured with EMG demonstrated a mean latency and amplitude of CMAPs of thenar muscles of 93% and 88% of the contralateral side measured at the elbow. Staining for neurofilaments in the thenar muscles was the same in the replant as on the nonoperated side. Vital motor end plates were found but were less than in the healthy side. Two of the three monkeys could easily pick up small articles between thumb and index; the third had more difficulties.

Concerning the allogeneic transplants, 12 partial hand transplantations were performed successfully [3]. Graft survival times were short in six cases (21–33 days) and long in another six cases (79–179 days). Five out of the 10 monkeys that showed rejection of their transplant were treated with steroids. In none of these cases could rejection reversal be obtained. The other 5 monkeys with rejection were treated with the combination of MAbs. In two out of 5 monkeys, treatment reversed their rejection episode. A second episode was treated successfully, as well. Consequently, MAbs therapy significantly prolonged allograft survival. Third-party blood transfusion did not induce a significant difference in the moment of onset of allograft rejection [35]. The remaining two monkeys were euthanised at 85 and 179 days postoperatively before rejection occurred (Fig. 2).

Blood Parameters

CyA through levels were above the minimal required dose of 400 ng/ml in 83% of all samples postoperatively and 92% of all samples after day 5. MAb serum levels varied but remained detectable 3–10 days after administration. After injection of MAbs in a rejection phase, leukocytes and lymphocytes nearly immediately decreased, which was not the case after a raise in steroids. Coating of peripheral lymphocytes remained for approximately 20 days. Elimination of Rhesus antigens lasted about the same time. If rejection was not reversed, this was of shorter duration.

General Complications

Although functional recovery was promising, complications were noted [36]. An average weight loss of 20% was observed (ranging from 8% to 40%). Seven monkeys died during the experiment. One monkey died due to an irreversible shock directly after the first injection of MAbs. Three monkeys died from multiple organ failure due to the presence of posttransplantation lymphoproliferative (PTLP) disorders. Three others died due to opportunistic infections. One monkey that died of sepsis also had PTLP disorder development at autopsy. In the monkeys that received MAb therapy, an enhanced predisposition to PTLP-disorder development was found [36]. All four PTLP disorders showed presence of simian T-leukemia virus (STLV) provirus at the DNA level in malignant tissue, indicating that this plays an aetiological role in combination with immunosuppression [37].

Fig. 2a-d. a Arm of recipient rhesus monkey (#3308) at operation. The radial unit of donor rhesus monkey (#2AC) is shown at the top of the photograph just before transplantation. The lower recipient thumb is discarded. **b** After completion of the radial hand transplantation (#2AC to #3308). **c** Rhesus monkey (#3308) 148 days after transplantation). Normal skin texture with normal appearance of the allograft. **d** Example of a transplant rejection in a rhesus monkey (#3439) 43 days after transplantation. Note the swelling and skin slough. From [1], used with permission

Sensory Recovery

In the short-term survival group (21–33 days), no sensory recovery could be detected [3]. In the long-term survival group (79–179 days), the first sign of sensory recovery was detected after a mean of 41.8 (range 27–64) days postoperatively in the median nerve area. The percentage of reinnervation increased in time. The maximal percentage reached for the six monkeys was 100%, 91%, 84%, 75% and 14% respectively. As soon as rejection occurred, sensory recovery decreased immediately, to restore again within a week following rejection reversal. In one monkey, the first sign of sensory recovery occurred 10 days after reversal of a rejection period.

Motor Recovery

The first sign of motor recovery with EMG was detected after a mean of 43.8 (range 31–56) days in four long-term surviving allografts [3]. In the two other long-term survivors, the first sign could not be established due to logistic reasons. In these two, EMG was first used at 72 and 79 days postoperatively with already present CMAPs as a sign of recovery. In two rejection-free monkeys, the ratio of amplitudes showed a similar pattern as in the replants. If rejection occurred, CMAP amplitudes decreased, with a fall of more than 40% in two monkeys. Antirejection therapy resulted in an increase again parallel to reversal of rejection to decrease

again after a new episode of rejection, with a fall of 20%. CMAP latency time decreased when relating it with time after the transplantation procedure.

Histology

In all cases where rejection was not reversible, the allograft was completely rejected within 5–10 days after onset of rejection therapy [36]. When MAb treatment was effective, rejection infiltrates and dermal hemorrhages were eliminated within 3 days. From the first to approximately the sixth day after onset of MAb therapy, coating of graft infiltrated lymphocytes, histiocytes and dermal and epidermal cells was observed. Re-epithelisation following (focal) epidermolysis developed within 5 days. In one case, necrotic parts of the skin after a second rejection episode were observed, and healing was seen within 2 weeks. Histological data confirmed muscle reinnervation. Vital motor end plates and the presence of axons were demonstrated [3].

Functional Tasks

Video recordings demonstrated long-term allograft-recipient monkeys as well as replants picking up small articles between thumb and index finger.

Summary

Our study provides data concerning technical, functional and immunological aspects of allogeneic transplantation of the radial side of the hand in the rhesus monkey [3, 32, 34, 36–38]. The monkey model was used, as it comes closest to the human situation regarding prediction of functional outcome under transplantation conditions and efficacy and safety of administered immunosuppressive drugs. For ethical and donor-side morbidity reasons, only the radial side of the hand was used as a functional unit. The rhesus monkey lives on the ground and therefore has a well-developed thumb, which closely resembles the human thumb. The human thumb is larger compared with its fingers in relation to the monkey and has more stability and independence. Therefore, our model consisted of the first ray with thenar muscles, nerves and major vessels. The model was enlarged with the radial forearm flap for monitoring and biopsy reasons.

Following replantation of our model, very good function could be achieved [32]. After allogeneic transplantation, sensory and motor function occurred. Rejection influenced functional recovery negatively but could be increased if rejection could be reversed. In nonrejected allogeneic transplantations, sensory recovery reached near normal levels when compared with the contralateral side. For motor recovery, amplitude of compound motor action potentials of thenar muscles and latency time approached the outcome of the contralateral side. Vital motor end plates were detected as a sign of motor reinnervation [3].

Six of 12 monkeys displayed long-term graft survival after transplantation with the use of continuous high doses of CyA and prednisone. An increase in steroids could not reverse rejection. MAb injection, however, could reverse rejection when it occurred. A significantly longer allograft survival was accomplished with this treatment when compared with an increase in prednisone. MAbs can eliminate various lymphocyte subsets in peripheral blood and in the allograft, therefore decreasing the rejection process [34].

Major complications were encountered, as seven of the 12 animals died during the experiment. Four of these were attributable to PTLP in which STLV was detected [36, 37].

Considering the aforementioned, promising results were obtained from technological, functional and immunological aspects. However, a less toxic immunosuppressive regimen was necessary at that time before actual allogeneic human hand transplantation could be performed.

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3. Ethics and Medico-Legal Implications

Section 3-a

Ethical Aspects of Non-Life-Saving Allografts with Special Regard to the Hand

Didier Sicard

Introduction

Ethical discussion is preferable to ethical judgment given prior to taking action. The issue is not to submit in advance medical or surgical protocols based on an arbitrary moral determination, acting as if the problem was already resolved by transcendental references (and what references?). This is even truer given that medical science is never satisfactorily based on the past and incurs as many hopes as fears, which are more or less justified. With respect to composite tissue allograft (CTA), four issues can be raised:

1. If at first, we had to fight against opposition to organ – kidney and, moreover, heart – grafts, these oppositions are now overcome. But is it possible to ethically think that the now ordinariness of these organ grafts will be, with time, considered as such for CTAs?
2. Shall there be conservative or liberal reasoning?
3. May therapeutic performance take over the well-being principle?
4. Is it possible to assimilate the CTA into usual clinical research?

In dealing with these four issues, we find four tentative answers, which are rather on the negative side.

1. No comparison is made possible since CTAs do not deal with the substantial mechanisms of life
2. Liberalism can be as dynamic as it can be adventurous; conservatism can be as cautionary as it can be apprehensive
3. Therapeutic performance only as a goal is not ethical
4. CTAs are not part of usual clinical research.

Ethical discussion should take place with the understanding that this is not going to be a match with a winner and a looser but one in which the outcome should leave us better informed. Rather than arguing in favour of or against something, which would give the impression of leaning to one side or another, we should raise questions knowing that it is always dangerous to determine an ethical judgment after the fact, such as endorsement once the fact is accomplished. This would be like having, in successive steps, the desire, the project, the research and animal experimentation, the writing of the protocol, the surgery and then the ethical reflection – a sort of "post" ethics? I will centre the discussion on CTAs related to body-part grafts.

Is Non-Life-Saving Allograft To Be Encouraged?

In this regard, several arguments can be made. Certain people think that autografts have been perfected and that in this surgical area, the allograft does not teach anything new. They argue that the delay in neuronal cortical reorganisation capacity due to peripheral stimulation is most unlikely or, in any case, the capacity for voluntary movement coordinated from a cortical command is unlikely. Even if we assume that "the

hand activates the brain" and not the contrary, this reasoning cannot apply to a graft. However, on the opposite side, experimental studies have shown – through functional magnetic resonance imaging (fMRI) – that cortical areas can change along with the postgraft evolution, and this unexpected information is encouraging. The core issue that remains is: are we dealing more with therapy or research, or neither? It is not therapy because it is not based on scientific certainty, and it is not research because a nonvalidated therapy does not automatically constitute research. But it is more a therapeutic performance in which natural effects are not small, whether regarding skin grafts for burn victims or the physiology of nervous regeneration.

Non-Life-Saving Allograft and Well-Being

Contributing to the well-being of individuals is a principle that is at the very core of all medical acts. In this particular context, is this principle being contradicted by a negative principle or is it just a balance between benefits and negative risks? The desire of a wounded individual to be cured is legitimate, but his or her hope to have his or her body back, as well as its motions, may sometimes be goals for which he or she is ready to take vital risks. It is in those terms of choices that consent must be sought without omitting to tell such individuals that the vital risk does not necessarily result in functional success even if there is a possibility of recuperating a function compatible with a normal life. Can a body-part graft be integrated in the body schema, whether on a short-term or long-term basis? The identity of a composite tissue donor cannot invade the identity of a receiver, except in movies (*Les mains d'Orlac*, from R. Wiene 1933 and E.T Greville 1950), but the permanent visibility of what is the most intimate, the most in-contact with the other, the most affectionate (we hold the hand of a dying person) may create a severe schizoid response. Integration or disintegration is not an issue raised with other organ or CTAs. In the case of a hand, the receiver, who slowly realises that it is not his or her own, may experience conflict that he or she would not experience with a mechanical prosthesis. It is true that in this domain, research remains to be done. Our ignorance is vast in terms of the human capacity to integrate "the strange foreigner". The tension between the physical aspect and the symbolic representation is maximal because of the hand's role in the economy of our organism. As opposed to organs grafts, the evolution of science – in terms of time – which will allow more and more satisfactory functioning of body parts will lead to less and less acceptable immunodepressive therapeutic constraints, and even more so because the hand has a conscious functioning based on will, unlike the liver, the heart and the kidney. Experimentation with continuous therapies for heart, kidney or liver grafts or for human immunodeficiency virus (HIV) infections shows the psychological limit of tolerance when having to constantly use medicine. We cannot hide from young, wounded individuals who will be undergoing surgery that they will receive immunotherapy for decades, which will eventually shorten their lives because of premature aging, accelerated atherosclerosis, infectious vulnerability and maybe lymphoma or melanoma. The issue is not one of a 5-year period but rather a 30- or 40-year period. Therefore, the principle of providing for the well-being of individuals has a counterpart of potentially serious drawbacks (such as the shortening of life). Thinking in terms of ordinariness for immunosuppressive treatments that are given in other types of illnesses or grafts may be considered more an opportunistic measure which prevents the patient from understanding completely and immediately the long-term stakes of the absolute necessity to undertake this therapy for life. Vulgarizing this kind of therapeutics finally deprives the patient of the opportunity of a real reflection making his consent rather uninformed.

Non-Life-Saving Allograft and Donors

The issue of the anonymous aspect of grafts, besides living donors, is a substantial principle. Here, it is removed for the receiver and also sometimes for the donor. Recently, a graft receiv-

er was thanking the family of a murderer who had committed suicide. The risk of dealing with morbid curiosity, or research by the family of the donor or, receiver of the hand cannot be excluded, and it may be the source of fantasies that a hand on the contrary to visceral organs holds the past of one's social and individual life. It would be tempting to choose body parts from isolated individuals with no families, but such specific research would raise major ethical issues. Can consent by a donor be implied or expressed in such a situation? Inasmuch as it is acceptable for most of human beings to consent to donate their liver, heart, etc., it is much more complex in our imagination to donate an arm or, specifically, one hand or two hands. In the case of CTA surgery, it is important to integrate this dimension immediately into questionnaires given to potential donors.

Conclusion

The composite tissue allografts also raise the issue of an indefinitely reparable human body. This functionalist, rational, mechanical vision is very exclusive and rejects everything that is not functional. Ethics remind us that life is not only about functionality. Ethics is also resisting functionality with tenacity, as our humanity should require, for we do not belong to ourselves, as self-respect cannot be separated from respect for others, to the body, to its integrity and unity. Therefore, if CTAs constitute an undeniable benefit for a large number of tissues, tendons, skin, cartilage, bones, etc., the issue of hand, arm or leg graft is still raised. Of course, the desire to recuperate a function, the quality of surgery teams and research – whether experimental or not – will give arguments to continue and encourage this type of activity. But ethical reflection implies that reflection on the human being be not limited to the technical success of surgery,even when it is spectacular. Non-life-saving allograft is ineluctable to insure their success to ethical reflections:

- The question of the real price to pay by the receiver: immunosuppressive therapy for life
- The question of organ donation considering that donation of a hand or a face and an invisbile organ for both receiver and donor is completely different.

Section 3-b

Ethical Issues of Organ Transplantation in Non-Life-Saving Situations

Mauro Cozzoli

Introduction

The issue of organ transplantation in non-life-saving situations is not new (consider, for example, transplantation of the cornea), but it has been rekindled in a new way by the increasingly complex interventions carried out today, for example transplant of the hand, knee or larynx. Here, I offer some general reflections on this topic, with the intent of setting the background and establishing a reference context in which we can consider the bioethical problems raised by these transplants and orient the development of guidelines and legislation. Considering that clinical policies regarding non-life-saving organ transplants are still being elaborated, with the aim of defining precise and shared medical guidelines, I do not wish to take a particular, clinical position but to proceed with an ethical analysis of the problem, offering useful points of reference, delineating conditions to respect, and recalling implications for those who have to contend with this new frontier of medicine and surgery.

The problem of organ transplantation in non-life-saving situations has moral implications from two perspectives: the surgical intervention and the donation of an organ.

The Surgical Intervention

It is *per se* accepted and incontrovertible that human and Christian morality approves of organ transplantation in life-saving cases, within the proper conditions [1]. In these situations, the life of a patient is at risk, so the operation is considered to have high priority and to be urgent; this is not the case for organ transplant in non-life-threatening situations. A life-saving operation legitimizes a level of "acceptable risk" higher than that for operations not carried out to save lives. Today, it is increasingly feasible to transplant non-vital organs: rather than being simple, these are particularly complex operations, not only considering the clinical profile but also for the multiple issues and implications for humankind. Do the same levels of acceptability and moral correctness apply?

If transplantation of a limb is not necessary to save life, it is nonetheless important for corporeal integrity, quality of life and physical liberty. Morality applies not only to the defense of life, but also to the care and promotion of health and psychophysical integrity of a person. Not having an organ, like the larynx, or part of a limb, like the hand, or suffering the devastation of an expanse of bodily tissue is not biologically normal: it is a *deficit*, a physical anomaly. The subject perceives this deficit as a deprivation, a handicap, a mutilation; he experiences it as a serious hardship. He is not considered a healthy person. He is an individual who needs care. Neither can one consider as equivalent a transplanted limb and a mechanical prosthesis: the first is a human organ that has biological continuity with the recipient, of the same physical substance as his body and therefore of the same

nature; the second instead is perceived by the recipient as an extraneous and succedaneous object.

The *principal of therapeuticity*, which legitimizes a surgical intervention meant to reestablish bodily integrity and functions, is therefore valid here. Organ transplantation for non-life-threatening reasons has justness and moral approval considering the care that we must have for our bodies and that leads us to cure our pathologies and handicaps. The intervention must be clearly therapeutic: aimed only at curing anomalies and reestablishing bodily integrity; it must not, instead, satisfy arbitrary, nonessential and superfluous desires. This would be the substitution of an organ motivated by pleasure, attractiveness, and efficiency, intended to promote or guarantee a "life of quality" rather than the quality of life of a person. Transplant surgery that forces the quality and duration of life is not part of the principal of cure but of mere desire, satisfaction and will. Every transplant really meant to cure falls within the order designed by nature, which constitutes both a criterion and a limit.

Transplant surgery is likewise part of the *principal of proportionality*, which leads us to consider the real benefit of the intervention for the recipient. For this to be called morally licit there must be proper balance (proportion) between the transplant procedure and the desired results, taking into consideration the patient's conditions. Considering that the issue at hand, in these transplants, is not survival but physical integrity and quality of life, the proportion tends to become more rigorous and demanding, establishing itself at a level or index lower than for life-saving transplants. The intervention cannot imply a greater risk than the hoped-for benefits. Curing a disability by provoking a more severe disability is not ethically acceptable, even less so if it poses a risk of death. The problem is related to the grade of functionality expected from the organ or limb transplanted, to the conditions and length of rehabilitation, to the impact and psychological reconciliation of the recipient in presence of a visible organ (like a limb) from someone else. In a particular manner, the problem is posed by the anti-rejection drugs and therefore by the state of immunosuppression required after transplantation: a clinically normal individual is transformed into an immunodepressed subject. What is the extent of this risk? What malaise does it represent? In the actual state of medicine and pharmacology, what is its real incidence on the clinical status and therefore health of the patient? In other words, is there a proper balance between the benefit of a live and living limb and the clinical and human costs of immunosuppression needed for its implantation? It is the role of ethics to pose these questions rightfully and inescapably, but it is not the role of ethics to decide the answer, because ethics does not have adequate knowledge to respond. This is role of medical science. The physician must establish – in general terms, considering biomedical progress, and case by case, considering each patient's condition – the proper balance and therefore formulate an adequate response.

Transplantation of the hand, a procedure that is becoming more common, is in particular a singular and special intervention. It is the grafting of a foreign body part, external and not hidden but tangible and visible, an organ of movement, relation and language. This may cause psychological problems, besides clinical ones; also these must be considered.

Obviously, the organ or limb to be transplanted must be obtained from a deceased person. It is not foreseen how one could licitly obtain a non-life-saving organ from a living person, even with his consent. It is not conceivable to restore the bodily integrity of one individual by severely mutilating another. Different is the case for life-saving organs, like the kidney. For non-life-saving organs, the donor must therefore be a cadaver.

There are essentially two conditions to be met for organ donation from a cadaver: the certain death of the donor (i.e. the donor is already a cadaver and does not become a cadaver), and the voluntary, gratuitous and informed consent of the donor regarding removal of the organs after death (thereby rendering unlawful explantation by force or for commercial purposes). For transplantation of a non-life-saving organ, there is a third condition: the exclusively therapeutic

destination of the donated organ, as already explained for the moral legitimization of a surgical operation. One must be careful not to expand into non-therapeutic applications, of vitalistic or aesthetic nature (like the previously denounced ones), which would no longer legitimize the donation and would justifiably dissuade subjects from donating their own organs after death. They would in fact see their will to help another person (not to satisfy abusive desires) contradicted and disregarded.

Organ Donation

This brings us from the moral problem of the medical operation to the act of organ donation that each of us is requested to make. The surgical intervention is more than a medical event (in technical sense). It is a profoundly human event. It is the meeting point and junction of an interpersonal relationship between donor and recipient. The surgical possibility and its widespread use today affects all human individuals, who are called to a singular and new form of availability and love for others. Transplant surgery is a call to give oneself: an organ from one's own body, postmortem, to another who finds himself in need.

The willingness to donate responds to the morality of solidarity, so emblematically expressed in common language by the saying "give a hand" to another. Today for the first time this expression may be true not only metaphorically. It is becoming true also in a real sense. This special form of solidarity has a high moral value and kindles a singular responsibility for donation. But for this to become effective, i.e. that individuals choose to donate their organs, it is necessary that the conditions of donation, in particular the respect for a strictly therapeutic destination of non-vital organs, are recognized and defended by the same physicians, are received and integrated into their deontological codes and are guaranteed by the law. Physicians must feel personally involved in arousing and ensuring the trust of donors. They cannot promise to satisfy desires, but only to cure handicaps, anomalies and diseases. Their *therapeutic faithfulness* is for donors a source of trust and incentive to donate organs. Transplantation of an organ for non-life-saving situations, while it enlarges the possibilities of surgical interventions, poses thus new requests for trust, on responses and guarantees to which the solidarity that motivates organ donation depends.

This is a problem that, for non-life-saving organ transplants, will continue to exist even when, for other types of transplantation, it will be possible to proceed with xenotransplantation or even the use of stem cells. Non-life-saving organ transplantation in fact cannot take advantage of transgenic animal organs, nor is it possible to procure a limb by cultivating stem cells. The possibility of non-life-saving organ transplantation is, and will continue to be tied only to postmortem organ donation. For this reason, it is necessary to stimulate, and not discourage by non-curative uses, the trust of donors.

The solidarity that leads to organ donation in the light of Christian faith has the biblical and theological sense of *charity*, the root of which – *karis* – signifies grace, gift. Charity is *karis*: to reproduce and effuse the free love of God, that here assumes a particular sense and a new possibility: the sense and possibility of "making a gift of one's self" to another with something profoundly personal, like an organ of one's own body that continues to live, giving life to another, a limb that continues to function giving liberty and possibility of action to a brother or sister. This reproduces the *gift of one's self of God in Christ to us*, that is the gift of love until death, that transforms death – a death dramatic like the cross – in event of life [2].

Transplant surgery permits this singular form of charity, in other words a love for life that continues after death. Therefore, the *bios* (Greek, life) in some organs is not immediately destined to decomposition but to continuity of life in another, who gains bodily integrity and liberty. This is a particular form of therapeutic charity – root and fruit of the therapeutic alliance between donor and physician – meant to cure another person with the offer of a good not external to us but part of us; I would almost like to state that the gift is ourselves. So that some-

thing of us continues to live in her, permitting a quality of life and a liberty of action in which the Creator's original project is reestablished. So which the recipient can open himself, reconciled, to the full praise of gratitude to God, principle and source of every gift.

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Section 3-c

Informed Consent, Medico-Legal Implications, Public and Private Insurance Issues and Quantification of Disability in Hand Transplantation

Umberto Genovese

"Well, space is there, and we're going to climb it, and the moon and the planets are there, and new hopes for knowledge and peace are there. And, therefore, as we set sail we ask God's blessing on the most hazardous and dangerous and greatest adventure on which man has ever embarked".

We Choose to Go to the Moon. Speech by John F. Kennedy, September 12, 1962

Introduction

The first hand transplant meant – apart from the extraordinary surgical procedure and due to the particular therapeutic purpose – an opportunity for a multidisciplinary (medical, juridical, ethical) consideration to support or contradict such a therapeutic procedure's legitimacy, which could be considered "*the most hazardous and dangerous and greatest adventure on which man has ever embarked*".

Disregarding the wide and uncertain problematic matters relating to the lawfulness of the medical-surgical act, when addressing this in relation to jurisprudence and bioethics, it is obvious that satisfaction of so-called "risks-to-benefits balance" is not necessarily obvious in a particular circumstance[1]. Upper-limb transplant, although set among allogeneic homoplastic organ grafts in which immunosuppressive therapy administration is mandatory, is considerably outside that category due to its "function-preserving" transplant definition. Meanwhile, side-effects yielded by antirejection drugs (in terms of increased sensitivity to infections and neoplastic forms) receive unanimous "justification" for "life-saving" grafts where benefits as prognosis *quoad vitam* exist.

While the collateral effects caused by antirejection medicines (in terms of increasing the susceptibility to infections and neoplastic forms) find approval in "safeguarding" transplants where a benefit in terms of *quoad vitam* prognosis exists, it is impossible to guarantee concisely in advance for transplants, such as that of the hand, which, in front of the feared collateral effects, offer a partial restoration of function that is prominent but not *vita*: is it really lawful to perform surgery that, while improving quality of life, puts at risk the health or even the survival of the individual who submits to it?

Apart from the current idea of health and illness, to which concepts such as "quality of life" and "well-being" are linked, and apart from the continuous extension of the limits and goals of medicine, it can be intuited how the legitimization of such a procedure can oscillate, searching for a balance between the ethical and scientific

duty of verifying the effectiveness of the procedure's therapeutic capacity and the need of taking into consideration the increasing health requests of the diseased individual and his or her right to free will.

Actually, some limits seem to be partially overcome, and a sort of "temporary legitimisation" has been conquered thanks to the addition, within such a surgical process, of two firm assumptions of the medical act: the experimental nature and informed consent. In a sense, the undertaking of an experimental project that addressed the verification of actual risks and benefits of the transplant of an upper limb enables not only the thorough examination of the validity of the scientific procedure but also fulfills the need of therapeutic capacity. In another sense, the drawing up of an informed consent, apart from being a procedure that cannot be disregarded in any experimentation, acquires in the actual case a further relevance, being a maximum expression of freedom and will of an individual who chooses his or her own health standards and quality of life.

Considering what the Italian experience has been, and based also on the same assumptions adopted internationally, hand transplants performed at the San Gerardo di Monza Hospital represent the success of an experimental surgical project that, with previous approval of the Ministry of Sanity and the hospital's Bioethics Committee, has been established as a result of the work of a multidisciplinary medical team composed of microsurgeons, anaesthetists, immunologists, psychiatrists and legal doctors. These last, in fact, have the task of formulating an informed consent form for the transplant of an upper limb that is not limited to addressing those aspects common to all surgical procedures (illustration of benefits, risks, postsurgical course) but, instead, is comprehensive of all issues, including those of an ethical, social and economic nature, that such a "not safeguarding" procedure evokes.

Proceeding then to the drawing up of diverse consent forms. The first illustrates the modalities of selection of individuals suitable for inclusion in the protocol for hand transplantation and is the expression of the free agreement to the diagnostic iter created for such an end. The second represents the insurmountable limitations of the execution of the transplant since it is the evidence of the full awareness of the patient regarding benefits, risks and possible repercussions (not only in terms of health) of the surgery and a contemporary expression of the will to submit to such an experimental procedure. In both cases, the informed consent forms are quite complex since upper-limb transplantation is so innovative as a "non-safeguarding" procedure and the multiple medico-legal problems to which it is correlated. Consequently, these forms are displayed here in their complete form, accompanied by explanatory comments in which insurance and social security aspects will also be taken into consideration.

INFORMATION PAPER TO CONSENT

THE DIAGNOSTIC PROCEDURE DIRECTED TO EVALUATE THE SUITABILITY TO THE POSSIBLE "NON-LIFE-SAVING" OPERATION OF UPPER LIMB TRANSPLANTATION (HAND)

This document exposes the terms and conditions of my consent and agreement to undergo a diagnostic procedure aimed to **preliminarily evalute and check my suitability**[2] for an upper limb (hand) graft coming from a corpse.

I hereby confirm that I directly got in touch with the medical equipe and that I **freely and willingly asked** to be included in the evaluation procedure for the suitability (recruiting phase) to a **possible** upper limb transplantation[3].

I have been preliminarily informed about the **kind of the operation** I could eventually undergo, about its **"non-life-saving" nature**, about the fact that **it cannot be guaranteed the success** of the surgical operation and of **the functionality of the graft which, in any case, will be only partial** and not comparable to the one of one's limb. I have also been informed about the **conservative therapy**, of a physical-rehabilitative and pharmacological nature, that will follow the transplantation and that this therapy could cause such complications to compromise my health[4].

It has been explained to me and I have clearly understood the multidisciplinary and multiphase features of the suitability evaluation procedure which I will undergo, that includes an interview and a surgical preliminary visit, a psychiatric verification, a haematochemical and microbiological evaluation, an anaesthesiological evaluation, an immunological evaluation and a medico-legal verification.

The execution of some of the above-mentioned verifications **will require some invasive routine practices** (for instance, blood sampling) for which, in any case, I will be asked **my specific consent** from time to time[5].

I am aware and it has been explicitly explained to me that such diagnostic phase is essential to the evaluation of my psychological suitability to graft, and, most of all, that **undergoing these evaluation procedures will not automatically lead to the subsequent performance of the tranplantation.** I have been also informed that **the suitability that might result from the procedure only represents a necessary condition, but it is not sufficient**, on its own, to the performance of the transplantation, as the operation also depends on other criteria, among which the morphostructural features of the possible limb graft and/or some possible clinical priorities, whose competence is exclusively of the clinical-surgical equipe[6].

I therefore confirm that **I have never**, in any moment, **been given the certitude as to the possibility to be the recipient of an upper limb graft.**
At the end of the multidisciplinary and multiphase diagnostic procedure, which **I willingly decide to undergo**, I will be informed of my possible suitability (which will derive from the criteria in use within this specific polyvalent equipe) to be included in a waiting protocol for this kind of transplantation[7].

Obviously, at any moment, I will be in the position to suspend my participation in this protocol.

I finally consent that the results of the verifications that I will undergo could be used to scientific aims (in accordance with the contents of the law concerning the protection of personal data).

INFORMATION PAPER FOR THE CONSENT
TO THE "NON-LIFE-SAVING" TRANSPLANTATION OF THE UPPER LIMB (HAND)

CONTENTS

This document exposes a **summary of the information collected from the interviews** with the members of the clinical-surgical equipe to which I applied and the terms and conditions of my consent and my agreement to undergo an upper limb transplantation (hand) coming from a corpse[8].

PREAMBLE

❑ I hereby confirm that I have **voluntarily** consented to undergo such operation and the conservative therapy that will follow it (of a physical-rehabilitative and pharmacological nature) and that I have been chosen after clinical, laboratory, instrumental and psychological evaluations for which I autonomously made myself available.

❑ I have been informed and I have fully understood that the operation I will undergo and the following therapy are carried out within an **experimental project**, whose aim is to evalutate the results. I am therefore going to undergo this procedure **both in order to fulfill personal therapeutical goals and to give a possible contribution to other patients**, who could benefit from the experience rising from my clinical-surgical case[9].

❑ I am also totally aware that the equipe I rely on cannot assure nor grant the good result of the surgical operation and of the functionality of the transplantation. I have also been extensively informed that the functionality will be partial and different from the one's own hand and that **a certain period of time will elapse after the surgical operation during which the transplanted part could be unable to function at the best** of the capabilities that have been proposed to me[10].

***FUNCTION-SAVING* TRANSPLANT AND ALTERNATIVES**

I have clearly understood that it is a surgical operation **with therapeutic aims even though it is not "life-saving".** I also know and I have been reminded and shown by the medical staff **the alternatives available at the moment** (mainly biomechanic prothesis) which I don't consider suitable for my case because:
- I have uselessly and repeatedly tried to use them in an effective way
- ... (other reasons)[11]

SOCIAL, ECONOMIC AND WORKING IMPLICATIONS

Nobody, at any moment persuaded me to undergo this procedure. I have never been proposed any personal or somebody's else interests and/or benefits of an economic or "image" nature[12].

I have also been informed that:

❑ **The improvement of my handicap could lead to the loss of privileged protections** of an employment and/or financial nature that are acknowledged to me[13];

❑ **The pharmacological therapy** that I will undergo to **could influence the signature of insurance contracts**, among which are the ones concerning the "disease" risk;

❑ The above-mentionned therapy along with the intense physical rehabilitation which I will have to undergo, could **compromise**, even for an undetermined period of time and not in the postoperative period, **my social and professional life**[14].

SURGICAL ACT AND DIRECT COMPLICATIONS

❑ Through some **interviews and the direct viewing of some slides and films**, I learnt both the realisation of the operation's details, during which a part of a deceased human being (forearm), considered by the equipe to be (immunologically, surgically and morphologically) suitable, will be transplanted on my body, and the present (local and general) conditions of the patient who preceded me[15].

❑ ..... (complications of the classical surgical procedure).
❑ I have been informed that important and/or long-lasting operations (like the one I will undergo), can cause **several complications including but not only, pulmonary embolism and severe infections**, which might have a mortal outcome[16].

ANAESTHESIA

I have been informed that the execution of the transplantation I will undergo needs to be carried out under general anaesthesia/block anaesthesia and with the use of specific techniques monitoring vital functions, of which I have fully understood the relating explanations. I have been informed that the anaesthesiological practices can cause **possible complications, some of which are extremely serious or even fatal.** I have been informed of the present risks related to these techniques and **I was given the possibility to gather more information privately and to obtain further direct explanations.** I have also been informed about the **possible haemorrhagic and transfusional complications ...**[17]

POSTOPERATIVE FOLLOW-UP

❑ I have clearly understood that the surgical operation will be followed by a hospitalised convalescence period and **a long phase of intense physical and rehabilitative therapy, the incomplete adherence to which I will compromise the result of the graft.** During this period, I will have to undergo routine verifications and specific tests which will include, among others, cutaneous biopsies, blood testing, reactivity and vitality tests on vessels, bones, muscles and nerves, and neurosychic evaluations[18].

❑ I have also fully understood that after the operation I will need to submit to an **antirejection protocol**; I have been informed that **it can damage among other functions, the blood production and my immune defence system,** leaving me generally more prone to infections and causing a possible increase of susceptibility to the onset of tumoral forms. The functionality of many other apparatus too could be damaged by this treatment[19].

❑ I have also been informed that, **as this kind of operation is at its first executions and is part of an experimental project,** there is the possibility of appearance of **well-known or unknown reactions such to suggest**, according to relevant medical advice and after my suitable information, **the amputation of the graft from my body** at any moment. I am also aware that these reactions, known or unknown, could also lead to my death[20].

PRIVACY

❑ **I am aware of the discussions, of ethical and moral nature**, related to the performance of this operation and its following pharmacological therapy and I have also been informed that, due to these factors and to the distinctiveness of the transplantation itself, **I could be the object of public attention. I engage myself**, on this subject, **to consult each time the medical equipe** who performed the operation and who will follow me, so that the information eventually given by me is, from a scientific point of view, as suitable as possible and not misleading to common people[21].

❑ Moreover, **I consent that my clinical-surgical experience and its images are used for scientific purposes** (with respect, of course, to the of law concerning the protection of personal data).

CONFRONTATION AND REFLECTION

❑ I attentively evaluated the terms of this consent paper and I had the opportunity to have an **independent advice** on its contents even from people different from those of the equipe who proposed it to me. **I examined this paper in particular with .....** (family members, family doctor, lawyer ...) who have no objections to it.

❑ I also had the chance **to make the decision to consent** to undergo the transplantation **after a** considered and in depth **discussion with my closest people**[22].

CONCLUSIONS

❑ I agree that clear and complete verbal information has been given by the members of the clinical-surgical equipe to which I spontaneously applied to and I have integrally understood the content of this paper, which represents the summary of the received information. At the moment, I do not have any further enquiry or question to ask, and I therefore consent to undergo the "non-life-saving" operation of upper limb transplantation (hand) coming from a corpse.

❑ It is understood that, should any doubt come to my mind, and I will have the chance to immediately consult any doctor of the equipe, and, at any moment, I will be able to interrupt my participation in the study (even in the postsurgical phase) without losing the complete collaboration of the same medical staff with the aim of protecting my health[23].

SIGNATURE

The patient .....

A doctor of the medical equipe .....

Testimonies [24].....

Notes

[1] Farneti A, Genovese U, European Consensus Conference on Replantation at the upper limb and the day surgery in surgery of the hand (1999) Hand transplantation: medico-legal implications. Rivista di Chirurgia e riabilitazione della Mano e dell'arto superiore 36(2-3)
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[2] The continuous use of certain terms and concepts within the form of consent is an intended and deliberate choice.

[3] Nevertheless, because of the experimental character of the surgery and the uncertainty of the therapeutic considerations between benefits and risks, it became immediately necessary to stress the presuppositions on which the actual form of consent is based. These are those of will and freedom to abide by a diagnostic iter addressed to the recruitment of patients that are appropriate for the transplant.

[4] The need for clarifying and illustrating the typology of the surgery, its functional purpose, the uncertainty of the results and the concrete possibility of complications arises from the demand of providing the patient with all the elements in order to judge the case. Ensuring the freedom of choice presupposes adequate, understandable and complete information provided by the doctor and the total awareness of the patient.

[5] Regarding a diagnostic iter to which an individual is exposed, not for the need of avoiding an event that could lead him or her to death, but instead, for gaining a better quality life, it becomes even more necessary to illustrate in detail the various phases of selection of an experimental sample, being attentive not to omit those invasive procedures for which an additional consent form is proposed.

[6] The "priorities" to which this refers are those correlated to peculiarities not only anatomical (e.g. bilaterality of the amputation, height of the same), but also psychosocial (strong motivation, family support).

[7] This is certainly the main passage of the present form of agreement to the phase of recruitment. Regarding an experimental protocol, the selection of the patient to submit to the surgery represents one of the most delicate and decisive phases, not only for the probable success or failure of the procedure, but above all for the custody of the individual that chooses to perform a transplant. Because of the inability to guarantee success, inappropriate selection would presuppose increasing the risk and reducing benefits, which are already, also when completely obtained, could not balance eventual side-effects of the immunosuppressive therapy. The decision to emphasize the lack of guarantees for the surgery arises as well from the need to respect those who are the therapeutical expectations of patients that, when proposing themselves to the innovative transplant, are strongly determined to regain their own physical integrity.

[8] The decision to highlight the fact that such a document displays a "synthesis" of the information provided to the patient intends to strengthen, in this specific case, the importance of interviews between the team of surgeons and the patient prior to the surgery. It is completely evident that a consensus given in written form cannot, and absolutely must not, leave aside dialogue, which represents an irreplaceable occasion of reciprocal interaction and clarification.

[9] The therapeutic experimentation, apart from obtaining the consent of the individual involved, finds it's own legality in relation to yielding a therapeutic benefit not only to the individual who submits to the experimentation, but also to the collective population that is needful of the same therapeutic goal. Regarding a consent that is formulated within an experimental project, and in order to guarantee to the patient a complete view of the procedure, it seems right not to omit pointing out the double objective to which it is addressed.

[10] Every experimentation, as such, implies an uncertainty regarding the result and, as a consequence, must be illustrated to the patient in order to make him or her completely aware. For the hand transplant, such therapeutic uncertainty becomes even more significant, for two series of reasons. First of all it concerns a procedure that attempts to increase the "quality" of life, and to which an individual chooses to agree to out of free will and is not forced by the necessity of avoiding a life-threatening situation. Secondly, the surgery guarantees restoration of the anatomical integrity of the upper limb but not its functional integrity, which can be restored only partially. Actually, the need to repeatedly address the "lack of guarantees" regarding the result, at first during the interviews with the patient and later during the drawing up of the informed consent, arises from the need to acquire a consent to the procedure based on the awareness of partial, and not even certain, therapeutic benefits expected from the transplant.

[11] A suitable informed consent must always foresee that the alternative to the surgical act proposed be addressed with the patient.

[12] It certainly regards a complete innovative and apparently inadequate relevance that the transplant of hand has had and the interest demonstrated by the mass media, in the present situation, it becomes necessary to include aspects that are not purely medical, such as those of economic and journalistic relevance.

[13] The anatomical–functional restoration of an amputated limb, even if partial, modifies in a positive sense the disabled condition of an individual. Considering that the Italian social security assistance system guarantees economic benefits to individuals who have permanent disabilities that affect their capacity of producing their own income, it is presumable that following the hand transplant, there could be a reduction in the benefits previously granted.

[14] The continuous administration of immunosuppressive therapy, with its repercussions in terms of increased infective pathologies and neoplasms, is the reason for refusal of private insurance companies to stipulate an insurance contract addressed to indemnify the economic prejudice derived from illness. The stipulation of "illness" policies and "refund of sanitary expenses", in fact, aims to safeguard the economic interests of the insurance company. These are known to be founded upon valuation of the amount of risk to which an individual is exposed: it can be intuited that the existence of a high risk will represent an economic disadvantage and reason for a "non-investment" from the insurance company.

[15] The use of images (photographs, videos) to show to the patient is essential in the procedure discussed. Apart from the importance of being able to concretely visualise the results obtained up to now, it is must also be taken into consideration the fact that in treating a transplant of an anatomical part that is not only visible but also full of relational and social significance, the patient will be able to "metabolise" what the result and new physical image could be.

[16] Leaving behind the peculiarity of the procedure, the matter regards a surgery, and therefore it cannot be excepted from the illustration of the most common, and above all, serious postoperative complications.

[17] This pertains to specifications that are commonly adopted by any consent form to a surgical act, not worthy of further inquiry.

[18] The postoperative aspects are rarely contemplated in an informed consent. However, regarding an experimental procedure, but most of all a procedure to which an individual freely decides to submit without there being a concrete life-threatening situation, it is essential to illustrate in a detailed manner the follow-up phases and the predictable postoperative course. In particular, the latter deserves a certain intensification of emphasis with the patient since, because it is a demanding rehabilitation program, it represents one of the possible causes of reduced compliance of the individual. Because it is not possible to concretely indicate the duration of the rehabilitative phase, the direct viewing of videos of the progress of previous individuals studied represents, for the patient, a useful instrument of judgment in that sense.

[19] This is probably one of the most relevant aspects in the present document of consent – being aware that the administering of immunosuppressive therapy, with its systematic repercussions, still represents the main "limitation" of such a procedure. It is even more crucial, therefore, that the patient be aware of the modality and, above all, the complications, of the pharmacological therapy.

[20] Besides not being able to guarantee the result in terms of functional restoration of the transplanted limb, it is necessary to point out to the patient that the conservation of the transplanted part is not guaranteed throughout time and that there are circumstances in which malfunction of the part or rejection could occur, leading to an inevitable amputation procedure.

[21] Once more matters of a "journalistic" nature are addressed. The patient is requested to make a commitment not to discuss the experimental procedure with the news media before having consulted with the surgical team. This request is focused on guaranteeing scientific trustworthiness of the information provided to the mass media.

[22] The fact that the patient consents to submit to the procedure after having consulted family or even the people to whom he or she is emotionally attached, is favorable. Apart from problems relating to the acceptance of immunosuppressive therapy, the procedure concerns a transplant of a physical part that is visible and is involved in gestural expressiveness and interpersonal hand contact, conditions surrounded by strong social and family values. The hand transplant does not exert psychological repercussions on the individual that undergoes the surgery only, but also on relatives, as does the long phases of rehabilitation. Lastly, it points out that the indication of characters, such as the family doctor and lawyer, is purposely inserted in order that the individual consults with technicians of specific sectors relating to the content of the consent form.

[23] The availability of the medical team does not cease in the event the patient interrupts the study. Instead it guarantees the full availability of the team to guide him or her toward a decision that will safeguard his or her health.

[24] Obviously, the signing of the form by the patient must be situated at the end of the document, after the conclusions, and in the presence of witnesses who acknowledge the same.

Section 3-d

Living with Two Different Fingerprints: Legal Implications and Identification Issues

Giuseppina Menna, Paolo Scarpis

Introduction

At the beginning of the twenty-first century, the news of the first hand transplant performed in San Gerardo Hospital of Monza, Italy, was widely announced and enthusiastically greeted by the press. The event was welcomed with amazement, as it reminded us of our deep emotions when Dr. Christian Barnard performed the first heart transplant in Cape Town, South Africa. At that time, the issue of any possible psychological changes in the recipient's personality was thoroughly debated: somehow, heart transplant could be experienced, sometimes with fear, as a different sense of self, a significant change in the peculiarity, individuality and identity of the human being.

As with the heart, hands "are undoubtedly one of the most interesting parts of the human body". They speak "about the individual's character (i.e. from handshaking), they can tell something about his or her work (the calluses if he or she works all day with shovels, spades or other tools), they express feelings (bitten nails)". Those who cannot talk "speak using their hands; you can paint or play music using your hands". Hands are the brain's direct prolongation, a means of communication with the outer world; they express feelings, they act and produce.

Who am I really if parts of my body can be replaced by parts belonging to other individual's? To what extent is my identity safeguarded? Who shall I be with someone else's hand? Could I still claim to be the same person when the hand is no longer my hand but a new limb bearing scars, lines, fingerprints, i.e. the distinctive features, of a different individual who has lived different life and experiences?

Thus, it is clear that hand transplant has crucial bioethical, social and psychological implications. But it can also have a dramatic effect on the daily routine procedures of antiterrorism, administrative and, above all, crime investigation police since the hand remains almost the sole part of the body to be investigated for the identification of human beings.[1]

From time immemorial, investigators and forensic pathologists have been concerned with identification; in particular, there has always been the need to link personal data to material entities, i.e. "to identify the person who bears the two segments (physical and administrative) needed to ascertain his or her identity. The person is identified when such elements are matched and known, but when one of them is unknown, then it is difficult to find it and therefore match it up with the other one". "Each human being has psyco-physical features that make him different, even unique and inimitable from the rest of mankind. These differences appear to be more significant whenever the human community of reference undergoes a major demographic development. All the more so whenever such community becomes a civil society subject to a legal system. In this case, the physical identification matches with the legal

one, and the virtual construction of the latter follows biunivocal models of reference pertaining to the anthropomorphic features and the personal details assigned - as acknowledged by the law system which the human being is subject to"[2].

In Italy, the forensic pathologist Prof. Ottolenghi (1861–1934), Cesare Lombroso's former assistant and a professor in Siena, put together a team of experts who created the fingerprint identification system (ten-finger system)[3]. In 1905 Giovanni Gasti (1869-1939), Police Superintendant and Forensic Science expert, made a special contribution to this system based on ten symbols (represented by figures from 0 to 9) and used until 1997, when A.F.I.S. (Automated Fingerprint Identification System*)* was introduced. In a computerized database, A.F.I.S. collects and manages photographing and fingerprint cards at national level[4].

A fingerprint is an imprint made by the tip of a finger pressed onto a smooth surface. Ridge lines are its most evident structural features: they run in parallel and circular lines thus shaping the fingerprint pattern. In certain finger areas ridge lines take on distinctive shapes and the whole pattern shows discontinuities, such as endings and/or bifurcations, known as "minutiae" and representing the elements on which fingerprint matching is more frequently based.

Nowadays fingerprinting is no longer limited to the traditional fields of investigations and legal proceedings, but it has a wider range of practical applications, from the security of logic access to data and services provided online to the physical access to restricted areas.

Moreover, dramatic events – from the terrorist attacks of 11 September 2001 to those recently involving the European Union – brought about a widespread demand for security. Thus, the individual identification or authentication is the only means which makes it possible to certify the identity of a human individual on the basis of his unique and inimitable features.

Many countries at high risk of terror attacks are thoroughly considering new types of papers bearing biometric data with a view to obtain a trustworthy identification. In the near future, the biometric passport as well as the electronic stay permit and card will bear both personal details and physical characteristics, usually fingerprints, coded as a digital picture or a mathematical model ("Template")[5]. Since 26 October 2005 new passports have been bearing a digital photograph and as of 26 October 2006 they shall also bear digital fingerprints[6]. Italian nationals will be issued with an Electronic Identity Card endowed with a microchip containing the digital representation of two fingerprints of its bearer, who is thus identified by comparing the template stored with the input template[7].

The use of fingerprinting has become widespread in all sectors of everyday life. Here are a few examples: employers' access to their workstation and to crucial computer applications where passwords could be replaced by fingerprints; access to smart cards by using a fingerprint instead of a PIN code. By simply pressing one finger against a scanner it could soon be possible to have access to contracts of sale and purchase, or get into one's house. An Italian territorial agency dealing with university students' rights decided to install fingerprint readers in restaurants and pizzerias, having an arrangement with them, after their employers' luncheon vouchers had been given to unauthorized people.

In order to provide an exhaustive exposition on the matter, it would be useful to make a few observations about "biometrics", a quite new discipline still being developed. It might be considered as a branch of biology which some people describe as a "science studying how to classify human beings on the basis of their physical characteristics, fingerprints, gestural expressiveness, veins map, iris [8], hand geometry[9] itself, biometric identification of their voices and signatures."

The biometric data[10] must be invariable for a long time, measurable, reliable and unique (that is, they must yield one single unambiguous result). The method of survey must not be invasive, the data must lead to results easy to be checked. While registering, the system detects a biometric element of the person and shows the measurement in the form of mathematical data; the following check step consists in the compar-

ison between the biometric data previously registered and the one being analized for either authentication or identification.

In this context, fingerprints are the most important element, they are the most stored and used widespread data. How will the changed pattern of papillary ridges affect a person's life?

Attention must be primarily drawn to the forensic investigation field.

As far as we know, the first hand transplant in the world was performed in Lyon and concerned a 48-year-old New Zealander who had his right forearm amputated in 1984, while he was detained in prison for tax fraud.

If we apply the Italian procedure protocols to this context, we presume that he underwent photo-fingerprinting by the police at the moment he was put in prison and fingerprinting, once again, on the register of the Roll Office personnel. As soon as the prisoner went to jail, a card was presumably drawn up with all his previous photo-fingerprints in chronological order, even in case he had declared a different name for each event. Such cards are the result of the fingerprint matching, made by A.F.I.S., which collects, as above mentioned, the photo-fingerprint cards over the whole national territory. Obviously, they are correlated not on the basis of the stated personal particulars, but exclusively on the basis of the fingerprints taken.

Going back to the New Zealandese patient, he will have, for the rest of his life, one hand which has never been fingerprinted before, unlike his, nor has ever appeared on any prison register.

If the new fingerprints of the patient who by ill chance commits a crime once again were later entered into the A.F.I.S. system, they would yield two separate alias lists, in the name of two different natural persons: the living patient and the hand donor. If someone being fingerprinted declares to be a person who underwent a transplant, the fingerprinting experts will steer their search of criminal records using exclusively the fingerprints of the natural hand; this search requires more time but the result will certainly be the correlation to the only photo-fingerprints taken before the transplant date. Moreover, this practice will protect the donor's right to remain anonymous.

The case of the patient of Monza (Italy) is of an ordinary man. During an interview he said: "My family has always been normal and close, I hope that there won't be any troubles in the future either." No problems of the kind described above arise in this situation.

Further questions could emerge if we merely consider the possibility that the transplanted hand had been of the corpse of a person photo-fingerprinted when alive. At a certain moment of his existence, Professor Lanzetta's patient could realize that he received the hand of a person with fingerprint records. Even not considering the unlikely conjecture of a patient who may become a thief and may leave fingerprint residue on the crime scene, a problem could arise under other circumstances. Let us think of a patient, victim of a theft in his house: the residue collected during the on-the-spot investigation, performed by the forensic police, will be immediately entered into the A.F.I.S. system; the search will allow to compare the relevant fingerprint card with the donor's one ... a burglar when alive.

The above hypotheses are paradoxical; however, they aim at explaining briefly the effectiveness of the A.F.I.S. system to people not belonging to the police force.

According to the actual procedure, the competent Police headquarters have to communicate to the Central Identification Registry at the Forensic Police Office in Rome, the names and addresses of persons with police or criminal records, deceased for any reason whatsoever. The date of death will then be added on the photo-fingerprint card of the deceased, so as to delete said data some time later. The alias card relating to the transplanted hand will lead back to the donor's card, where the date of death will definitely appear.

In any case and provided that the utmost respect of privacy is ensured, it is absolutely necessary to take the fingerprints of the donor's corpse when, obviously, there are reasons to believe that photo-fingerprints of that person had been taken during his lifetime.

We assume therefore that hand-transplant must be performed immediately and this excludes the possibility of fingerprinting before the transplant. Yet, fingerprints can be taken

some time later. The print of just one of the fingers of the non-transplanted hand will suffice for A.F.I.S. purposes. The tip of one finger will be inked and pressed against a scrap of paper. The finger will then be cleaned in respect of the dead donor's dignity.

As a matter of fact the donor is always a fully identified person; therefore it might be enough to delete the cards referring to the donor's personal data in the A.F.I.S. system. Of course photo-fingerprints generally pertain to fictitious names declared during one's lifetime so as to hide the true identity; hence, search of personal data might not suffice. Instead, the positive issue of the donor's fingerprint matching would entail the immediate and definite erasing of all the relevant input photo-fingerprint cards.

In this way the donor's right to remain anonymous would also be safeguarded.

Another matter of interest concerns the devices used to take fingerprints for any authentication purpose.

"Systems performing different functions were developed according to various needs. Some data processing platforms are based on access control and are meant for a quite restricted number of users; thus, their database is limited accordingly, while other kinds of database are real client recipients, that is they store and file biometric data for purposes which can be even decided later. In this last case, at times, some black lists are set up. They consist of groups of users who, from a certain moment on, are unwelcome in the organization structure. Therefore the system will exclude those users or forbid them to have access to information or reserved areas"[11].

Making use of optical instruments, it is possibile to process and adequately compress the image of a fingerprint in order to hold it on a database and make it available for further matchings each time it is necessary to identify a person. The positive identification practice entails a link between a person whose identification data are held on the system and the user. It is the result of a comparison between the pattern being examined and one or more stored templates. The person to be identified is (or is not) included in a group of people known to the system; the ultimate aim is to prevent any single person from using several identities as well as to avoid access by someone not properly authorized[12].

When someone dies and their fingerprints were entered as key to access the system, as well as when an employee is transferred to another branch, we can easily assume that the administrator of the database will delete the data no more usable.

The transplant patient himself will require, if necessary, a new storage of his biometric data when related to the fingerprints of the transplanted hand, together with the erasing of any data stored prior to said transplant.

We would like to conclude this reflection with Francis Galton's words said in London in 1888 and published in a serious scientific journal of that time:

"I do not now speak of the large wrinkles in which chiromantists delight and which may be compared to the creases in an old coat or to the deep folds in the hide of a rhinoceros, but of the fine lines of which the buttered fingers of children are apt to stamp impressions on the margins of the books they handle."

This passage from Galton's speech briefly recalls in other words what we have been trying to explain. Hands are not only a set of nerves, bones and muscles, but they define individuals as regards their expressions and, more technically, their biometric characteristics.

A patient waking up in hospital with a new hand will certainly be given a renewed quality of life. As time goes by, the patient will hopefully be able to give his new hand not only his own blood and energy, but also his own indisputable identity.

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Notes

[1] **Art.4 *TULPS* (Consolidation Act of Police):** "Police forces have the authority to order a dangerous or suspected person, or a person who is unable or unwilling to give evidence of his identity, to undergo identification tests", i.e. descriptive, photographic, fingerprint and anthropometric tests.

Art.11 Law Decree 21.3.1978 no.59 turned into **Law 18.5.1978 no.191** – Penal and procedural rules relating to crime prevention and repression – art. 11, paras. 1 and 2: "Police officers have the authority to escort to their Offices a person who, being requested, refuse to give personal details, and to hold him for the time necessary to identify him and, in any case, for a period of time not exceeding 24 hours. The same rule is to be applied where there is evidence suggesting that said person gave false personal details or showed false identity papers".

Art.349, para.1, Code of Criminal Procedure: Criminal Police identify the person under investigation and anyone able to report circumstances relevant to the reconstruction of the event. The person under investigation can also be identified, where necessary, by means of photographing, fingerprinting, anthropometric tests or any further tests".

Regulation (EC) no.2725/2000 – Asylum-seekers and persons who have crossed a frontier in an irregular manner are subject to photographing and fingerprinting. Under the Dublin Convention, each Member State will be able to determine whether a foreign national found illegally present in its territory had previously submitted an asylum application in another Member State (**Eurodac** programme started in 2003)

Law 9.10.2002 no.189 containing urgent provisions on the regularization of non-EU nationals' illegal work. It provides that the foreign national must be photographed and fingerprinted before applying for the issue or the extension of a stay permit.

[2] In ancient Babylon, debtors were asked to leave their fingerprints on clay tablets as a guarantee for their creditors. But it was the ritual itself that strenghtened the guarantee. In the 17th century in France, criminals were branded on their skin with the initial of the crime committed. It was an ineffaceable mark through which criminals could be identified. This procedure was abolished in 1832. In the 19th century in Campania, at the *Questura* of Naples, an Anthropomethric Laboratory was founded by Professor De Blasio, an anthropologist of the University of Naples. Photographs of "brigands" were taken and their comparison started being used in 1892.

[3] **Marcello Malpighi** (1682–1694), a professor of anatomy at the University of Bologna, is thought to have been a pioneer of fingerprint research: in 1686 he described the different layers of the derm and the patterns on fingertips. In one of his works, published in 1823, **Jan Evangelista Purkinje** (1787–1869), a professor at the University of Breslau, Germany, made a systematical fingerprint classification into 9 basic patterns. In France, **Bertillon** (1853–1914), the director of the archives of the criminal identity division at the *Préfecture de Police* of Paris, suggested a filing of the records based on 11 figures referring to measurements of body parts, thus creating the anthropomethric classification which is still in use, in some cases, for technical investigations. **Henry Faulds** (1843–1930), a Scottish missionary who practised as a surgeon in Japan, collected and analysed fingerprints left on glasses by servants and customers in bars and lounges thus trying to discover who had drunk from them. In England, Dr. **Francis Galton** (1822–1911) introduced the fundamentals of fingerprint science with his studies on papillary ridge lines and their patterns, grouping them into four basic types: whorl, left loop, right loop and arch. In exchange for money, visitors to the London World Exhibition in 1884 were taken fingerprints, with which he created an archive for his office. The first fingerprint classification in the world was

drawn up by **Juan Vucetich** (1858–1925), a Dalmatian responsible for the Argentine Police statistics division including the identification office in La Plata, and was officially adopted on 1.1.1896. In 1900, **Edward Richard Henry**, a British officer on duty in the British Indies, worked out the most widely used fingerprint classification.

[4] It is an automated fingerprint identification system, through which minutiae can be coded and joined together so as to obtain separate patterns whose corners, sides and surfaces are subsequently matched with those stored in the database. A list of possible candidates is selected from the Central Identity Database and then checked by fingerprint experts.

[5] Non EU-nationals will be allowed to enter Europe only if in possession of a biometric visa with fingerprints stored on a chip, as envisaged by **Regulation (EC) no.334/2002** set forth by the European Council on 18/02/2002. The **Board Crossing Card,** introduced in the U.S. in 1998, is a sort of biometric visa bearing fingerprints: it allows border workers from Mexico to be promptly identified, thus speeding up inspection procedures. The **U.S. Green Card** – in use since 1988 – and the **Permanent Resident Card Canada** – in use since 2002 – are typical examples of permanent stay permit bearing the holder's fingerprints, photograph and signature.
As to stay permit and card, see **Regulation (EC) no.2252/04** set forth by the European Council on 3/12/2004 and **Regulation (EC) no. 1030/02** set forth by the European Council on 13/6/2002.

[6] **Regulation (EC) no. 2252/04** set forth by the European Council on 13/12/2004.

[7] According to art. 4 of ***D.P.R.* no.437 dated 22/10/99** the Electronic Identity Card may contain data and applications required for the digital signature in compliance with technical rules set forth by ***D.P.R.* no.513 dated 10/11/1997** as well as the elements needed to obtain the biometric code. The text of the regulation is stated in very general terms; the necessary operational data will be dealt with by future regulations. **D.P.R. no.445 dated 28/12/2000** - the Consolidation Act of rules and regulations relating to administrative documentation – provides for the requirement of digital signature. See also the ministerial decree dated **2/8/2005,** which amended the previous decree dated 19/7/2000, containing "Technical and security rules relating to electronic identity card and identity papers". Article 7, vicies ter, of **Law no.43 dated 31/3/2005** orders that as of 1/1/2006 current paper identity cards be replaced by electronic ones when applying for issue or extension.
[8] One of the gates in **New York City** airport is checked through the iris identification of the personnel passing-by; in case of non-identification of the authorized staff, the safety device snaps shut and the door will not open. The devices installed in the airports of **Toronto**, **Vancouver**, **Amsterdam** (Schiphol) and **Tokyo** (Narita) work in the same way.

[9] The hand geometric characteristics, unlike fingerprints, are not sufficiently descriptive to be unique; therefore they cannot be used for personal identification, but at the same time they are descriptive enough for identity verification. The sensor of data entry has to be particularly resistant; this method is suitable for frequent use, i.e. check of physical access and sensing staff present at work. San Francisco airport in **Canada** and several industrial plants in the **United States** make use of the same system. In 1996 during the Olympic Games in **Atlanta,** this method was adopted to identify athletes, staff and participants (about 150.000 people). The same system has been operating at ***Tel Aviv*** airport since 1988 for the identification of frequent travellers (about 50.000 workers) entering Israel from Gaza every day.

[10] **Legislative decree no. 196 of 30/6/2003** containing the "Personal data security Code", art. 4 letter b) which defines "personal datum" as any information concerning a natural person, a juridical person, agency or association, identified or identifiable, also indirectly, through reference to any other information, a personal identification number being included. This definition might be referred to the biometric datum. Consequently, the above said code will be applied to data processing and data requirements, ensuring fulfillment of the obligation of information and respect of the minimum security measures. On 01/08/2003 the Group of European Guarantors adopted the following definition: "The biometric data can always be considered as information regarding a natural person since they provide in themselves details about a given person".

[11] The application possibilities are various: they range from access to presence control, from authomatic surveillance to valuables safety, from security of computer nets to safe transactions on the Internet. In **Germany,** the "Videotake 24 Fingerprints" allows to rent VHS or DVD. In **Japan**, a great number of financial trusts give their business associates a special card, so that they can make money movements and Exchange transactions. The biometric password was introduced in **Italy** in 2002; it allows to record the position of the seat and the attitude of drive in one's car. At last we shall cite **banks** which have the greatest number of biometric two-stage doors installed.

[12] It has to be considered that the Guarantor for personal data protection establishes that the use of biometric data is legitimate only in case of proven necessity, proportionality and purposes (necessity does not exist when the data processing purposes can be achieved using anonymous data or identifying codes; proportionality does not exist in situations free from actual risks or when recognition is not really necessary; the purposes depend on the decisions made by the person who orders the biometric detection).

4. Organization of Hand Transplantation

Section 4-a

Selection of Candidates and Waiting List, Dealing with the Media and the Public, Setting Up a Pilot Study, Clinical Trial Organization, Staff Requisites for Hand Transplantation: The Lyon Experience

Jean-Michel Dubernard, Xavier Martin, Palmina Petruzzo

Introduction

The hand transplant programme conducted by the Department of Transplantation in Lyon, France, is based on a clinical trial programme approved by the Minister of Health, which allowed only bilateral hand transplantation after evaluation of the protocol by regulatory, scientific and ethical authorities (Comité Consultatif de Protection des Personnes participant à la Recherche Biomédicale Lyon I, Comité Consultatif National d'Éthique, Agence Française de Sécurité Sanitaire des Produits de Santé, Agence de Biomédecine, Comité d'Éthique de l'Université Claude Bernard, Lyon I).

Selection

Hand allograft is a composite tissue allograft (CTA) consisting of different tissues, which should be used for functional and cosmetic restoration of patients with severe tissue loss. Although grafted hands improve patients' quality of life, the impact of the transplantation has been objectively measured. We have to consider the delicate balance between risks and benefits when evaluating a patient for limb transplant. In fact, there are some main questions: (1) Is the potential recipient able to understand the risks and to make appropriate judgements? (2) Are patient's expectations realistic? (3) Is patient's psyche prepared for daily required immune medications, repeated controls and the possibility of rejection and/or infective episodes? For all these questions, the potential recipients are submitted to several interviews with psychiatrists and team members [1]. He or she has to be informed of all procedures, the need for lifelong immunosuppression and the correlated risks. This period of conversations and careful deliberation by the patient lasts about 3 months. During this period, the patient's family is involved.

Recipient evaluation starts only when the team is sure of his or her understanding, autonomy and compliance with future medications and physical therapy. It includes the same pretransplantation screening performed in solid-organ transplantation and assessment of technical feasibility [2]. Patients must also employ prosthetic alternatives before transplantation. When all results are satisfactory, there is a final interview, and the recipient must sign a complete informed consent form. At present in our centre, exclusion criteria are:

- Unilateral amputation
- Age <18 years
- Remission from cancer <5 years
- Severe hypertension
- Kidney failure (creatinine>150 μmol/l)
- Patients with score >2 on the basis of American Society of Anesthesiology (ASA)
- Patients with score >1 on the basis of the New York Heart Association (NYHA) classification
- Mental disease.

The ideal recipient is a 20- to 40-year-old patient with traumatic bilateral amputation at wrist or distal forearm level for a period ranging from 3 to 6 months to 2 to 3 years who employed without satisfaction prosthetic alternatives.

Waiting List

At present, there is no waiting list in France. Each case was considered individually, and a dossier including recipient and donor characteristics was submitted to the Agence de Biomédecine. Obviously, for the particularity of the grafted anatomical parts, matching of size and skin colour must be performed in order to choose an appropriate donor. It is important to note that we decided not to use in case of CTA the "presumed consent" regimen but to obtain a clear and full consent from the relatives after explaining the exact nature of retrieval and technique of body restoration. In fact, although there is no legislation concerning CTA to assure "a decent body restoration", in our experience, we always prepare and attach a prosthesis before returning the donor body to the relatives [3].

Dealing with the Media and the Public

Ethical appropriateness of an innovative procedure is often assessed on Moore's six criteria [4], which are:

1. The scientific background of the innovation
2. The skill and experience of the team
3. The ethical climate of the institution
4. Open display
5. Public evaluation
6. Public and professional discussion.

When we performed the first hand allograft, many commentators affirmed that we failed to fulfill some of these criteria, particularly those of "open display and public evaluation" [5]. On the contrary, we consider any CTA a promising therapeutic research area and consequently publish our results in scientific journals. Some degree of controversy within the scientific community is normal; however, some publicised overreactions have created, *via* biased media coverage, suspicion against CTA and much inaccurate information. For these reasons, it is important to avoid excessive media coverage but at the same time yield clear and essential public information. Well-designed clinical trials, however, may avoid many polemics inside the scientific community. It is also important to respect anonymity principles for the recipient and particularly for the donor. For the first hand transplantation, it was unfortunately impossible to avoid excessive media coverage, and the team was obliged to communicate a great deal with the media due to leakage of incorrect information.

Setting Up a Pilot Study and Clinical Trial Organization

Hand transplantation as well as other CTA is an experimental procedure, and for this reason, it should be performed in a well-designed protocol study. Consequently, approval of a pilot study by institutional review boards is essential. In the organisation of a clinical trial, some points are important:

- Finding the appropriate recipient by means of a thorough screening process with inclusion and exclusion criteria
- Finding the appropriate donor with the same criteria
- Carefully assessing all procedures and establishing the immunosuppressive protocol
- Performing an accurate and lengthy rehabilitation programme
- Organising rigorous follow-up.

In our experience, it has been difficult to find the ideal recipient, as only bilateral amputatation at wrist or distal forearm level were included. When a possible candidate was identified, the screening process [6] involved the transplantation team with immunologists, psychiatrists, hand and transplantation surgeons, physiotherapists and the neuroscience team with experts in functional magnetic resonance imaging (fMRI).

The Agence de Biomédecine was involved in finding the ideal donor, who was always a multi-organ cadaveric donor in France. In the phase of retrieval of upper extremities, 4 surgeons and nurses were required, in addition to the staff usually involved in organ harvesting. Organization of the transplantation was complex, and almost 34 persons were required. Hand surgeons and reconstructive microsurgeons constituted a team of 18 persons who simultaneously had to prepare both stumps and procure upper extremities, and then perform the actual transplantation procedure. Fourteen nurses and 2 anaesthetists were involved in this procedure, which lasted almost 12 h.

After transplantation, both forearms were supported by a volar splint. As the recipient needed intense monitoring, several nurses were involved in the postoperative management phase. The immunosuppressive protocol started in operating room, and from that moment, the patient was submitted to a series of controls. Indeed, a careful follow-up is required for recipients of a CTA, which is a non-life-saving procedure.

One of the most crucial points in hand transplantation is the rehabilitation programme, which began 12 h after surgery and must be undertaken for several years. The first year it included physiotherapy, electrostimulation and occupational therapy, then it continued twice weekly over the entire follow-up period. At present, we believe that an appropriate programme should be performed to teach the recipients all movements necessary to perform daily activities because they seem to have "forgotten" how to carry them out over the several years following amputation. On the basis of our experience, muscular power and range of motion should be improved with steady and targeted exercises. Patients need strong motivation, not usually required in solid organ transplant recipients, as the results follow a rigorous protocol of physiotherapy.

Staff Requisites for Hand Transplantation

As with other CTAs, hand transplantation is a complex procedure, and its success is conditioned by the team's composition and motivation. Each member is indispensable: transplantation and hand surgeons are involved in recipient and donor selection, surgery, organisation of the rehabilitation programme and follow-up; the transplantation team is involved with immunologists in pretransplantation screening, the immunosuppressive protocol and management of the grafted patients during follow-up; the dermatologist is involved in detection of rejection and histological study of grafted hands; the neuroscience team has demonstrated that peripheral input can modify cortical hand organisation in sensorimotor regions [7], and they play a pivotal role in our staff; and finally, psychiatrists are indispensable in each phase of this project for its particular context. They must evaluate the recipient's understanding, autonomy, motivation and compliance to future medications and physical therapy in the pretransplantation period; then they must to support him or her in the postoperative period and during the follow-up to accept the continuous visibility and contact with the "foreign hands" of a cadaveric donor, allowing their integration into the body schema.

In conclusion, although the initial success of our hand transplantation programme was based on the enthusiasm of all members of the team, hand transplantation must be carried out under strict and ethical research guidelines.

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Section 4-b

Selection of Candidates and Waiting List, Dealing with the Media and the Public, Setting Up a Pilot Study, Clinical Trial Organization, Staff Requisites for Hand Transplantation: The Milan Experience

Marco Lanzetta, Roberta Nolli, Ilaria Radaelli, Rosella Coletti, Felice Paleari, Anna Cappellini, Franco Uggeri, Mario Scalamogna, Alessandro Rampa

"Experimentation … is justified primarily by the individual's and not by the community's interest. However, this does not exclude that, provided that one's own substantial integrity is preserved, the patient could legitimately bear a part of the risks to contribute with his/her initiative to the progress of medicine, and in this way, to the welfare of the community. Within the community, the purpose of medicine is to free the human being from the infirmities that block him, and from the psycho-somatic fragilities that humiliate him".

Pope John Paul II

October 27, 1980. From the address to the participants to two surgical meetings in Rome, Italy

Introduction

Hand surgeons and reconstructive microsurgeons have been "transplanting" composite tissues for a long time. Every time we perform a free flap, we are actually performing a true transplant. Most of the time there is no choice other than to adapt a flap coming from a certain donor area to a recipient area with different characteristics. Normally this happens in cases of complex multitissue defects or when the tissue that needs reconstruction carries some unique features. So while we are now exploring the area of prefabrication or prelamination of flaps, we are aware that we are far from perfection, hence the need to look at using possible allogeneic tissues [1]. With the advent of microsurgery, the long-term results of autologous replantation of the upper extremity, especially after a clean-cut amputation, have become extremely good. In a recent long-term review study, it was shown that replanted hands and forearms had a mean loss of range of motion of 17.5% [2]. When standard microsurgical criteria are met, replanting/transplanting a body part can be a long-term cost-saving procedure that produces superior functional results to amputation and the use of a prosthesis [3–5].

The Italian Hand Transplantation Programme

Our three patients were all men, aged 35, 32 and 33 years, who lost their dominant right hand respectively 22, 4 and 10 years previously. The original amputation was due to a crush injury while working in a farm in the first case, an explosion in the second case and a car accident in the third case (Fig. 1). All patients had considered the option of an artificial device and tried a number of aesthetic, mechanical or myoelectric prostheses, which were eventually refused because the patients' functional and cosmetic expectations were not met. The patients underwent a series of routine pretransplant investigations, including an angiogram, computed tomography (CT) scan, muscle and nerve charts, magnetic resonance imaging (MRI) of the stump, functional MRI (fMRI) of the brain, a comprehensive battery of psychological tests and ophthalmic, dermatologic, allergologic and general surgery evaluations. They were seen by a hand therapist and a prosthesis consultant who assessed the number and quality of independent activities the patient could perform with and without the different prostheses.

Donors were men aged 43, 25 and 16 years, respectively. They had the same blood group as their respective recipient, and there were 6 human leukocyte antigen (HLA) mismatches in the first case, 3 in the second and 5 in the third; cross match was negative in all cases. The harvested hands were aesthetically as close as possible to the recipients' own hands (Fig. 2). In all cases, according to our protocol, after harvesting the right hand and forearm, a custom-made aesthetic prosthesis was fitted to restore the donor's body integrity.

Fig. 1. Italian recipients. *RHD*, right-hand dominant

Fig. 2. Italian hand transplantation: donor characteristics, size and colour matching, surgical and ischaemia times

Surgical Procedure

Average time for transplant was 12 h 20 min (range 12–13 h), with a total average ischaemia time of 11 h (Fig. 2). Forearm bones were transversely cut so that the grafted limb would be of the same length as the contralateral limb. Bone fixation of radius and ulna was achieved by means of compression plates and 4.5-mm screws, and autologous cancellous bone graft from the iliac crest was placed around the osteosynthesis site to improve healing. Most of the deep flexor and all extensor tendons were repaired. The hand was then revascularised by anastomosing the radial and ulnar arteries and as many veins as possible. Upon tourniquet release, the hand quickly regained a colour and temperature. At this stage, median and ulnar nerves were repaired, and the remaining more superficial flexor tendons were sutured. The skin was closed in layers (Fig. 3). Upon completion of the transplantation procedure, the donor's skin was grafted to the left hip of the recipient (*see* Section 8-c).

Immunosuppressive Regimen

Patients were given 250 ml of dextran 40 before declamping and 20 ml/h for 7 days. Aspirin 150 mg was administered for 7 days and wide-spec-

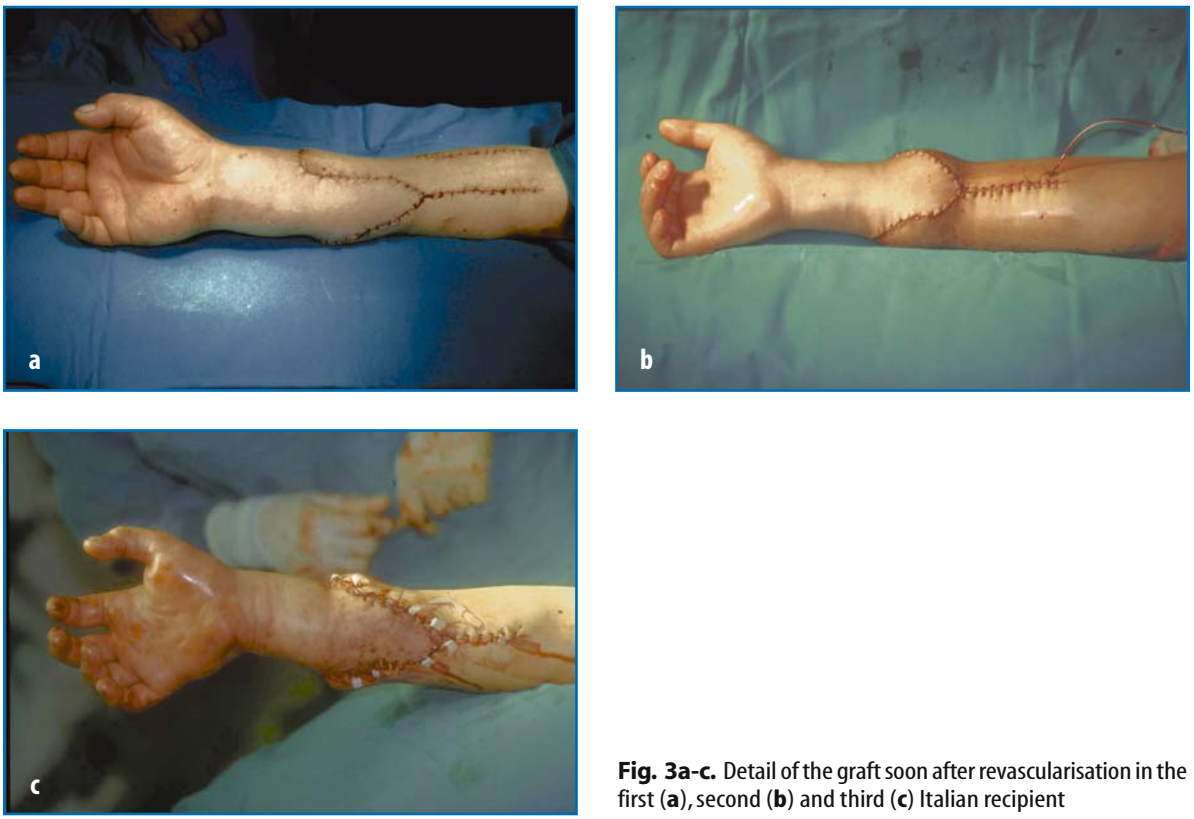

Fig. 3a-c. Detail of the graft soon after revascularisation in the first (**a**), second (**b**) and third (**c**) Italian recipient

trum antibiotic therapy for 10 days. The induction immunosuppressive protocol consisted of 20 mg of monoclonal antibody anti-CD25 [basiliximab (Simulect)] 2 h before the operation and on days 4 and 45 postoperatively, FK506 [tacrolimus (Prograf)] adjusted to maintain blood concentration between 15 and 20 ng/ml for the first month, mycophenolate mofetil (MMF) (Cell Cept) 2 g/day) and steroid (prednisone) 250 mg on day 1 and rapidly tapered to 20 mg/day. Maintenance therapy consisted of FK506 (blood levels between 5 and 10 ng/ml), MMF 1 g/day in 2 cases and 1.5 g/day in the last case, and prednisone 10 mg/day [6–8].

Postoperative Regimen and Functional Rehabilitation

Routine laboratory blood tests were repeated daily for the first 5 months and included evaluation of blood concentration of FK506. Monthly assessments included C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum ferritin, immunoglobulin dosage, Epstein-Barr virus antibodies, cytomegalovirus (CMV) antigen, serum proteins electrophoresis and reticulocyte count. Every 3 months, lymphocyte subpopulations were assessed.

Every month, a chest X-ray was carried out and a colour Doppler ultrasound was performed to evaluate blood flow at vessel anastomotic levels and in the peripheral arterial system. Monthly radiological assessment of the hand and wrist evaluated bone callus formation. An electrocardiogram (ECG) was repeated monthly to check for heart problems, as well as a dermatologic assessment for skin lesions. Every 3 months, an ophthalmic assessment included evaluation of the fundus oculi, enophthalmic pressure and visual field and an oto-rhinolaryngeal (ORL) evaluation included an audiometric exam. A chest CT scan and an abdominal ultrasound examination were conducted every 6 months. Possible occurrence of chimerism was assessed twice in the first year postoperatively.

Physiotherapy started as soon as swelling subsided and was performed twice daily for 180

days and once daily thereafter as the patients returned to work. It included a standard rehabilitation programme for flexor and extensor tendons, sensory reeducation and cortical reintegration. Electrostimulation was started on day 60 and carried out twice daily since. Occupational therapy focused on sensory, visual and motor stimulation of the grafted hand. A psychological support programme was started on day 1 and continued on a daily basis for the first month, twice weekly until day 90 and then once weekly.

Functional outcome of the transplanted hand was assessed at different intervals by clinical examination, including sensory and motor tests, multichannel surface electromyography (EMG) and a computerised data analysis system connected to an instrumented keyboard, an instrumented mouse-like support and a sensor glove. Skin biopsies were not taken at prefixed intervals but only when deemed necessary

Results

General Considerations

No surgical complications were seen in the three reported cases. All wounds healed normally, and the skin allografts took entirely. Steroid-induced hyperglycaemia required insulin administration in two cases, which was slowly tapered down and then substituted with oral hypoglycemic agents and eventually withdrawn. Mild anaemia required blood transfusion and iron supplement therapy in two cases. The patients were discharged from the hospital at 24, 30 and 35 days, respectively. CMV activation without clinical signs needed to be treated in two cases, and in one case there was also a concomitant severe serum creatinine increase, intestinal *Clostridium* infection and weight loss, which required a second, prolonged, hospital stay. Beginning of nerve regeneration was evident as early as 2 weeks postoperatively, with Tinel's sign progressing from the nerve repair site towards the periphery. At approximately 3 months, in all patients sensation reached the metacarpophalangeal joints of the long fingers. By 8 months, sensation reached all fingertips, and two patients could distinguish between various thermal stimuli. At the latest follow-up, the first patient showed some signs of discriminative sensation in the little finger only at 29 months postoperatively, the second regained some discriminative sensation in all fingers at 18 months postoperatively while the last patient is only 7 months postoperative and is still in the early process of nerve regeneration. Intrinsic muscle activity was detected as early as 6 months postoperatively by a multichannel surface EMG. Strength and movement evaluation of the hand and fingers with a series of instrumented tools and a sensor glove showed that the patients could activate fingers in an independent fashion and modulate the strength of each fingers depending on the task performed. At approximately day 90, the patients were allowed to drive to the hospital every day using the right transplanted hand. They returned to work at day 138, 150 and 270 postoperatively, respectively.

The patient transplanted 4 years after the original trauma shows the best functional outcome from both sensory and motor points of view. He can drive, eat, write, shows a great deal of manual dexterity both at work and at home and was able to obtain a hunting licence for game shooting. The patient transplanted after the longest interval since the injury (22 years) shows only partial motor recovery; nevertheless he is very satisfied with the results, and his hand is never excluded from his daily activities and duties. He was able to obtain an unrestricted driving licence and is now completing the necessary steps to obtain a truck-driving licence.

Hair and nail growth is normal in all three transplanted hands, with little aesthetic difference between the two hands in terms of size, colour and morphology (Fig. 4). Psychologically, the patients are stable and satisfied with the results (Fig. 5). An fMRI of the brain has shown that sensorimotor activations have shifted from an area close to the face representation to the classical cortical hand area [9–11].

Quality or Quantity?

Quality or quantity? This is the main issue raised by these operations. At the beginning of the third millennium, could we (as a society) consider transplantation not only to prolong or save life but also to improve quality of life [12–18]? In the

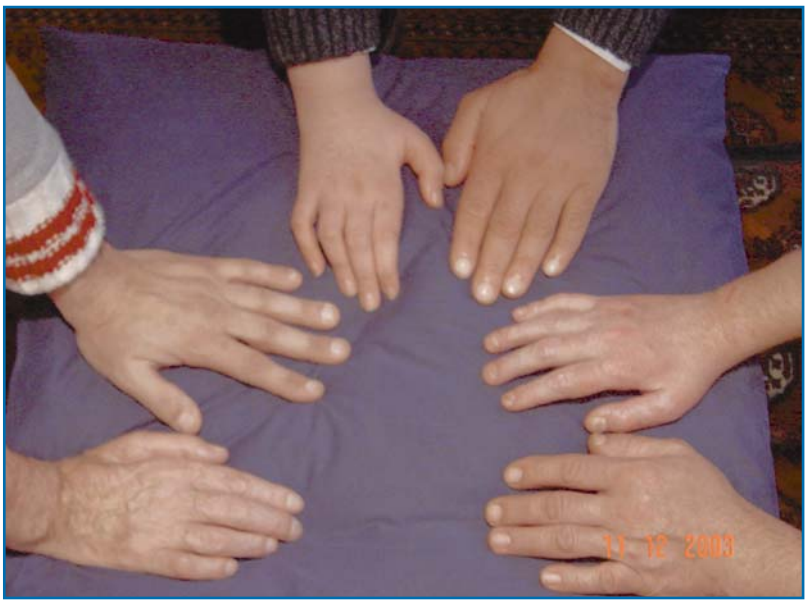

Fig. 4. Examples of colour and size matching in Italian recipients

Fig. 5. Italian recipients at a follow-up of respectively 3, 1 and 2 years (from left to right)

Western world, this concept may well be justifiable, as our society tends to encourage and legitimise the expectations of individuals for a better home, better health and a good job – in short, for a better life style free from material and health problems – so that we can fulfill our projects in life, whatever they are. Living with any type of deficit or deformity has become increasingly difficult in modern societies that value appearance, which reflects on our emotional, physical and social well-being. Health is found to be grounded in a sense of self and a sense of body, both of which are tied to conceptions of past and future actions. It seems as if there is no place for less-than-perfect people, hence the obsessions with fitness, body image and, in general, with the way we appear to others [19, 20]. In case of an upper extremity traumatic amputation, today's goal should include restoration of a socially acceptable presentation of the constantly exposed hands [21].

Candidate Selection

This is probably the most important issue in hand transplantation. Strict criteria must be applied to select the ideal candidates to these procedures. Selection must involve a collegial evaluation of the patient involving not only the surgeons, but also the physicians, a clinical psychologist, an immunologist, a hand therapist, an anaesthesiologist, a lawyer and the patient's general practitioner (Fig. 6). At the moment, we recommend the following inclusion criteria: age comprised between 18 and 50 years, traumatic amputation, dominant hand or bilateral at the wrist level, tried and refused different prosthetic alternatives, otherwise healthy and mentally sane, able to give informed consent, resident in the country, available to follow-up and with support of family and local medical practitioners (Tables 1–3).

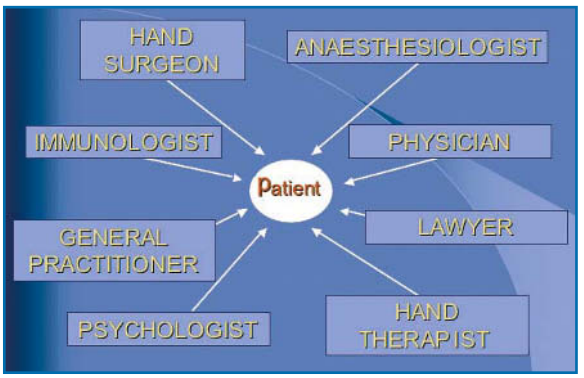

Fig. 6. Professional figures involved in selecting the suitable recipient for hand transplantation

Table 1. Inclusion criteria for hand transplantation

- Age between 18 and 50 years
- Traumatic amputation at the wrist level, either bilateral or involving the dominant hand
- Maintained awareness of the missing part (nonpainful phantom representation)
- Unsuccessful trial of prosthetic alternatives
- Otherwise healthy
- Mentally sane
- Fully aware of the possible complications and adverse effects (consent form)
- Totally available for routine follow-up (living in the country)

Table 2. Exclusion criteria for hand transplantation

- Age less than 18 and more than 50 years
- Congenital amputation of one or two hands
- Amputation of a nondominant hand
- Severe painful phantom representation of the missing part
- Amputation at forearm, elbow or arm level
- Happy with a prosthesis (aesthetic, mechanical, myoelectrical)
- Unhealthy (i.e. cardiovascular or systemic disorders, heavy smoker)
- Mentally unfit
- Unable to sign a consent form
- Not available for routine follow-up (not living in the country)

Table 3. Ideal psychological profile of hand transplantation recipients

- Age between 25 and 45 years
- Married with children
- Well inserted in family, social and working context
- Average intelligence
- High performance score
- High index of adjustment to reality
- High frustration threshold

Age and Hand Dominance

According to Italian law, a minor (i.e. under 18) may be subjected to a particular medical procedure if consent for that procedure is given by his/her parents or guardian. We felt that even in case of a decision to proceed by the parents, a minor could not be subjected to a new pioneer operation without his or her own will and consensus, due to the impossibility of providing enough information to a child and receiving informed consent.

In case of a patient older than 50 years, we felt that the reasons for a hand transplantation were not strong enough, as this individual was sufficiently close to his/her retirement to lose some of the most important benefits of restoring his/her functionality. Also, results in hand replantation are less satisfactory in older people for their decreased capability to recover function and sensibility due to less effective nerve regeneration.

Exclusion criteria were congenital deformity or limb absence not only in infants or minors but also in adults within the age range (18–50 years). In fact, in case of congenital amputations and malformations, we do not yet know enough to proceed with transplantation. In these cases, the missing part has never been lost; it simply has never been there. This means that no information has ever been transferred from the absent part to the brain, and no commands have been imparted to it. Although it would be logical to conclude that the cortical representation of this part is missing, there is some evidence that this might not be the case. This relates mainly to the incidence of phantom-limb sensations in people with in congenital (aplasic) absence of limbs. Aplasic phantoms are based on the existence of specific neural circuitry associated with innate motor schemas, such as the neural matrix responsible for early hand–mouth coordination [22]. It is therefore hypothesised that the neural network, or "neuromatrix", that subserves body sensation has a genetically determined substrate that is modified by sensory experience [23]. If transplanted from a donor, nerves would probably advance in the allograft, given the tremendous potential for regeneration in children. However, would these nerves then transfer meaningful data to the central nervous system and into which area? The clinical experience with toe-to-hand transfers in congenital cases confirms that cortical plasticity allows for complete integration of the added part. Therefore, it might be hypothesised that composite tissue allografts for reconstruction of congenital malformations should be performed very early so as to benefit from the greater capacity for integration. However, if risks associated with the immunosuppressive therapy greatly decrease in the future, these procedures could be considered for congenital malformations.

Our protocol allows for bilateral or dominant-hand transplantation. It is felt at this early stage that a nondominant hand is not sufficiently important in the global manual activities to undergo a difficult and potentially risky operation.

Lower Limbs

In our view, a lower limb amputee is definitely not a good candidate at this moment in time unless he or she is in a special situation, i.e. lost his or her hands and lower limbs, cannot use a prosthesis for some reason or received a solid organ transplantation in the past and is on immunosuppressants. Lower limbs have a different "value" and function to the arms. Their main function is to permit weight bearing and ambulation. They do not have additional social and emotional functions or a strong symbolic value. Current prostheses are excellent at restoring stability, weight bearing and walking ability [24, 25], and clothes can disguise them so that they are not apparent to the others. The benefit of a transplanted leg does not presently outweigh the drawbacks of current immunosuppressive therapy.

Level of Amputation

We have considered and will consider in the near future only patients with an amputation at the wrist level or very distal forearm. This decision is based on a very simple fact: when transplanting a hand, the working extrinsic muscles are present in the recipient's forearm and will just need to be redirected at a peripheral action to be useful and provide strength to finger flexion and extension. Only the intrinsic muscles will need to be reinnervated by peripheral nerve regeneration to contribute their strength and fine movements. If the transplanted part contains extrinsic muscles, such as flexor and extensor muscles in the forearm, they will require a long time to be reinnervated and will show some degree of atrophy, even in case of an excellent recovery. Results of hands transplanted at the wrist level should therefore be superior in terms of movement compared with those transplanted with the forearm, or even worse, with the elbow joint.

Prosthetic Alternatives

A number of prosthetic replacements for lost hands and forearms are currently available. They range from purely aesthetic to those with an emphasis on function, which have less cosmetic value. The vast majority of traumatic amputees consider the option of a prosthesis, and some actually have one custom made according to their wishes (functional or aesthetic). The patient's acceptance of a prosthesis varies considerably. For a very few, it may become routine to wear it every day in all sorts of situations. These patients are normally those with low expectations either from an aesthetic or a functional point of view. When the patient's expectations are not met, even by the best-crafted and custom-made prostheses, then they are refused. Sometimes this is because the prosthesis cannot provide sensation, at other times it is because of less-than-satisfactory aesthetic matching to the contralateral hand. It is at this stage that the patient might seek further medical advice and explore other possible surgical options. It is therefore imperative that any patient contemplating the possibility of a hand transplant should have considered, and possibly tried, two prostheses, and refused them.

Ability to Consent and Freedom of Choice

Is there a limit for an individual to make responsible decisions about his or her own body and health? One criticism advanced against hand transplantation is the assumption that a non-life-saving allograft could transform a healthy individual into a chronically diseased patient. First of all, somebody who has suffered a traumatic amputation is not a "healthy" individual. He or she might be fit in general, but the deformity must be considered as a permanent, chronic condition. From a medico-legal point of view, loss of a dominant hand is considered to cause a 65% loss of total body efficiency while the loss of both hands causes a 100% permanent disability, exactly the same as that attributed to a blind person.

Freedom of choice is directly proportional to the degree of information the individual has been given on his or her state of health and the possible solutions for his or her condition [26]. Informed consent is even more important for experimental procedures, as the possibility of an unforeseeable outcome must be considered [27]. In our cases, a combined team of lawyers prepared a very detailed consent form, which included all aspects of the hand transplant as well as possible known and foreseeable complications, the possibility of terminating the transplant if a serious complication develops, risks associated with the surgical procedure and anaesthesia, risks of immunosuppression therapy in the short, medium and long term and finally the necessity for a supervised rehabilitation programme and long-term psychological support. Once the patient has been given every piece of information in the most complete and accessible manner then, provided he or she is mentally sane, he or she can truly make a responsible decision for the sake of his or her own health and future.

In the case of a limb transplant, a potential recipient would have to evaluate the risks associated with the procedure against the benefits. For most, the risks will be far too high and will outweigh the benefits. However, others would consider the possibility because of the lack of satisfactory prostheses and their desire to regain anatomical and functional integrity, even considering the risk of the possible complications. Paradoxically, an amputee might accept the risk of decreasing his or her life expectancy in terms of years in exchange for improvement in life quality. In other words, can somebody be prevented from saying: "I'd rather die earlier but with two hands because my present quality of life is miserable?"

Another important point that we contest is the suggestion that a limb transplantee needs to be considered as chronically ill. As individuals, we are all potentially ill depending on a number of factors, including the environment we live in, our profession, our stresses, our life style and our habits and leisure activities. For example, if we start smoking cigarettes as teenagers, we can reduce our life expectancy by as much as 20–25 years. In developed countries as a whole, tobacco is responsible for 24% of all male deaths and 7%

of all female deaths. A smoker is 5–6 times more likely to develop cancer from the smoking addiction than is a transplant recipient on immunosuppressive therapy [28]. Yet even if the adverse effect of smoking is largely negative, we are free to take up this habit, and similar considerations apply to the consumption of alcohol. Hence, we all freely make decisions that affect our life expectancy.

Staff Requisites and Organisation of the Clinical Trial

The Italian Hand Transplantation Group was formed about 24 months before the first hand transplantation was eventually carried out in the country (17 October 2000). The group met once every 2 weeks and prepared a detailed protocol that was presented to the Ethical Committee of the hospital for approval. At the same time, the protocol was submitted to the appropriate sections of the Italian Department of Health in order to obtain the necessary government authorisation. An inspection by Health Department officials of the hospital operating rooms and wards led to minor changes and some instrument acquisition. The group of about 60 people included hand surgeons an microsurgeons, anaesthesiologists, transplantation surgeons and physicians, immunologists, neurologists, physiotherapists, legal medicine doctors, psychiatrists and psychologists, pathologists, a lawyer, hospital officials, nurses, laboratory technicians, representatives from the organ procurement institute, representatives from local service clubs, a photographer and a cameraman. The first task of the group was to establish the inclusion criteria for the project.

At the same time, a number of patients were interviewed, and an informed consent was prepared according to the special characteristics and needs of such procedure. Patients who were thought to fit the inclusion criteria were seen on a regular basis (approximately once a month), given all the available information and updated on the progress of patients who had the procedures carried out in France and the USA [29–32].

At this time, contact was established with the patients' local family doctors and when possible their lawyer to include those who were serving as counsellors in the process of reaching a final decision. A total number of around 400 possible candidates were seen over a 2-year period, and according to the selection process and inclusion criteria, 12 were thought to be preliminary candidates for single or double hand transplantation. These 12 patients proceeded to a formal hospital admission after signing part one of the informed consents in order to sustain detailed evaluation, including invasive diagnostic tests (i.e. full blood tests, angiography, MRI, psychological tests, muscle and sensory evaluation, ultrasound, HIV and hepatitis tests). Of the 12 patients, only 3 were found to be acceptable to be put on a waiting list for hand transplantation. They were asked to sign part two of the informed consent, and a final meeting was arranged with their families at large and the family doctor. The leading Italian manufacturer of helicopters made available to the hand transplantation group a helicopter in their fleet for a quick dispatch in case of need.

Rehearsal and Hand Transplantation Simulation

About 6 months before starting the clinical trial, the Italian Hand Transplantation Group was put on full alert for a simulation of the hand transplantation procedure. After about 2 weeks of on-call period, at 5 p.m. on 1 March 2000, a call was made to the coordination centre, and a group of surgeons flew to a nearby hospital where a harvesting procedure was carried out on a plastic body. The harvested arm was then carried back to our hospital, and transplantation was carried out again on a plastic body. By midnight, the operation was concluded. The next morning the group met, and the logbook of the procedure was analysed. A list of possible operational improvements was done, and actions were taken to solve the remaining problems. A press statement was released to the Italian media.

Long-Distance Monitoring of the Transplanted Hand

Once the patients left the hospital, they were housed in a nearby apartment equipped with long-distance monitoring equipment. This equipment, based on the Propaq Encore 206EL, monitored the hand's temperature and continuous $Sp0_2$ pulse oximetry sensor. The system was connected via modem to the main office of the Hand Surgery Unit. An alarm would activate in case of variation of set parameters, and an automatic message would be sent to the surgeon on call *via* his or her pager.

Donor/Hand Selection

In order to select the best possible hand for each patient on the waiting list, careful measurements were taken of their contralateral hand (length of the middle finger from base to fingertip, circumference at the palm, circumference at the wrist). A skin colour analysis was conducted by a colour analysis and cosmetic specialist using technology derived from the cosmetic industry, and a colour chart was prepared for each patient. This information was stored at the Organ Procurement Centre of North Italian Transplant, the organisation coordinating all transplantation procedures in northern Italy. The Intensive Care Units alerted for hand transplantation were advised not to cannulate the radial artery on the right wrist (as our patients were all right-hand amputees) to avoid problems in revascularisation and vascular supply of the transplanted hand.

A variable number of donors were excluded by the treating intensive care physicians at participating hospitals directly without consulting the hand transplantation group based on a number of agreed upon criteria (age mismatch, race, gender, previous hand trauma or injuries, vascular complications or infection, unstable patient and need to proceed quickly with solid-organ harvesting). Only if the general characteristics were met, then size and colour of the hand were used as final selection criteria. Even using these strict criteria for selection, an average number of 3 donors were refused for each patient. This decision was made by the hand transplantation surgeons directly on site for a number of reasons normally due to particular clinical observations (i.e. peripheral oedema, previous scaphoid fracture with nonunion, initial trapeziometacarpal joint arthritis) or, more frequently, due to refusal to donate the hand by the relatives. Although Italian law would have allowed us to proceed without the relatives' permission, we felt that at this stage, we had to be careful to avoid any possible bad publicity from imposing our decision in a relatively new context.

Prosthetic Replacement of the Harvested Limb

For each patient on the waiting list, a cast of the contralateral limb was made in order to prepare an aesthetic prosthesis to be used for the donor. As the donor was obviously unknown, we decided to use the patient's skin colour and hand size to obtain a prosthesis as close as possible to the donor's own hand and forearm, which would be used to reconstruct the body after the harvesting procedure. Respecting the donor is essential in Italian society but also throughout the Western world, and it is not conceivable that a cadaver is given back to the family for the funeral with a visible missing body part.

Dealing with the Media

From the beginning of the programme, we tried to prepare the Italian public to the upcoming operations. This was accomplished by providing regular updates to the leading press agencies, newspapers and media channels to avoid misinformation and possible confusion. The goal of this strategy was threefold: we wished to raise the consensus for such an operation within the nation, including the medical community; we wished to reach the people in their homes and explain our project in plain lay terms; we wanted to start selecting patients by prompting amputees to come forward and be available for

an initial medical and psychological evaluation. We believed it important that when facing a difficult decision such as allowing the harvesting of somebody's hand, relatives of the brain-dead person would already know that the operation was legal, authorised, part of a serious programme and likely to be successful. We wanted people to form an opinion in their minds about our programme at a moment when they were unaware that they may unfortunately be called upon to have to make a decision later. We knew the moments immediately before the decision were not suitable for explaining ex novo the reasons supporting our medical programme, as pain and grief tend to be too overwhelming, and the reaction can be of plain refusal even to discuss options.

We were proved to be right in at least two of the three cases. In the first case, the donor was a 45-year-old manual worker from a city in the north of Italy who suffered a few years before a left upper-limb posttraumatic palsy. When watching a television interview, he had said to his family that he knew exactly the disability of not being able to use a hand and that he would be willing to donate his own hand in case he was in the unfortunate situation of being a brain-dead donor. Similarly, the second donor had expressed the same thought to his family, as he belonged to an organisation promoting the culture of organ donation. In the third case, following the first two successful operations, we had clearly shown by then that the procedures had been medically justified, as return of function was clearly seen.

Discussion

Are we ready to accept that almost anything could come from a cadaver, even if it is not a life-saving organ, to reconstruct missing parts and restore anatomical integrity and, especially, function? Organ transplant surgeons have already provided some answers to this question. Most kidney transplants and solitary pancreas transplantations are currently performed not necessarily to save lives but to improve quality of life. Permanent dialysis is not incompatible with life, but it interferes greatly with the patient's professional, social and emotional environment. When three parameters for quality of life are considered (life satisfaction, well-being and psychological effect), kidney transplant recipients have a higher quality of life than patients on dialysis and compare well with the general population [33]. Similarly, solitary pancreas transplantation is performed to make diabetic patients insulin injection independent, and diabetes is not immediately life threatening. Successful pancreas transplant patients perceive their health as good and have a greater ability to function socially, with life quality becoming better over time [34, 35].

Based on these facts and following the concept of function-saving transplants, a number of isolated muscle, bone, joint, tendon, nerve and vessel allografts have been reported [36–40]. More complex composite tissues, other than the hand, have also been successfully transplanted, such as the larynx, face, knee joint, abdominal wall and uterus. Results are very encouraging if patients are determined to follow the postoperative drug regimen and necessary rehabilitation, when indicated, and if we do not lower the guard on possible complications or potentially severe side-effects.

While we learn every day from our own experiences and from our patients who bear the risk of facing the partially unknown, I do believe we should now come to terms with the fact that this new area of surgery will certainly expand in the near future. We need, therefore, to move forward and be ready for the many challenges that lie ahead of us.

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Section 4-c

Selection of Candidates and Waiting List, Dealing with the Media and the Public, Setting Up a Pilot Study, Clinical Trial Organisation, Staff Requisites for Hand Transplantation: The Innsbruck Experience

Gerald Brandacher, Stefan Schneeberger, Raimund Margreiter

Introduction

Composite tissue allotransplantation (CTA) represents a therapeutic option after the loss of a hand, forearm or digits. Even if the level of immunosuppression seems to be comparable to that after pancreas, heart, or kidney transplantation, a high incidence of rejections and loss of two transplanted hands for immunological reasons have been observed so far [1]. Hand transplantation is a complex and multidisciplinary treatment and should only be considered in carefully selected patients. Not so much the risks of the surgical procedure but, rather, that of the associated long-term immunosuppression needs to be weighed against the expected benefits of such a transplant.

Ethical considerations and debates were often based on the assumption that hand transplantation is a therapeutic option for all patients after loss of a hand [2–5]. Interindividual differences were rarely taken into consideration. Instead, the potential benefit or disadvantage in a nonselected group of patients (not even requesting a transplant) was evaluated [2]. Actually, the patient's wish for such a transplant should be the prerequisite for any evaluation process. Various inclusion and exclusion criteria have been defined, and a large number of patients were denied a hand transplant because one or more of those criteria were not met [6–8].

The principle goals of hand transplantation are to achieve a motor function superior to myoelectrical prostheses together with a discriminative sensation. Moreover, patient satisfaction and an improved social integration are components justifying such a cost- and labour-intensive procedure. As hand transplant programmes are expected to be initiated in several institutions throughout the world, we feel that attention needs to be paid to optimise organisation of this logistically demanding procedure. In this chapter, we give an overview of the assessment of potential candidates and donors as well as organisational aspects in hand transplantation. In this context, donor and recipient selection, regimens for induction and maintenance of immunosuppression, rehabilitation programmes as well as psychological and ethical issues are discussed based on the Innsbruck experience in hand and forearm transplantation.

Required Resources

Donor and recipient selection as well as a detailed plan of the surgical procedure, an individually adjusted immunosuppressive protocol, postoperative monitoring and a specifically designed rehabilitation programme need to be established and discussed in a multidisciplinary fashion well before transplantation. Preparations should be made aiming for maximisation of functional outcome, long-term graft survival, minimisation of drug side-effects and risk of

developing malignancies. Therefore, to facilitate successful hand transplantation, close co-operation between fully trained and experienced transplant and hand surgeons, immunologists, pathologists and physiotherapists is a prerequisite. In addition, Moore's criteria, defining the standards for therapeutic innovation, have to be observed [9]. There must be adequate scientific background and considerable skill and expertise among team members. The institution should be willing to foster this type of transplant and create an ethical climate that should be available for public scrutiny [10].

Candidate Selection and Waiting List

Recipient Selection

Careful evaluation of all potential candidates will leave only a few "good candidates" who meet the criteria required to achieve an outcome satisfactory for both patient and physician. As detailed selection criteria are not available from all centres, inclusion and exclusion criteria for hand transplant recipients at our centre are listed in Table 1. In summary, a patient after loss of both hands or forearms with amputation at a level distal to the elbow can be considered an appropriate candidate when he or she is between 18 and 55 years of age, physically and mentally healthy, capable of understanding the complexity of such a procedure and being highly motivated to undergo at least 1 year of intensive rehabilitation in addition to a painstaking perioperative period.

In this context, it is of utmost importance to obtain the patients informed consent. Every single potential problem that might be associated with the procedure and, in particular, with pharmacologic immunosuppression, must be extensively discussed with the candidate. Patients need to be apprised of every imaginable clinical scenario, including possible consequences. Furthermore, the patient must be made aware and understand that a daily intense training during the first postoperative year is a prerequisite for reintegration of the hand in the cerebral cortex and recovery of hand function. Each hand transplant candidate has to cope with that and for the time being the additional stress associated with media coverage. A sound sociofamilial background and considerable psychological stability are therefore considered essential.

We particularly feel that early and thorough information provided to the patient about methods and intensity of the planned rehabilitation programme is necessary to obtain compliance and motivation. Only highly motivated patients actively requesting a hand transplant should be accepted. It is important that patients be given time to weigh the anticipated improvement in quality of life against the potential risks associated with a transplant.

Donor-Recipient Match

Donors need to be matched for blood group, gender, age, bone size and texture as well as color

Table 1. The "Innsbruck Criteria" for inclusion and exclusion of potential hand transplant recipients

Inclusion criteria	Exclusion criteria
Patient's strong desire	Malignant tumour in the past 10 years
>18, <55 years	Infection (temporarily)
Bilateral loss of hand or forearm	Neurinoma proximal of amputation level
Normal function of all vital organs	Blindness
Psychologically healthy and stable	Insulin dependent diabetes mellitus (IDDM)
Intact sociofamilial background Capable of understanding the complexity of the procedure and any potential consequences	

of the skin with the recipient. Blood groups may be compatible or identical. For practical reasons, human leukocyte antigen (HLA) match may be disregarded, but the lymphocytotoxic crossmatch needs to be negative. Serological testing for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), lues and toxoplasmosis has to be negative in all patients considered as donors for CTA. Cytomegalovirus (CMV) matching of donors and recipients is desirable, as the risk of a CMV infection in hand transplantation is high, and complications have been observed in a considerable number of patients [11]. Despite presumed-consent legislation in some countries, consent for hand donation should be requested from the donor family in any circumstance.

Pretransplant Examinations

To facilitate exact planning of surgical procedures for each individual case, the recipient stumps should be carefully investigated. Bone length, muscular, vascular and nerval status should be assessed by means of computed tomography (CT) scan, CT angiography, magnetic resonance imaging (MRI) and ultrasound. A neurinoma far proximal of the level of amputation is considered an exclusion criterion, as nerve regeneration cannot be expected in such a case. Assessment of the cardiovascular and pulmonary status should include routine tests such as chest X-ray, electrocardiography, echocardiography and spirometry. For exclusion of comorbidities that would contraindicate transplantation, such as malignancies or infections, patients undergo gastroscopy, colonoscopy, dental and oropharyngeal examination.

Psychologic Status

As mentioned, profound psychological stability together with the ability to understand all facets of the therapeutic modality is of crucial importance. Therefore, candidates are assessed for their mental and social status. These results need to be confirmed by an independent expert not related to the transplant team.

Pretransplant Assessment of Patient Satisfaction

Prostheses without motor function may help to improve gross appearance of the patient but are of no functional value and interfere with motor imagery. In contrast, myoelectrical prostheses are valuable tools for performing daily activities but are of very limited use for social interaction, as they lack sensitivity. Even if some transplanted hands might not perform better than a prosthesis in terms of motor function, they can be of significant benefit for the recipient because they facilitate sensory awareness. Measurement of range of motion, grip strength, Semmes-Weinstein monofilament or two-point discrimination are helpful tools for measuring motor function and quality of sensitivity after hand transplantation; however, definition of the benefit of such a procedure is much more complex, and additional aspects need to be taken into consideration. Patient satisfaction with or without his myoelectrical or cosmetic prostheses need to be assessed prior to transplantation and provide the basis for outcome evaluation. Activities of everyday living, social integrity, self-esteem, body image and the ability to interact by touching, hugging, caressing or any form of intimacy with a partner should be evaluated with adequate questionnaires. The ability of objective judgement of these activities, feelings and impressions, as well as personal satisfaction, however, is limited, as interindividual differences do not allow for sufficient comparability.

Planning the Surgical Procedure

No standardised protocols can be followed by the institutions carrying out hand transplantation, and surgical strategies have to be adapted to the level of amputation as well as the exact length and quality of structures in the recipient's stump. Thus, for each individual case, a precise plan and, in some cases, specific surgical training of novel reconstruction techniques might be necessary. The surgical procedures must be planned and, if necessary, practiced on

cadavers prior to putting patients on the waiting list.

Simultaneous preparation of donor limb and recipient stump in adjacent operating theatres is recommended whenever possible since this keeps cold ischaemia short. When organising a hand transplant procedure, particular emphasis should therefore be given to such logistical aspects that allow for minimisation of ischaemia time. There is growing evidence that prolonged cold ischaemia might significantly impair short- and long-term graft function as well as functional outcome [12–15]. Hence, allocation with long-distance transportation should be avoided. Muscles are sensitive to ischaemia, and damage such as interstitial oedema, microvascular constriction or damage of myocyte membranes may result in muscle dysfunction after 2.5 h of (warm) ischaemia only [16]. Although cold flush and preservation with University of Wisconsin (UW) or similar solutions might limit myocyte damage, the shortest possible ischaemia time should be aimed for. To minimise ischaemia, donor and recipient operations are performed simultaneously at our centre. Such an approach permitted ischaemia to be kept at 150–170 min.

In terms of planning the surgical procedure itself, a "routine hand transplant" includes at least the following steps: All anatomic structures such as tendons, nerves, and vascular structures are dissected under tourniquet control in donor and recipient. After release of the tourniquet in the donor and when haemostasis is achieved, the forearm is then perfused with cold UW solution through the brachial artery. Next, all structures are transected and both bones osteotomised at the midforearm to allow enough length of all structures to be joined with the recipient. After wound closure, the recipient's cosmetic prostheses are fitted to the donor. Donor and recipients bones are trimmed and fixed with appropriate metal plates. The radial and ulnar artery are then anastomosed. After cephalic and basilic vein anastomoses are completed, the graft is reperfused. Subsequently, hand and finger flexors and extensors are repaired. Ulnar, median and superficial sensory branch of the radial nerve are then sutured above the wrist followed by skin closure. Simultaneously, the same procedure is performed on the contralateral side. After dressing, both arms should be placed on long-arm splints.

Ethical Considerations

Few developments in healthcare have created as much discussion as has hand transplantation and, in particular, ethical issues associated with the procedure. Therefore, ethical guidelines have to be defined before such a clinical programme is launched. Such guidelines should be delineated in a formal protocol by professionals with appropriate expertise in designing, implementing and evaluating such a programme. Two major differences between hand and solid-organ transplantation – which is widely accepted as treatment for end-stage organ failure – are the subjects of the public debate: (1) transplantation of a limb, tongue, larynx or, as recently performed, of a face are not life saving, nor do they improve patient survival; (2) in contrast to solid organs, a hand or a face is visible to the patient as well as the public, including the donor family.

Controversy over limb transplantation has peaked after the first clinical cases, a debate focusing on whether this procedure is ethically correct, and whether the benefits justify the risks of surgery and immunosuppression were discussed in the medical press and even more so in the lay press [3, 5, 17–21]. There is no doubt that some, perhaps even the majority, of traditional transplants directly save lives, but it would be a mistake to conclude that transplantation could only be justified in situations in which individuals are at risk of death. Nowadays, organ transplantation aims at various goals, such as improving quality of life and cost savings in addition to lifesaving efforts. These goals are essential elements in the full ethical justification of transplantation although they receive little or almost no attention in the media [22].

Most of the current public discussion is based on early reports of the outcome after hand transplantation as well as on "probabilities" of possible outcomes; however, an assessment of the individual quality of life is often lacking. It is surprising that despite the number of published

works on hand transplantation even in the scientific community, a patient's personal view, opinion or feelings has not been properly addressed. After loss of one or both hands and/or transplantation, a patient's individual state of mind is of interest. We feel that the scientific press as well as the lay press lead a debate on a very select group of patients without taking personal views into consideration. Major progress, especially in pharmacological immunosuppression but also surgery and rehabilitation, has been made over the past decade, and we wonder if it is still justified to categorically withhold such a therapeutic option from patients. As answers to many questions in human hand transplantation can only be given years later, we feel that a decision to put a patient on the waiting list must be made on an individual basis. The "benefit-to-risk ratio" needs, therefore, to be assessed on a case-by-case basis with respect to the patient's hopes, fears and expectations. It goes without saying that ethical guidelines and good clinical practise have to be respected. There is general agreement in the transplant community that organ transplantation is ethically justified only in situations in which there is an acceptable relationship between cost and benefit and which is to expect that quality of life can be attained. Survival under seriously compromised conditions, without a satisfactory quality of life, cannot be an ethically justified goal of organ transplantation. Because organ donors and especially donors for CTA are scarce and transplantation costs are high, selection as aforementioned of the best candidates is an additional ethically important aspect of this procedure. Such considerations allow a conclusion that hand transplantation or other CTAs that do not directly save a life are ethically justified if they favourably meet cost–benefit and Quality-of-Life outcome standards.

One of the expenditures associated with hand transplantation is the need for lifelong immunosuppression. The intensity of immunosuppressive therapy not only determines side-effects but may be responsible for infectious complications and tumour development. Overimmunosuppression can prevent rejection but might be associated with an unacceptable risk for those complication. From early experiences, it seems that immunosuppression for hand transplantation is similar to that used in kidney transplantation. This, of course, might be true for most but not all patients [23–25]. Progressive acute or chronic rejection may develop despite severe immunosuppression, and at a certain point, the question arises as to when to stop immunosuppression and to give up the graft. These decisions can also only be made on an individual basis, including the patients' view with their expectations and fears being taken into consideration. However, we would rather accept the loss of a hand than overimmunosuppress the patient, for instance, by giving repeated doses of deletional antibodies or by maintaining tacrolimus trough levels over 15 ng/ml long term.

Future Clinical Trials

The organisation of prospective randomised clinical trials for this kind of transplantation might be limited by the small number of transplants performed worldwide for the time being. Despite the fact that only a few centres have experience with this type of transplant, it would be important to establish standardised clinical protocols in order to obtain meaningful data and allow for comparison of results. Such protocols should identify specific inclusion and exclusion criteria and include data collection and ongoing assessment and surveillance of medical and psychosocial risks, complications and adverse events as well as assessment of outcomes. The main challenges of composite tissue transplantation, as to minimisation of immunosuppression and prevention of chronic rejection, are also the main problems associated with solid-organ allotransplantation. The goal of such pilot studies or clinical trials must therefore be to collect enough comparable clinical data to decide what are reliable inclusion and exclusion criteria for selection of "good" candidates, what are the best immunosuppressive protocols with minimal toxicity and side-effects to achieve long-term graft survival and which rehabilitation programmes provide optimisation of functional outcome. One additional key aspect that should be

addressed in studies of hand transplantation is monitoring the recipient's immune status. Composite tissue transplants present a unique immunological challenge, and issues such as development of mixed chimerism or even graft-versus-host reaction have not been thoroughly studied. Donor-derived cells in the blood and bone marrow of the recipient should therefore be monitored and quantitated using sophisticated appropriate laboratory techniques such as flow cytometry with HLA monoclonal antibodies, DNA typed molecular probes, real-time polymerase chain reaction (PCR) and ELISPOT and immunostaining at various defined times after transplantation. However, currently, the organisation of prospective randomised trials seems very unlikely. Therefore, retrospective data must serve as the basis for developing future clinical protocols. Of utmost importance, therefore, is an international database (www.handregistry.com) including all hand transplant patients that allows independent reviewers to more objectively evaluate functional results, incidence of chronic rejection and risks of long-term immunosuppression.

Generating Immunosuppressive Protocols for Hand Transplantation

There is no question that the ideal situation and ultimate goal in hand transplantation is to induce antigen-specific tolerance, making immunosuppression unnecessary. The induction of immune tolerance, however, has not been achieved so far, and it is not very likely to become a clinical reality in the near future. Nevertheless, recently introduced protocols demonstrate that long-term immunosuppression can be minimised [26–30].

Specific immunosuppressive protocols have to be designed for the early postoperative period (3 months), the first 2 years as well as for long-term immunosuppression. For the time being, immunosuppressive protocols similar to those used in solid-organ transplantation are applied and have been shown to be effective in preventing rejection, as reflected by a 1-year graft survival of 100% [1]. When designing immunosuppressive protocols for hand transplantation, new developments in solid-organ transplantation, experimental limb transplantation and experiences derived from clinical hand transplantation have to be taken into account.

Differences to organ transplantation need to be considered as: (1) hand amputees are otherwise healthy recipients and usually lacking comorbidities that might increase the risk of infection and possibly to develop malignancy; (2) transplantation of a hand is not life saving, nor does it positively influence long-term patient survival; (3) the hand is constantly visible, and rejection can be detected in most cases just by inspection.

Since most acute rejection episodes occur during the first postoperative year, the level of immunosuppression during that time period seems to be crucial. Tacrolimus has been shown to be more effective in preventing rejection in comparison to other calcineurin inhibitors; therefore, most centres use the drug as the cornerstone in their immunosuppressive protocol. Furthermore, the stimulatory effect of tacrolimus on the synthesis of axotomy-induced growth-associated protein (GAP-43) might be relevant for nerve regeneration [31, 32]. For long-term immunosuppression, a low toxicity profile together with an inhibitory effect on chronic rejection would be desirable. Hence, the following recommendations for immunosuppression after hand transplantation can be given:

1. Induction therapy with antithymocyte globulin (ATG) or Campath-1H may help to prevent early rejection and keep maintenance immunosuppression low
2. Tacrolimus should be part of the immunosuppressive regimen during the first 2 years, not only for its immunosuppressive potency but also for its nerve-regeneration accelerating properties.
3. During the first 2 years, a triple therapy including steroids and mycophenolic acid seems to be effective in preventing acute rejection. Steroids should be tapered and finally withdrawn within that time period
4. At 2–3 years after transplantation, calcineurin inhibitors should be replaced with a target of rapamycin (TOR) inhibitor for their low toxicity profile, their protective effect on chronic rejection and their antitumor properties.

Preparation and Organisation of Rehabilitation

Systematic rehabilitation based on an individually tailored programme is essential to obtain an optimal functional result. One has to keep in mind that rehabilitation after hand transplantation is a team effort! Our team consists of 3 physical and one occupational therapist and is headed by a specialist in physical medicine and rehabilitation. In addition to coordination and performance of physical and occupational therapy, team members evaluate the patient's condition and healing process. The progress of graft function is discussed with surgeons and neurologists in frequent intervals or whenever clinically indicated.

Outcome assessment is an essential tool for reporting, monitoring and evaluating functional results of the rehabilitation progress. Choosing the most reliable and valid instruments is crucial for measuring function, treatment effectiveness and rehabilitation. Therefore, standardisation of these tests is necessary in future studies to facilitate comparison of functional outcomes. The rehabilitation programme should also be designed with respect to different time frames of healing among reconstructed tissues (bone, blood vessels, tendons, nerves and skin). Goals must be to support the healing of each component and induce and accelerate cortical reintegration and reorganisation of the transplanted limbs.

The main focus of early rehabilitation is to control swelling and pain, prevent joint stiffness and adhesion and achieve good motility without jeopardising the healing process of joined structures. As "early protective motion" (EPM) after hand replantation has shown favourable results, we introduced a similar programme after [33]. Long-term treatment aims at motoric re-education and sensibility training. Nerve regeneration was assessed for the presence of Tinel's sign.

Diverse programmes for rehabilitation have been applied at various centres with different emphasis on either maximisation of range of motion and strength or on sensory re-education and cortical reintegration [33–37]. In Innsbruck, sensory re-education is given high priority, and therefore the specific cognitive exercise programme described by Perfetti is an important part of our rehabilitation programme [33, 34]. This programme is especially designed to introduce a new cerebral concept (motor imagery) to the patient's reprogramming of a motoric action through targeted tailored tasks. The programme encompasses 3 levels: (1) the patient learns to control the abnormal stretch reflex of the involved muscles; (2) the patient aims for control over common mass joint-muscle movement and the proper formatting of the movement parameter; (3) the patient learns to avoid nonparticipating muscle movements and to perform specific contractions of the structure of trained muscle units. All three levels were integrated in the training of the tactile and kinaesthetic cognition in both our patients.

Conclusions

In summary, to date hand transplantation can be performed successfully with an acceptable risk profile. However, the potential patient's benefits must be carefully weighed against the enormous effort and risks associated with such a complex procedure. Therefore, meticulous planning and organisation according to standardised protocols will be a prerequisite when considering establishment of a hand transplant programme.

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Section 4-d

Selection of Candidates and Waiting List, Dealing with the Media and the Public, Setting Up a Pilot Study, Clinical Trial Organization, Staff Requisites for Hand Transplantation: The Brussels Experience

Frédéric Schuind, Carlo Van Holder, Daniel Abramowicz

Introduction

Hand transplantation is controversial and raises serious ethical issues. Many hand surgeons remain critical, fearing the complications of immunosuppression and considering that the indications of hand transplantation are few, if any. In contrast to these pessimistic views, the world experience of hand transplantation has been quite rewarding, raising much enthusiasm [1–22]. Based on our experience of one successful hand transplantation in Brussels (Fig. 1), [23], and based on the international registry of hand transplantation [22], we are now better able to define the indications and contraindications of this exceptional procedure. We will then discuss the clinical organisation to successfully achieve a hand transplantation.

Indications and Contraindications

Type of Amputation

The ideal recipient is the patient presenting the sequelae of a "clean-cut", bilateral, mid- or distal forearm amputation, representing 33.3% of the indications in the world experience. In such a case, the extrinsic muscles are preserved in the amputation stumps, allowing – after solid tendon-tendon suture – immediate postoperative active mobilisation of the wrist and fingers. The vessels are in good condition, avoiding patency problems following arterial and venous anastomoses. The median, ulnar and radial sensory nerves are intact, presenting usually big distal neuromas: in this situation, it is easy to recut the nerves at appropriate lengths in order to obtain a perfect microsuture without deleterious tension. With such a good nerve suture, which can never be attained in traumatologic situations (with retraction of the nerve extremities), except in case of associated bone shortening, the nerve regeneration possibilities are at best, and, in combination with the beneficial axonal-regenerating effects of tacrolimus [4, 16, 24], quick and satisfactory recovery of sensibility and hand motor function can be expected. All patients end up with at least good protective sensation, many with excellent finger discriminative sensation [22]. The Brussels patient regained two-point fingertip sensory discriminative sensation of 6 mm at the thumb and index and could almost perfectly localise each finger and each hand territory with his eyes shut [23]. Most patients also regain voluntary activity in the hand intrinsic muscles (thenar and hypothenar muscles, hand lumbrical and interossei muscles), which is essential for good hand function. The first evidence of intrinsic recovery occurred in the Brussels patient at 6 months. At 37 months, the patient had metacarpophalangeal (MCP) flexion of long fingers and good thumb palmar abduction; however, the abduction/adduction possibilities of the long fingers remained limited [23].

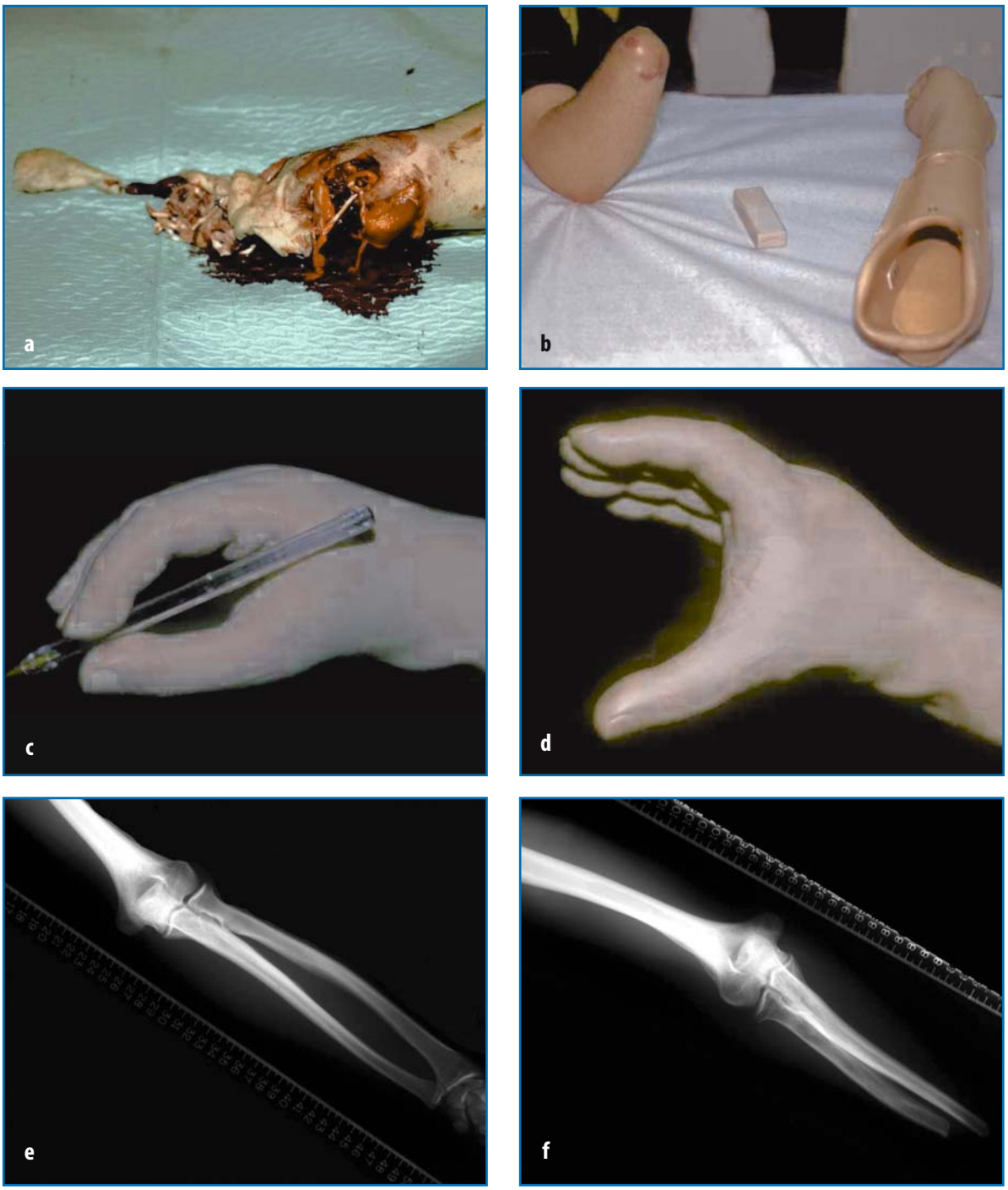

Fig. 1a-l. *Brussels hand transplantation case*. A 22-year-old man suffered from a traumatic amputation of this right dominant hand at the junction of the middle and distal thirds of the forearm (**a**). **b-d** Before the transplantation, he had been using a myoelectric prosthesis and had regained good function with it. Pretransplantation workup including comparative scanograms of both forearms (**e, f**), magnetic resonance imaging (**g**) and arteriogram (**h**). Hand transplantation with quick disarticulation of the donor limb at forearm (**i**), preparation of the hand on a side table (**j**) and aspect at the end of surgery (**k**). Appearance of the hands at 3 years after transplantation (**l**)

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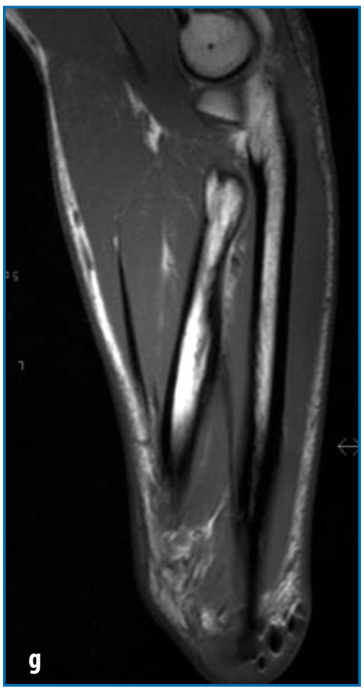
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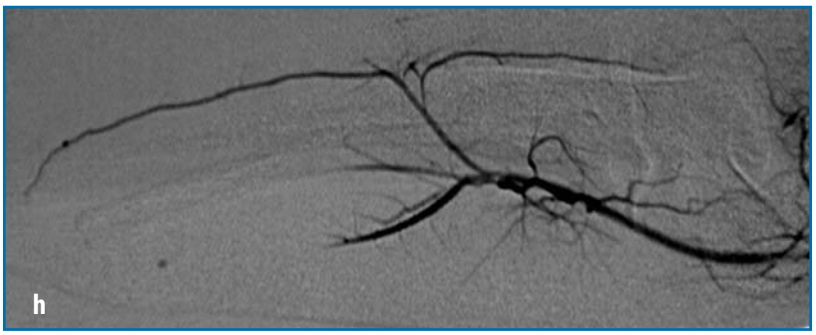
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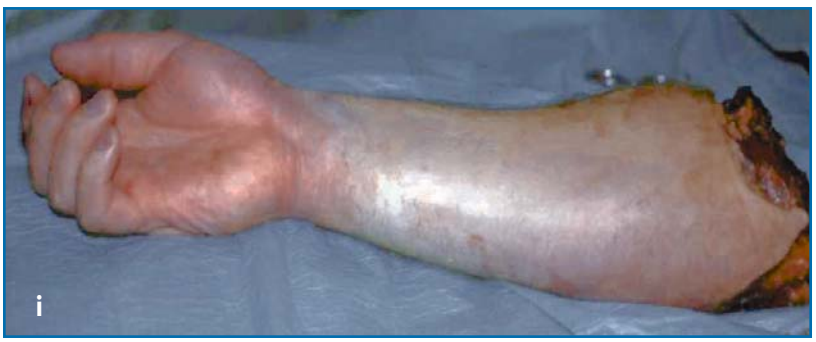
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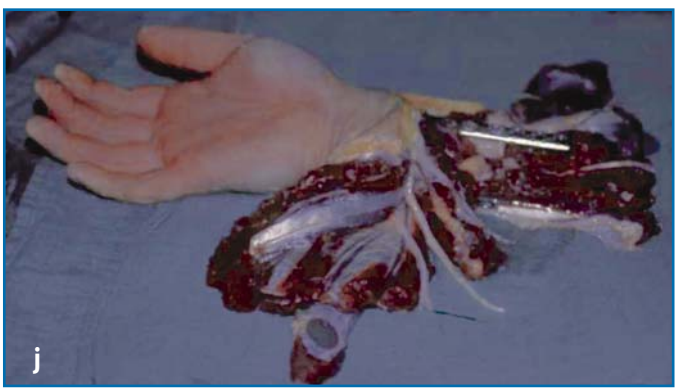
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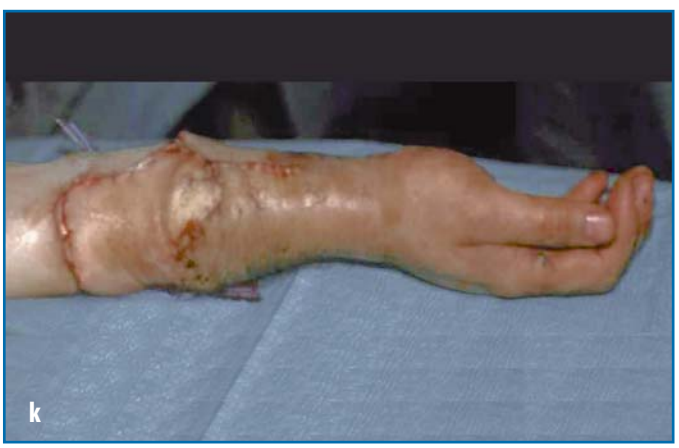
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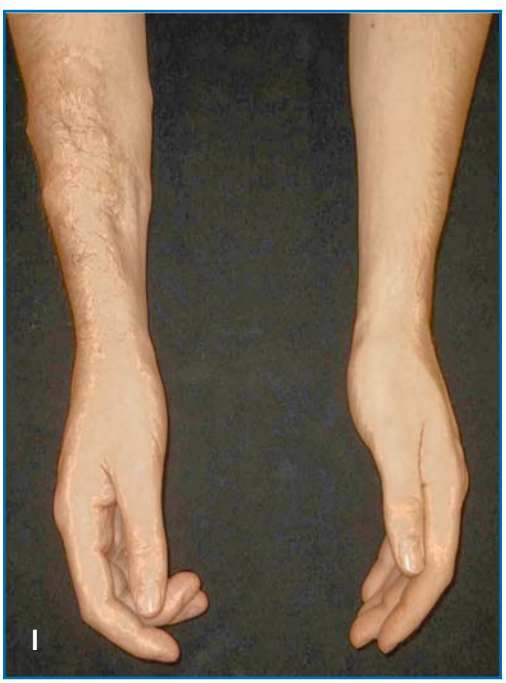
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A more controversial indication of hand transplantation is the unilateral amputation of the dominant hand, with a similar amputation stump as discussed above, representing 61.1% of the world experience of hand transplantation, including the Brussels case. Some ethical committees accept only bilateral forearm amputations, considering that in the case of a unilateral amputation, the functional deficit is not so serious to justify the risks associated with a transplantation and the subsequent medical treatment. However, they do not consider the moral sufferance related to the amputation of the hand. This problem is underestimated by most of the medical community. The loss of body image due to the amputation is variable from patient to patient but may have disastrous psychological effects [25–30]. As written by Klapheke et al. [31], "amputation of the hand is a tremendous physical and psychological trauma that can precipitate powerful conflicts regarding loss of autonomy, guilt/punishment, and potency". Many amputees are willing to take risks to recover their body image and function, to hold their wife or husband in their arms, and so on. The personal views of the patient candidate to a transplantation should be taken into consideration.

Finger amputation, in particular thumb amputation, can be treated by transplantation of a normal ray. While it is feasible, and indeed has been already done in China, we consider at the present time that there are, in the majority of cases, other satisfactory techniques of reconstruction available, for example, pollicisation of a neighbouring finger (Fig. 2), stump lengthening (Fig. 3) or toe transfer, and that the risks of

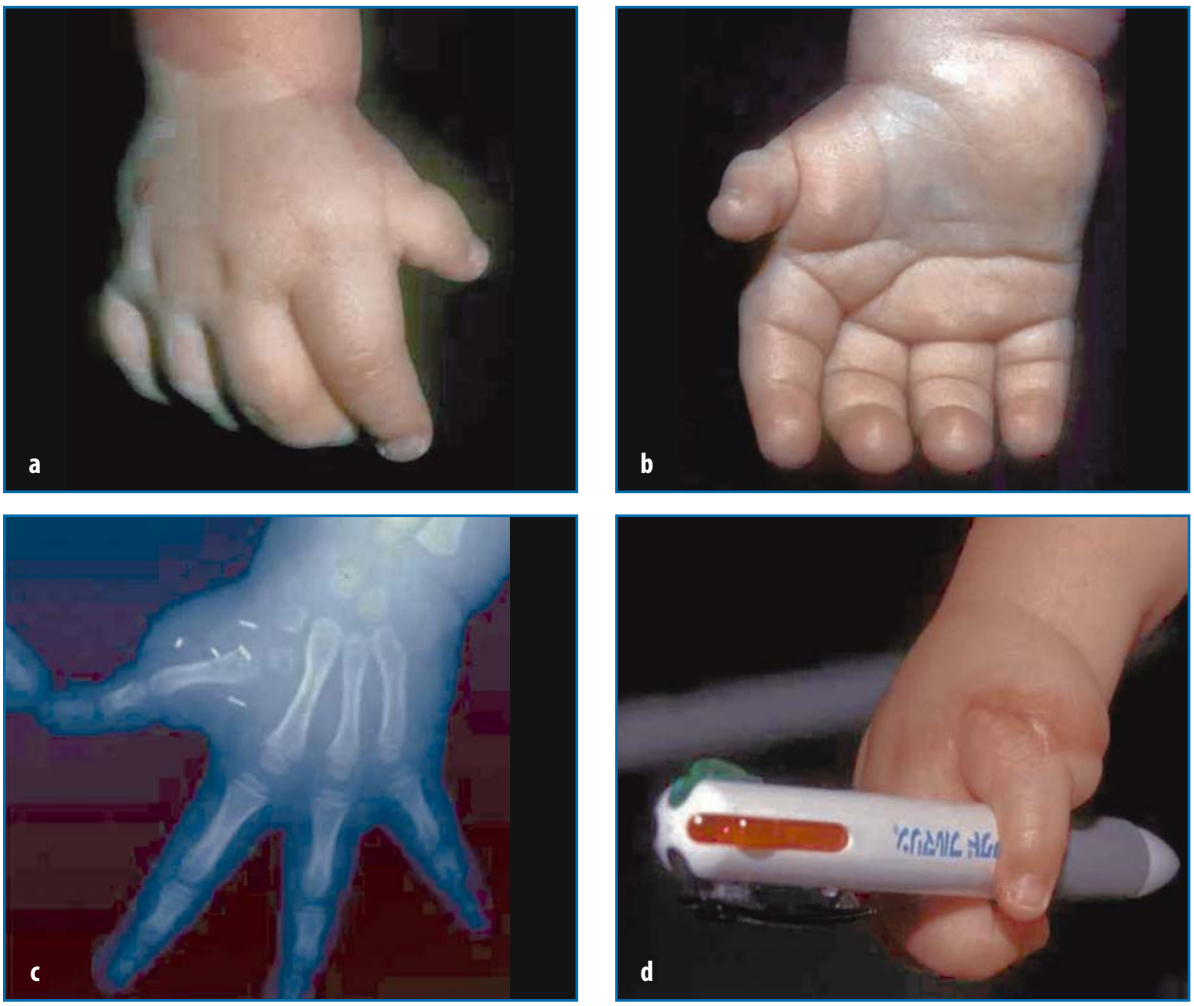

Fig. 2a-d. a, b Young child suffering of VATER syndrome with hypoplastic thumb. **c, d** Pollicisation of the index finger at 2 years. Early result. The child ended up with an excellent functional result

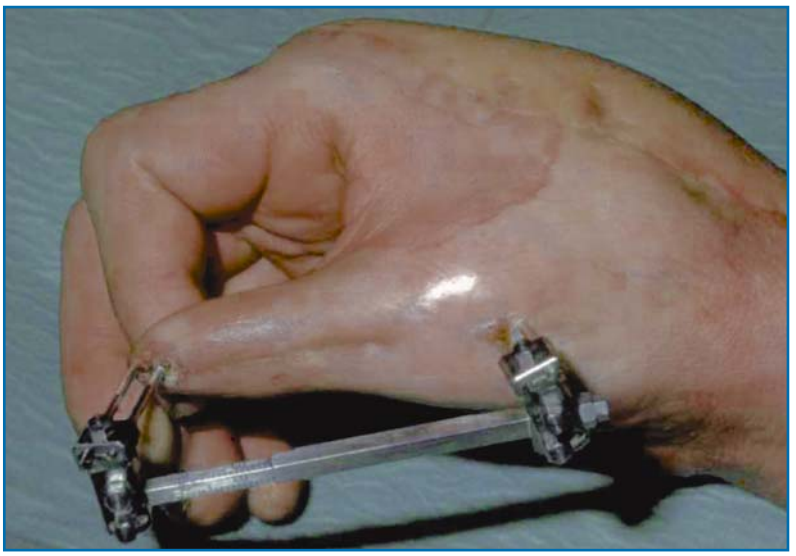

Fig. 3. Posttraumatic hand sequelae with thumb amputation at mid-diaphysis of the proximal phalanx. Thumb lengthening by mini-external fixation. The patient regained excellent function

immunosuppression are not justified in such cases. However, if the patient is already under immunosuppression, for example, for a previous renal transplantation, then finger transplantation could be indicated. In the future, when we will be able, with reasonable risks, to induce allogeneic tolerance (defined as indefinite donor-specific immune allograft acceptance independent of chronic immunosuppressive therapy), then finger transplantation could be indicated in selected cases.

Transplantation has been considered as not indicated in very proximal amputations because all the hand function will then depend on nerve regeneration: there are no remaining extrinsic muscles for early active wrist and finger motion. However, the last Innsbruck transplantation (just under the elbow) involved forearm extrinsic muscles reinnervation. If this patient attains a good functional result, transplantation could, as well, be proposed for proximal amputations, even, maybe, for some above-the-elbow amputations. One potential problem is then the important volume of transplanted tissues, with the immediate risks of muscle ischemia and renal failure and the early and late potential immunological problems related to the amount of allogeneic tissue. The last Innsbruck patient had, indeed, many rejection episodes [32]. On the other hand, it is classically stated that a very large volume of grafted tissue could, indeed, favour immunological tolerance.

Finally, we see many patients presenting with bilateral stiff, insensitive, and almost totally non-functional hands, for example, after war injuries. The only reconstruction that could provide those unfortunate patients some function is the amputation of the hands followed by a bilateral transplantation. Until now, this has not yet been attempted because, in case of vascular failure or of uncontrollable rejection, the patient will be left with no hand.

Patient Personality and Motivation

A hand transplantation represents for the patient and for the medical treating team a very heavy therapeutic programme of long duration. The patient has first to wait, frequently a long time, before there is a good donor. The ideal brain-death donor is young; with blood matching; with a good human leukocyte antigen (HLA) compatibility (ideally about three or four HLA-antigen mismatches and, of course, a negative lymphocytotoxic crossmatch); with an acceptable match for gender, bone size, hand span, and skin color; and intact hand(s) – including, if possible, the absence of arterial or venous forearm catheters. Usually, the agreement of the family should also be obtained, and such approval is less evident than for internal organs. After the transplantation, the recipient (and his or her family) has to accept the new

hand(s), which is not so easy psychologically. The patient is under life-long pharmacologic immunosuppression, which implies taking many pills every day, consulting frequently – in the beginning every week – the medical team, having many blood tests and other medical investigations in the routine follow-up of the transplantation, not taking into account the possible rejection episodes or the possible medical complications. Most patients need also one or several reoperations to improve some aspects of the new hand(s) – for example, tenolysis, tendon transfer, hardware removal, and so on. The patient must also live with the constant risk of irreversible rejection and the possibility of late transplant dysfunction or severe complications of immunosuppression, including serious infection or cancer. Finally, the patient has to follow daily intensive physiotherapy for at least 6 months, preferably 1 or 2 years, to attain maximal hand function. The Brussels patient initially had 3 h of physiotherapy per day, 5 days a week. All these efforts need a well-motivated patient, with a strong personality, able to understand the complexity of the therapeutic programme. The existence of a strong sociofamilial background is quite helpful. Preoperative thorough psychological investigation is indispensable, and patients with weak personalities or poor motivation should be discouraged. A good test of the motivation and collaboration of the patient is to see how he or she accepts, in the preoperative period, myoelectric prostheses and how he or she learns to use them. Postoperative psychological follow-up and support is indispensable as well.

Age Considerations

Although paediatric limb transplantation is possible; although growth would probably be observed in transplanted limbs of children (in experimental limb transplantation performed in growing animals, the epiphyseal plates maintained their growth potentials [33–36]; although the indications could be quite frequent if we consider congenital amputations; hand transplantation is probably not acceptable at the present time in children because of the impossibility of obtaining from a child an informed consent [37], not even considering the limited number of paediatric donors. It should, however, be mentioned that medically, neonatal transplantation could possibly be achieved without immunosuppression (neonatal tolerance induction of the immature immune system by exposure of the recipient to donor cells [38–44]. Although in adults there is no clear limitation of age, there is a consensus that a hand transplantation should not be offered to an elderly adult (for example, older than 55) mainly because the possibilities of brain adaptation to the new hand(s) have probably become insufficient.

Medical Contraindications

Contrary to cardiac or liver transplantations, a hand transplantation does not save the life of the patient. Immunosuppression represents significant risks, including increased susceptibility to infections and cancers, and an increased risk of cardiovascular disease in the long term. Therefore, before considering a hand transplantation, a general workup is necessary to rule out medical conditions that would preclude organ transplantation, such as cancer; significant abnormalities of renal, pulmonary or cardiac function; and carriage of HIV or HCV virus. If the patient has no Epstein Barr (EB) antibodies, a hand transplantation from an EB-positive donor is formally contraindicated, as the risk of developing a posttransplant lymphoproliferative disease is then quite high [45, 46]. The transplantation of a cytomegalovirus (CMV)-positive graft is also relatively contraindicated in a patient who is CMV-negative, as there is a high risk that the postoperative course will then be complicated by recurrent episodes of CMV infections, even in the case of prophylaxis at transplantation. In addition to the symptoms of CMV disease and the complications of medical treatment of this infection, there could be an association between viral replication and rejection episodes [47]. Glucose intolerance is a relative contraindication, as tacrolimus may induce diabetes mellitus.

Clinical Organization of a Successful Hand Transplantation Programme

Selection of the Hand Transplantation Centre

A hand transplantation programme should be set up only in a centre closely associating with the following areas of expertise: (1) a solid programme of transplantation, including specialists (usually nephrologists) of immunosuppression (including monitoring of rejection); (2) psychologists involved in amputation, moral sufferance evaluation and support; (3) surgeons specialists in all aspects of hand surgery (particularly microsurgery, osteosynthesis, tendon repair, palliative procedures, skin reconstruction); (4) physiotherapy and occupational therapy departments with specialised sections in hand rehabilitation and in cortical reprogramming (for example, mastering the method of Perfetti [48]). Although not mandatory, other areas of expertise are quite helpful: pathologists specialised in rejection phenomenons, immunologists, neurologists specialising in brain plasticity, and so on. Ideally, there should also be an associated research facility investigating some fundamental aspects of limb transplantation. For all these reasons, the hand transplantation centre is usually the academic hospital affiliated to a university. Our institution in Brussels meets all these criteria, as it is characterised by a long tradition of close collaboration between orthopaedic and plastic surgeons, particularly for hand surgery and microsurgical reconstructions, possesses an excellent centre for hand physical and occupational therapy, and has associated areas of expertise in psychology in traumatic sequelae and aesthetic problems, in pathology, immunology and functional magnetic resonance imaging (MRI). Our hospital is one of the leading Belgian institutions in transplantation. In addition, our centre has associated funded laboratory research on limb allotransplantation.

Medications necessary to maintain the state of immunosuppression and postoperative medical monitoring are quite expensive. There should be preoperative agreement from a third-paying party, as most patients cannot afford such expenses, not even considering preoperative investigations, aesthetic prosthesis for the cadaver, the transplantation itself or the treatment of potential postoperative complications. If such financial agreement is not obtained, surgeons should not embark in a hand transplantation.

Preoperative Information of the Patient and Ethical Committee Approval

We believe that the decision to perform a hand transplantation is to be made by the patient himself or herself based on his or her own perception of *balance* between *quality* and *quantity* of life – although it is clear that such a decision will be based on nonrational elements [49, 50]. To be able to make the best decision, the candidate to a hand transplantation must have full information about the risks of the transplantation and of immunosuppression. Such information should be given several times, orally and on paper, including by colleagues independent of the transplantation team. In the end, the patient should sign an informed consent document. It should be noted that we are presently unable to provide such complete information, as we do not know the long-term results of hand transplantation or the risks related to long-term immunosuppression in healthy patients. Therefore, the patient should understand the uncertainties that we face [29].

In addition to the information given to the patient, we believe that the patient's file should be reviewed by an independent Ethical Committee with the ability to carefully analyse all issues regarding the proposed transplantation.

Preoperative Planning

Preoperative investigations must first rule out any medical contraindication to the transplantation (*see above*). In addition to these routine pretransplantation investigations, the surgeons should consider all information regarding the state of the amputation stump(s). We recommend the following preoperative investigations (Fig. 1): scanograms of both forearms; MRI of the amputation stump(s); Doppler and, if necessary, arteriograms to objectify the preserved permeability of

the donor arteries; electromyogram, to study the preoperative muscular function. Preoperative functional cerebral MRI may also be interesting to study the postoperative cortical recovery of sensibility [51] and motricity [52]. An aesthetic prosthesis, based on the patient's normal hand in case of unilateral transplantation, should be fabricated in advance and placed on the cadaver of the donor after all organs have been harvested.

The ischaemic time is probably critical, especially for very proximal transplantations. To reduce this time as much as possible, it is better to harvest the hand(s) prior to the solid organs [23, 53]. This can be done very quickly, under tourniquet, by elbow disarticulation (Fig. 1). Such a sequence needs, of course, preoperative consultation with the other transplantation teams. In Brussels, this was done at meetings of the Transplantation Council. Also, for the same reason, to reduce the ischaemic time as much as possible, and for reasons of good organisation, it is probably better to harvest the hand(s) and to operate the recipient in adjacent operating theatres of the same institution, if it is feasible.

It is likely that there will then be in this centre several transplantations at the same time. We had in Brussels a separate on-call list for our unique case of hand transplantation, including surgeons, nurses and anaesthesiologists to be sure there was sufficient human workforce to achieve, in optimal conditions, the hand transplantation. Other centres have relied on staff from neighbouring or foreign hospitals. We have also found it helpful to write a detailed operative protocol before the transplantation in order to prepare in advance all necessary hardware for the surgical procedure.

Dealing with the Media and the Public

There should be strict confidentiality, and neither the transplantation team nor the direction of the institution should take the initiative to contact the media. However, experience shows that the media are quite interested in medical achievements, especially those raising ethical discussions such as hand or face transplantation, and that the transplantation team and the patient are not able to avoid public exposure. These matters should be discussed well in advance with the candidate recipient. The transplantation team should provide objective and limited information to the media, in agreement with the patient, preserving the anonymity of the donor and the recipient, and providing no picture. It is helpful to discuss these matters as well with the Ethical Committee.

Discussion

Before 2001, the official position of the International Federation of Societies for Surgery of the Hand (IFSSH) was "caution before proceeding with more transplants"; at its Istanbul 2001 congress, the Federation agreed "to proceed with more transplantations in specialized departments". At present, the best indication is probably a bilateral traumatic hand amputation with good stumps, in a motivated patient, devoid of psychological problems [10, 18, 20, 53–56]. Potential candidates should understand not only the risks of transplantation but also the need for a long-lasting and tiring rehabilitation programme. They must have strong will and determination, and the single most important factor for a successful transplantation is probably the personality of the candidate recipient. Relative counterindications include unilateral amputation on the nondominant side, a long delay amputation-transplantation (though Lanzetta performed a hand transplantation after 25 years), anticipated psychological problems, insufficient motivation and/or poor stump (vessels, muscles).

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Section 4-e

Patient Management and Follow-up

Palmina Petruzzo, Stefano Lucchina, Clara Dezza, Giovanna Lucchini

Introduction

At present, clinical hand transplantation is still considered an experimental procedure, and consequently, carefully monitoring the transplanted patient is required. From our experience with hand transplantation, we stress the importance of preoperative screening [1], including fully informed consent; patient compliance to postoperative medication and physical therapy and, above all, careful follow-up of the recipient.

Postoperative Period

In the postoperative period, the recipient must be considered as a patient who has undergone surgery lasting an average of 12 h and who has received induction therapy and sometimes several blood transfusions. Thus, during this critical period, the patient must undergo a daily blood test, including tacrolimus blood level, and a weekly routine, including viral markers, fungal antigens, antihuman leukocyte antigen (HLA) antibodies and lymphocyte subsets [2].

Follow-Up

Follow-up time points are at 1, 3, 6 and 12 months and at each anniversary of the transplantation. On the basis of our experience [2–4] and that of the International Registry on Hand and Composite Tissue Transplantation [5], the follow-up must include the routine evaluation performed in solid-organ recipients and the functional evaluation regularly performed in hand replantation (Table 1).

General Management

The immunosuppressive protocol used in hand transplantation [steroids, tacrolimus, mycophenolate mofetil (MMF)] is comparable with that used in kidney transplantation. Therefore, it can be hypothesized that the risk of immunosuppression associated with a hand transplant is similar to that of a kidney transplant [6]. Consequently, follow-up must include careful control of possible complications, such as posttransplant diabetes, hypertension, nephrological complications due to tacrolimus nephrotoxicity, gastrointestinal toxicity due to MMF, osteoporosis, cataract, opportunistic infections (herpes virus, cytomegalovirus, Epstein-Barr virus, viral hepatitis, candida, aspergillus, toxoplasmosis) and malignancies, particularly skin cancer and lymphoma. In addition, chest X-ray, abdominal ultrasounds and echocardiography are performed for global evaluation. Early detection of side-effects is important, as the majority is reversible following dosage reduction of immunosuppressive drugs. In

Table 1. Schedule of the follow-up of hand grafted patients

Time	Evaluation
1, 15, 30, 90 and 180 days and as needed	Biopsies taken from the hand or DSSG
Daily	Tacrolimus blood concentration
Each time point of follow-up	Lymphocyte subsets by FACS
	Microchimerism (PCR analysis of DNA from blood leukocytes)
	Immunoglobulin assay
	Anti-HLA antibodies
	Viral and mycosis markers
	Chest X-ray
	Abdominal sonography
	ECG and echocardiography
	Kidney function examination
	Eye test
	DEXA evaluation
	Venous and arterial Doppler
	Upper-extremity X-ray and bone scintigraphy
	Dermatological evaluation and biopsy
	EMG and neurological evaluation
	fMRI

DSSG, distant sentinel skin graft; *FACS*, fluorescence-activating cell sorting; *PCR*, polymerase chain reaction; *HLA*, human leukocyte antigen; *ECG*, electrocardiogram; *DEXA*, dual-energy X-ray absorptiometry; *EMG*, electromyogram; *fMRI*, functional magnetic resonance imaging

addition, it is imperative to perform prophylaxis for *Pneumocystis carinii* pneumonia and cytomegalovirus infection.

Immunologic Follow-Up

Although hand transplantation is a form of allografting that behaves in many ways similarly to solid organ transplantation, the developing a greater immunologic understanding of such new allografts awaits greater clinical experience. Therefore, blood lymphocyte subsets by fluorescence-activating cell sorting (FACS) and microchimerism by polymerase chain reaction (PCR) analysis of DNA from blood leukocytes should be routinely monitored at each time point of the follow-up, and immunohistochemical studies of skin biopsies should be performed [2].

In transplantation, either solid organ or hand, the most common form of rejection is acute rejection, and its incidence is highest in the first 3 months posttransplantation after the first week. Acute rejection episodes are characterized by the presence of cutaneous lesions; therefore, clinicopathological monitoring of the skin is the most reliable way of detecting early allograft rejection. During the first month, clinical inspection of the skin should be performed daily and then weekly for the first 6 months. In addition, skin biopsies should be performed routinely 7 and 15 days after transplantation; at 1, 3, 6 and 12 months posttransplantation and then once every 6 months. Skin biopsies must always be performed when cutaneous lesions appear [2, 8].

Evaluation of Hand Transplantation

At each time point of the follow-up, x-rays and bone scintigraphy are used to study bone healing, and venous and arterial Doppler are used to explore vessel patency; moreover, an angiogram may be performed at 1 month and/or 1 year after transplantation. The first electromyography should be planned 6 months after transplantation and then at each anniversary.

Psychological Assessment

Psychological evaluation and assessment during follow-up is essential, particularly in the first postoperative period when the recipient worries about the outcome of the transplantation procedure and the grafted hand has not yet developed function and sensitivity. Hand transplant patients undergo different psychological phases after before accepting the "visible" grafted hand as one's own [9]. For this reason, they need psychological support, which must occur daily during the hospitalisation period, weekly for the first 3 months, then periodically and, if necessary, during the follow-up.

Neurophysiological Evaluation

Integration or lack integration of the transplanted part into the cortex does not occur in solid-organ transplantation while in hand transplantation, lateral motor cortex sites, which were active for hand movements in the pretransplant period, were not active following the graft, and hand representation shifts from the lateral to medial region in the motor cortex. This phenomenon has been shown by functional magnetic resonance imaging (fMRI) [10], which might be performed before transplantation and then at 1, 3, 6 and 12 months to monitor neural integration of the grafted hand.

Rehabilitation Program and Functional Evaluation

Recipients must perform a rigorous programme of rehabilitation [11], including physiotherapy, electrostimulation and occupational therapy. Currently, there are no standardized protocols for hand-grafted patients, and the majority of teams applied the same protocols used after replantation procedures. It is very important that physiotherapy is started 12 h after surgery twice a day then twice a day for the first year post-transplantation and that active exercises are added as soon as possible to avoid finger and wrist stiffness during the healing period. On the basis of our experience, another important point is that occupational therapy and physiotherapy must focus on sensory, visual, motor and haptic stimulation of the hands.

Specific tests to assess sensitivity and motion recovery must be performed each month for the first 6 months and then at each time point of the follow-up. Protective sensibility, two-point discrimination and touch sensation (Semmes-Weinstein test) are the principal investigations to be performed. Active range of motion of wrist, metacarpophalangeal and interphalangeal joints must be registered and grip and pinch strength evaluated. Tests, such as Minnesota and Carroll tests, may be planned at each anniversary of transplantation to evaluate functional results.

Conclusions

Hand transplantation is the allografting of several heterogeneous tissues, which constitute a very special organ with a unique level of function and versatility. Indeed, hand transplantation requires an integration of sensory input and fine motor control; moreover, being an external organ, it carries obvious emotional investment. Finally, hand transplantation is not a life-saving procedure; it may improve the recipient's quality of life, but recipient compliance to medication and rehabilitation is indispensable. For all these reasons, patient education, careful management and rigourous follow-up play a pivotal role for a successful transplantation.

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5. Surgical Technique of Hand Transplantation

Section 5-a

Instruments, Sutures and Needles for Hand Transplantation

Giovanna Lucchini, Francesca Magni, Marco Lanzetta

Introduction

A complete range of instruments to face any possible situation during a hand transplantation procedure is mandatory. The material and instruments needed for hand transplantation should be carefully selected and prepared in advance. Their division into practical and ready-to-use sets is essential in a scenario where wasting time must be minimised. Our list of instruments and materials as well as the additional items to be used in the operating theatre is as follows:

- Surgical instruments trays
- Suture stitches tray
- One table with sterile sheets
- Two tables, one of which prepared with a tray for harvesting an iliac crest bone graft
- Two squared tables: one for the osteosynthesis set; one for the harvested limb (including iced solution)
- Operating microscope with videocamera and monitor
- Portable image intensifier.

A. Surgical instruments trays:
   - Two basic hand trays
   - One iliac crest bone graft tray
   - One bone tray
   - One tendon tray
   - One microsurgical tray.

B. Additional individually stored instruments/items:
   - One power drill
   - One saw blade (size 1-2-3)
   - One electric dermotome + blade + 10-cc syringe with Vaseline
   - Two Lambottes, large size
   - Two bowls, large and medium size
   - One basin
   - One preset colour-tagged mosquito for each individual flexor/extensor tendon (Fig. 1).

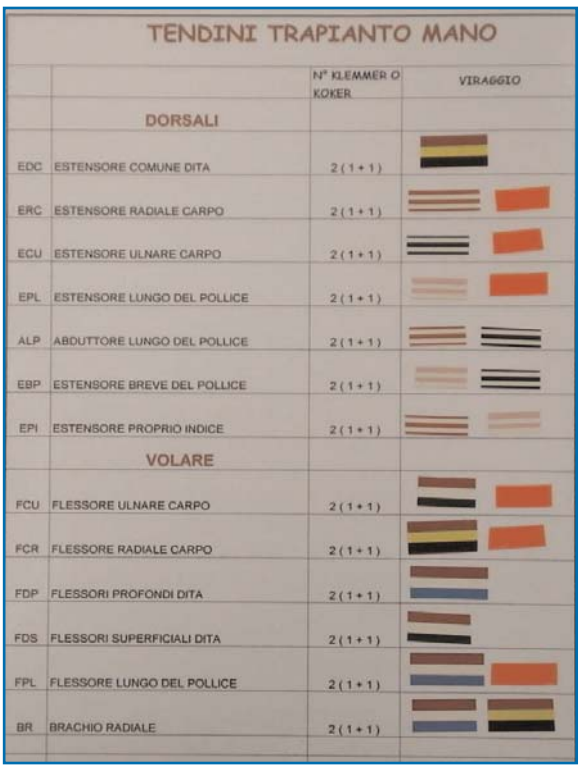

Fig. 1. Preset coloured tags for each individual flexor/extensor tendon

- Two Kilner skin retractors, double ended, medium size
- Two Kilner skin retractors, double ended, large size
- One lead hand
- One osteosynthesis (forearm) set
- One elastic bandage (Esmarch).

C. Complementary items:
- Six scalpel blades, no. 15
- One beaver scalpel blade
- Two marking pens
- One electric scalpel
- Various towels TNT repellent
- One operating microscope towel
- Bone wax
- Aspirator
- Cannulated needles of various sizes
- One 20-cc syringe with local anaesthetic and adrenalin 1% (for skin preparation in iliac crest bone graft harvesting)
- One hypodermic needle
- Four 10-cc syringes: two with NaCl 0.9%, one with heparin, one with local anaesthetic
- Suction drains
- Silicon drains
- Skin stapler, small and medium size
- Tapes of various sizes
- Three plaster splints, sponge-cloth and elastic bandages.

D. Sutures (Fig. 2):
- Subcutaneous and muscles: Polysorb 1/0, 2/0; Monocryl 3/0, 4/0
- Skin: Dafilon or Ethilon 3/0, 4/0
- Ligaments: Ethibond 3/0
- Flexor tendons: Ti-Cron 3/0, 4/0; Prolene 4/0, 5/0
- Extensor tendons: Prolene 4/0, 6/0
- Microsurgery: Ethilon or Monosoft 8/0, 9/0
- Safil loops: 3/0

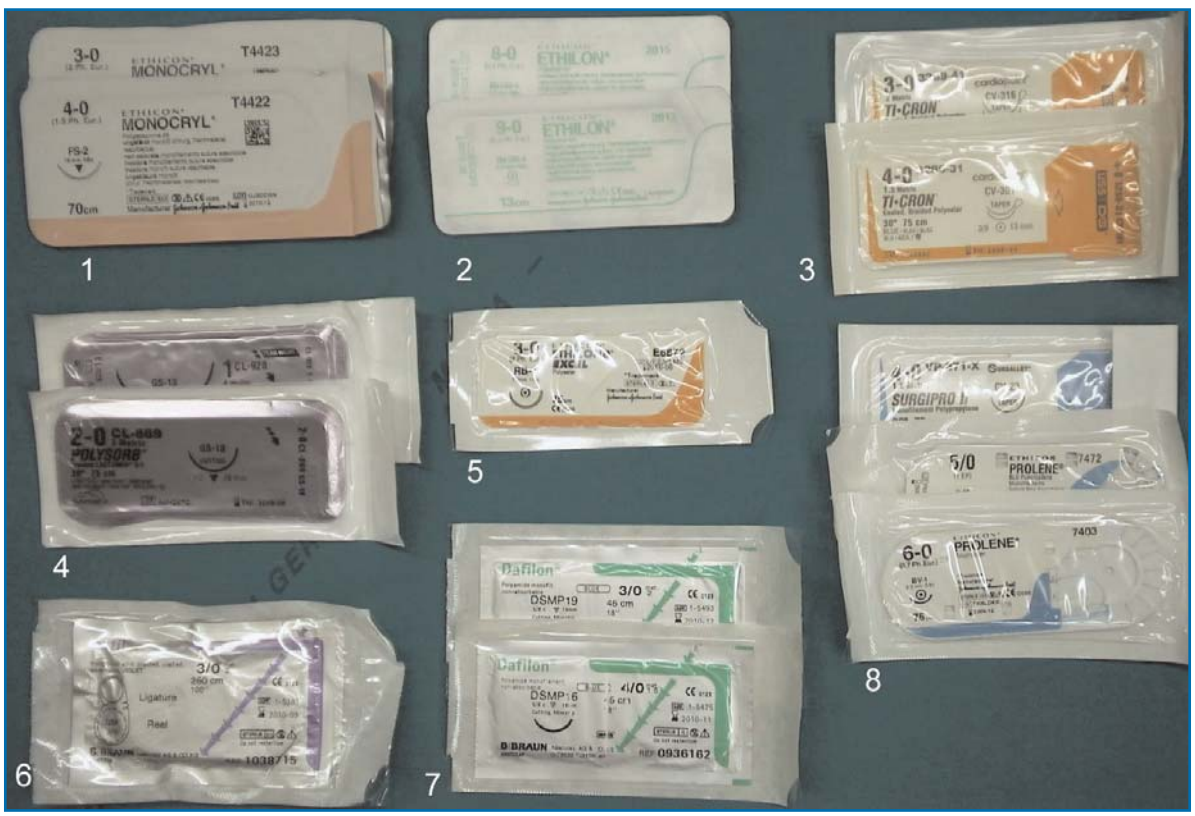

Fig. 2. Sutures: *1* Monocryl 3/0, 4/0; *2* Ethilon 8/0, 9/0; *3* Ti-Cron 3/0, 4/0; *4* Polysorb 1/0, 2/0; *5* Ethibond 3/0; *6* Safil 3/0; *7* Dafilon 3/0, 4/0; *8* Prolene 4/0, 5/0, 6/0

E. Basic hand tray (Fig. 3):
- Marking pen
- 10-cc syringe for irrigation
- Scalpel with no. 15 blade
- Beaver blade handle with no. 64 blade
- Adson dressing forceps, 12 cm plain
- Stevens tenotomy dissecting scissors, 11 and 13 cm
- Sutures scissors, sharp/sharp, 12 cm
- Adson tissue forceps, 12 cm with 1?2 teeth
- Tendon hook, blunt
- Gillies skin hooks, 17.5 cm
- Farabeuf skin retractors, double ended, small size
- Freer dissector sharp/blunt, 15 cm
- Kleinert-Kutz periosteal elevator
- Curette, soluble ended, small
- Ruler
- Hegar needle holder
- Towel clips
- Self-retaining retractor
- Bone/synovium rongeur, double action, 15 cm
- Dressing scissors, sharp/blunt, 15 cm
- Dressing scissors, sharp/blunt, 15 cm
- Metzenbaum scissors
- Mosquito forceps, straight, 12.5 cm
- O'Shaugnessy forceps
- Mosquito forceps, curved 12.5 cm
- Bipolar coagulation forceps
- Needle holder with fine, smooth jaws
- Small bowl for irrigating saline.

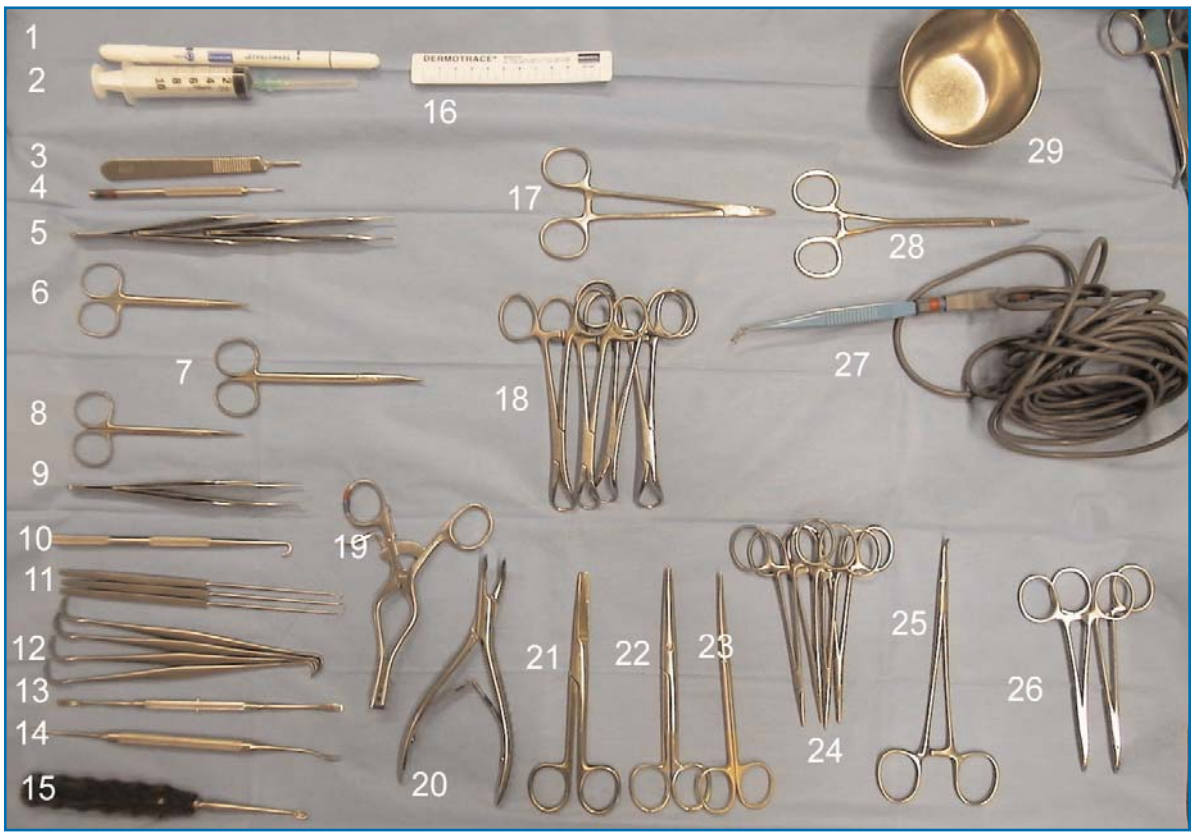

Fig. 3. Basic hand tray: *1* marking pen; *2* 10-cc syringe for irrigation; *3* scalpel with no. 15 blade; *4* beaver blade handle with no. 64 blade; *5* Adson dressing forceps, 12 cm plain; *6* Stevens tenotomy dissecting scissors, 11 cm; *7* Stevens tenotomy dissecting scissors 13 cm; *8* suture scissors, sharp/sharp, 12 cm; *9* two Adson tissue forceps, 12 cm, with teeth; *10* tendon hook, blunt; *11* two Gillies skin hooks, 17.5 cm; *12* Farabeuf skin retractors, double ended, small size; *13* Freer dissector, sharp/blunt, 15 cm; *14* Kleinert-Kutz periosteal elevator; *15* curette, soluble ended, small; *16* ruler; *17* Hegar needle holder; *18* towel clips; *19* self-retaining retractor; *20* bone/synovium rongeur, double action, 15 cm; *21* dressing scissors, sharp/blunt, 15 cm; *22* dressing scissors, sharp/blunt, 15 cm; *23* Metzenbaum scissors; *24* mosquito forceps, straight, 12.5 cm; *25* O'Shaugnessy forceps; *26* mosquito forceps, curved, 12.5 cm; *27* bipolar coagulation forceps; *28* needle holder with fine, smooth jaws; *29* small bowl for irrigating saline

F. Tendon tray (Fig. 4):
- Button with straight needles eyelet
- Link forceps
- Tunneler
- Tendon-pulling forceps
- Kocher clamp
- Tendon stripper
- Tendon hook, blunt, 10 and 12 cm.

G. Bone tray (Fig. 5):
- Bone spreader
- Metzenbaum scissors
- Bone curettes, double ended
- Bone hook, sharp
- Kleinert-Kutz periosteal elevator
- Freer dissector sharp/blunt, 15 cm
- Two small Hohmann retractors
- Set of small straight osteotomes, 2- to 15-mm diameter
- Mallet
- Bone compression/distraction device
- Goniometer
- Awl
- Bone rongeur, double action
- Liston bone cutters
- Bone-holding forceps
- Bone-reduction forceps
- Metallic wire, various sizes
- Wire cutters
- Wire pliers.

H. Microsurgical tray (Fig. 6):
- Fine-toothed forceps, Pierce design, 10 and 12 cm
- Fine, nontoothed forceps, Vicker's design
- Fine jewellers forceps, nontoothed
- Straight suture-cutting scissors, Vicker's design
- Curved dissecting scissors
- Needle holder, Vicker's design
- Needle holder
- Microvascular clamps, double and single
- Background material
- Ikuta approximator
- Clamp holder.

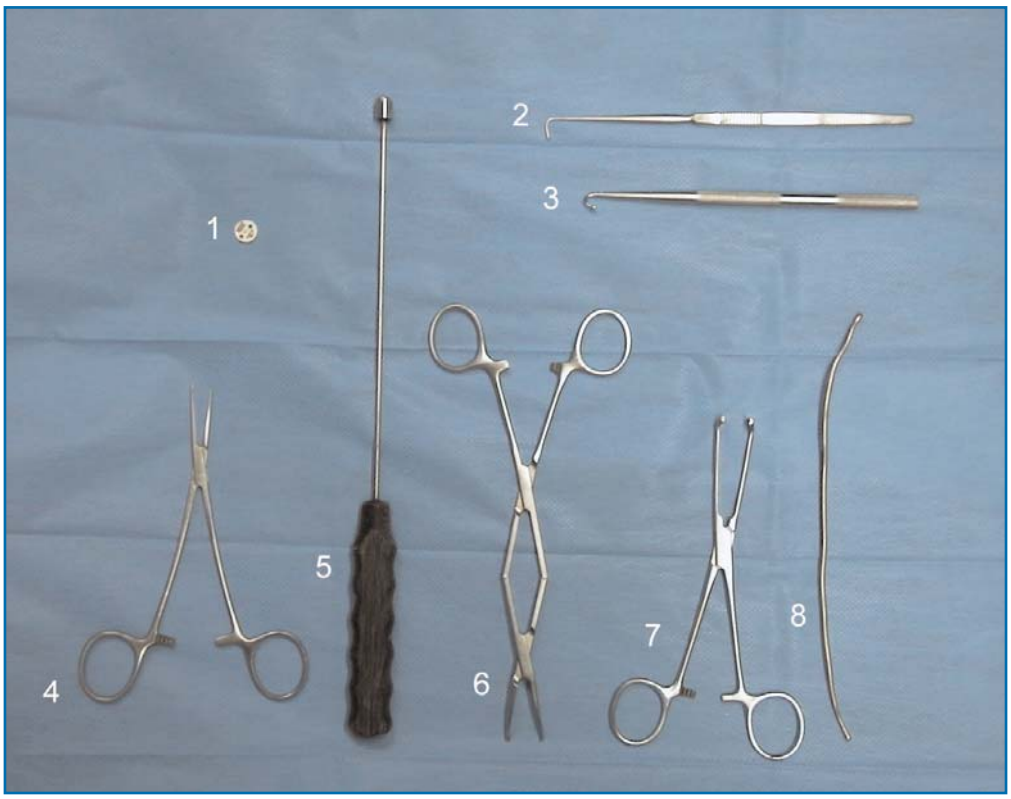

Fig. 4. Tendon tray: *1* button with straight needles, eyelet; *2* tendon hook, blunt, 12 cm; *3* tendon hook, blunt, 10 cm; *4* link forceps; *5* tendon stripper; *6* Kocher clamp; *7* tendon-pulling forceps; *8* tunneler

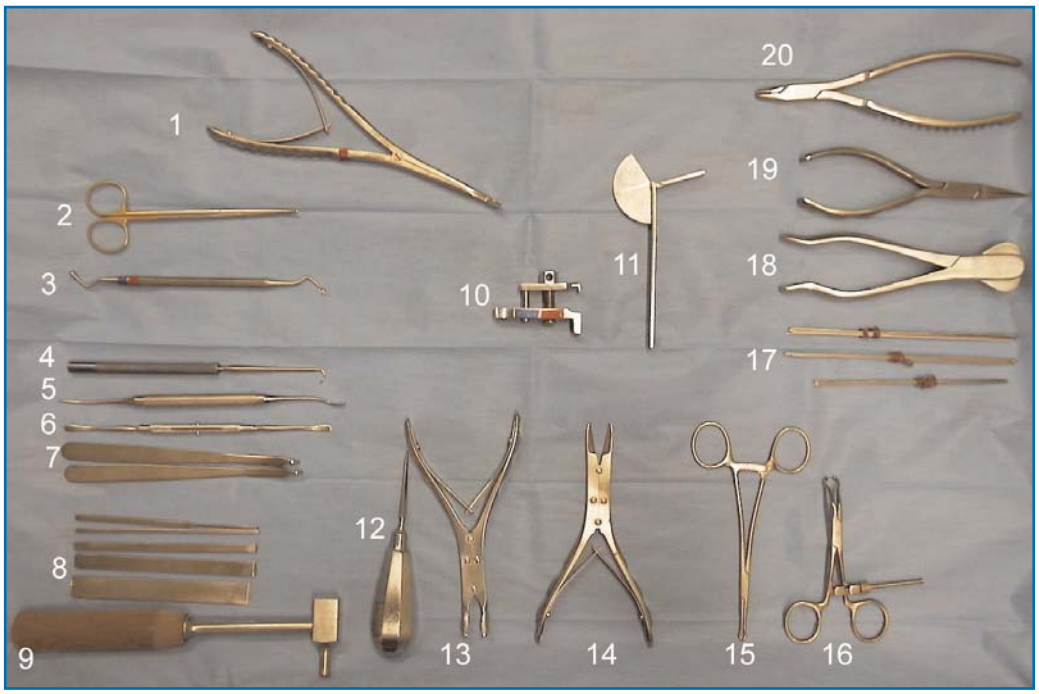

Fig. 5. Bone tray: *1* bone spreader; *2* Metzenbaum scissors; *3* bone curettes, double ended; *4* bone hook, sharp; *5* Kleinert-Kutz periosteal elevator; *6* Freer dissector, sharp/blunt, 15 cm; *7* two small Hohmann retractors; *8* set of small straight osteotomes, 2- to 15-mm diameter; *9* mallet; *10* bone compression/distraction device; *11* goniometer; *12* awl; *13* bone rongeur, double action; *14* Liston bone cutters; *15* bone-holding forceps; *16* bone-reduction forceps; *17* metallic wire, various size; *18* wire cutters; *19* wire pliers; *20* wire pliers

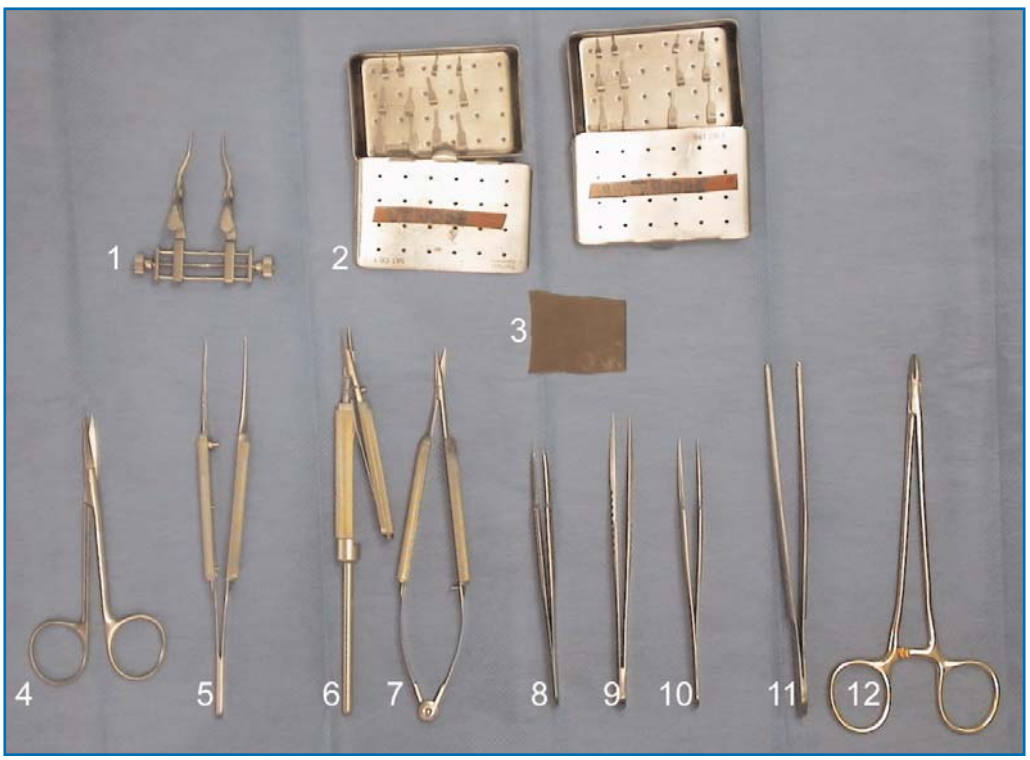

Fig. 6. Microsurgical tray: *1* Ikuta approximator; *2* microvascular clamps, double and single; *3* background material; *4* curved dissecting scissors; *5* fine, nontoothed forceps, Vicker's design; *6* needle holder, Vicker's design; *7* straight-suture cutting scissors, Vicker's design; *8* fine-toothed forceps, Pierce design, 10 cm; *9* fine-toothed forceps, Pierce design, 12 cm; *10* fine jewellers forceps, nontoothed, 10 cm; *11* clamp holder; *12* needle holder

I. Iliac crest bone graft tray (Fig. 7):
- Needle holder
- O'Shaugnessy forceps
- Self-retaining retractor
- Mayo scissors
- Scalpel with no. 15 blade
- Fine-toothed forceps
- Heavy-toothed forceps, Gillies
- Suture scissors
- Metzenbaum scissors
- Bristow periosteal elevator
- Set of large osteotomes
- Heavy mallet
- Large curette
- Farabeuf retractors, medium and large size.

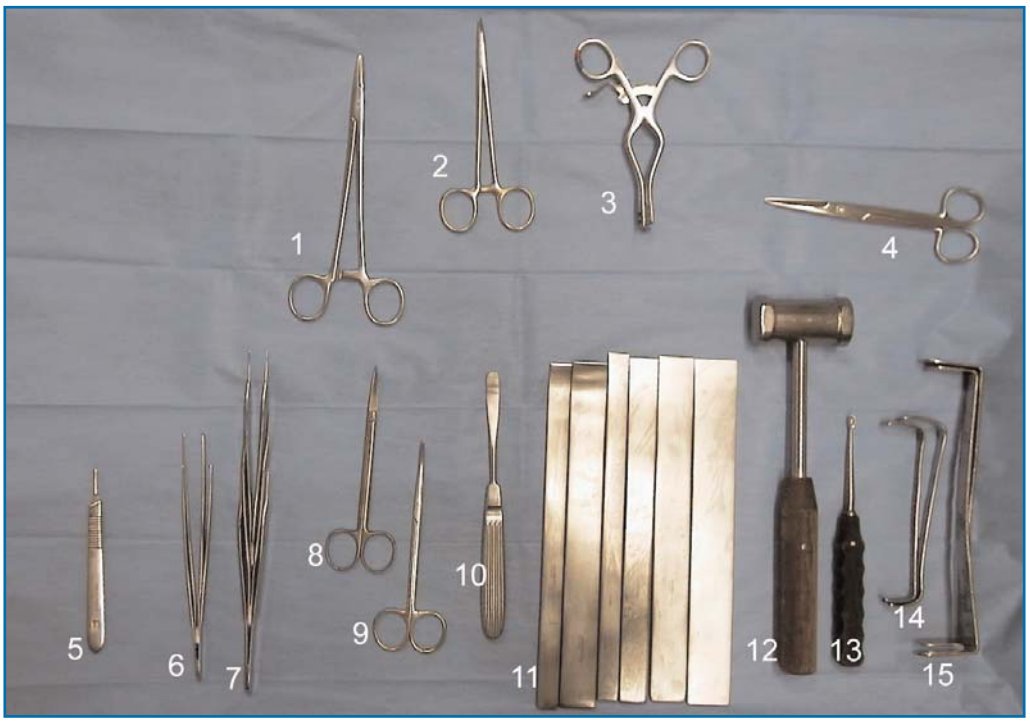

Fig. 7. Iliac crest bone graft tray: *1* needle holder; *2* O'Shaugnessy forceps; *3* self-retaining retractor; *4* Mayo scissors; *5* scalpel with no. 15 blade; *6* fine-toothed forceps; *7* heavy-toothed forceps, Gillies; *8* suture scissors; *9* Metzenbaum scissors; *10* Bristow periosteal elevator; *11* set of large osteotomes; *12* heavy mallet; *13* large curette; *14* Farabeuf retractors, medium size; *15* Farabeuf retractors, large size

Section 5-b

Anaesthetic Management

Giovanni Vitale, Ettore Martinez, Paolo Maisano, Lorenzo De Marchi, Maurizio Saini, Giacomo Bellani

Introduction

Reconstruction of skin, muscle and bone loss due to previous traumatic injuries or congenital defects by transferring free vascularized tissue (free flaps) has been made possible by recent and continuous improvements of microsurgical techniques and is the background on which hand transplant surgery stands. Surgical skill is a well-recognized factor of success in free flap surgery, but hypoperfusion and subsequent necrosis of transferred tissues are important problems [1] which challenge both surgeon and anaesthesiologist. Anaesthesia influences central haemodynamics and regional blood flow and therefore may affect blood flow in the flap [2] or in the transplanted limb. Changes in blood volume and use of vasoactive drugs during anaesthesia may influence free flap perfusion as well [3]. Therefore, anesthesiologists are confronted with several problems: procedure duration; maintenance of haemodynamic homeostasis and regional blood flow favourable to the free flap; intraoperative blood losses and fluid volume shifts; patient comfort and adequate intraoperative and postoperative pain control; postoperative prevention of thrombosis and risk of bleeding. This chapter reviews the pathophysiology of several problems faced by anaesthesiologists during hand transplant procedures and reports the management of 3 cases.

Factors Influencing Patency of Microvascular Anastomoses

Extraluminal Factors

Extraluminal factors can generate variations of diameter of the vascular lumen. Smooth muscles of the vascular wall respond to sympathetic stimuli; flap tissues are denervated and therefore maximally vasodilated. Still, vasal spasms due to surgical handling or to cold temperature may occur. Sympathetic control, however, is intact before the transferred tissue. Extravasal pressure due to oedema, ischaemia and inadequate venous drainage may exert an obstructive effect on flap small vessels. If vessel diameter is reduced due to increased sympathetic tone or compression, intraluminal pressure necessary to keep the vessel open needs to be high due to Laplace's law [4], and the driving pressure necessary to maintain flow through the vessel needs to be increased, due to Poiseuille's law (Fig. 1). These general principles suggest that maintaining maximal vasodilation of anastomosed vessels is critical in order to provide adequate flow to the transferred flap. Equally critical is maintaining driving pressure, or systemic blood pressure, without triggering compensatory mechanisms of vasoconstriction, and this can be achieved only by providing a normovolemic status.

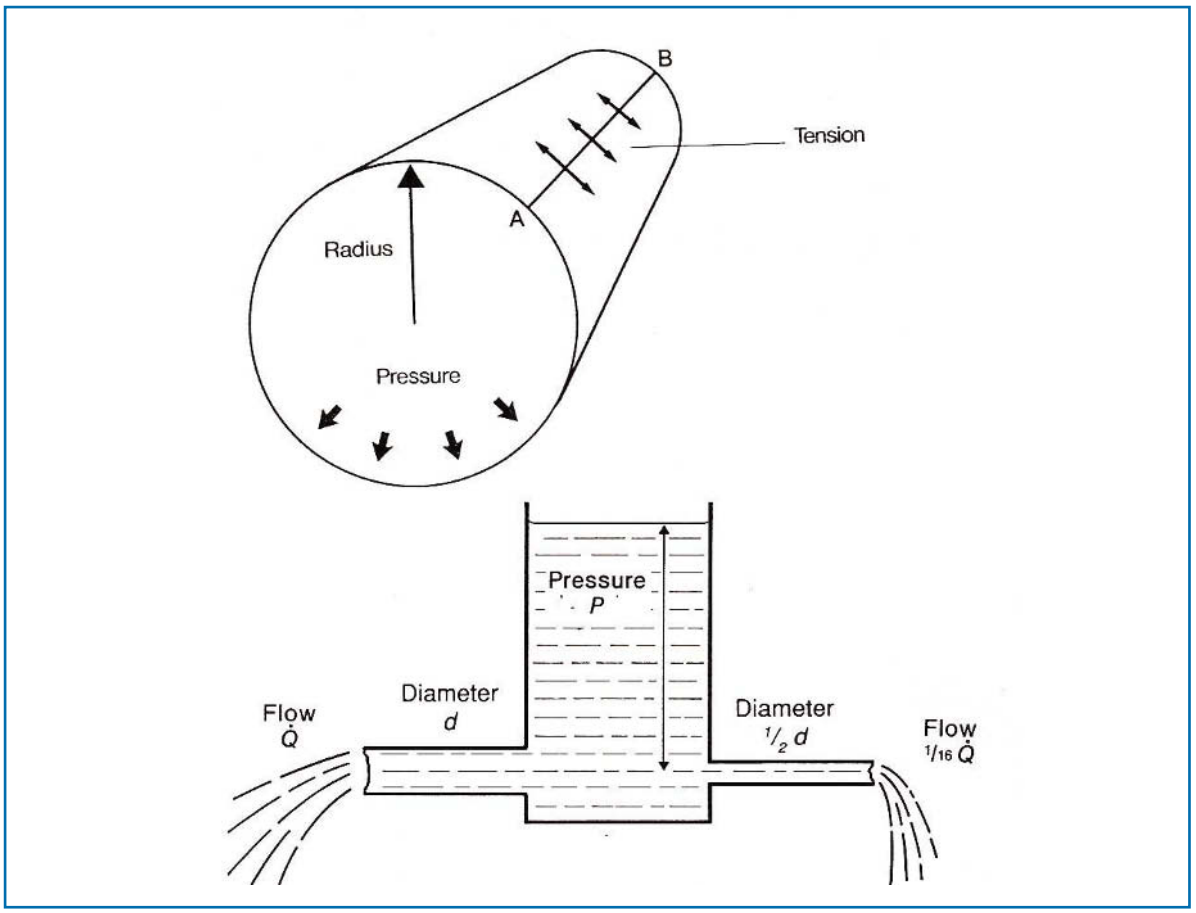

Fig. 1. *Upper panel*: Laplace's law describes the equilibrium of forces in a distensible vessel: pressure=tension/radius, where tension is related to sympathetic tone and/or extravasal pressure while radius represents vessel patency. *Lower panel:* Poiseuille's law: $Q = \frac{\pi \cdot r^4 \cdot \Delta p}{8 \cdot \eta \cdot L}$ Flow is directly proportional to driving pressure (Δp) and fourth power of radius (r) and inversely proportion- al to viscosity (η) and length (L)

Intraluminal Factors

Based on Poiseuille's law, blood viscosity greatly influences blood flow. Blood viscosity depends upon haematocrit and increased plasma levels of fibrinogen, albumin and macroglobulin. Among these factors, haematocrit can be artificially lowered by normovolemic haemodilution, and a lower viscosity can be achieved in order to provide maximal capillary flow. Microvascular thrombosis jeopardizes blood flow in the flap. Thrombi start with platelet adhesion to injured subendothelial structures. During platelet aggregation, arachidonic acid metabolites produced by platelets (thromboxane A2) mediate vasoconstriction while metabolites produced by the vascular wall (prostacyclins) mediate vasodilation and inhibition of platelet aggregates. Prevention of platelets adhesion and aggregation can be achieved by acting pharmacologically on the metabolism of arachidonic acid in order to provide adequate microvascular blood flow.

Anaesthesia Protocol

Candidates for hand transplantation were evaluated in preparation to anaesthesia. Blood count, plasma electrolytes, hepatic and renal function tests, haemocoagulation screening, electrocardiogram (ECG) and chest x-ray were obtained. Written informed consent was obtained regarding anaesthesia procedures and related risks and anonymous data processing. Three recipients received the following anaesthesia protocol:

1. Continuous regional block: either an infraclavicular or axillary catheter was positioned before starting the procedure according to the site of intervention. Catheters were placed through an electrostimulated needle [5–7]. Twitches corresponding to motor innervation of median, musculocutaneous, radial and ulnar nerves were individually detected applying a 1-mA electric stimulus to the needle tip, subsequently decreased to 0.5 mA to confirm proximity between needle and nerve. Identification of nerves in amputated patients is obviously not based on distal movement of the hand; we mainly relied on proximal movement at forearm level and on patient perception of movement at the level of their phantom hand. Anaesthetic dose was equally divided among the four nerves, and a catheter was left in place at the site corresponding to the medial or radial nerve. Ropivacaine 0.7% 30 ml was used as starting dose. Doses equal to 60% of initial bolus were repeated at 2.5-h intervals intraoperatively through the perinervous catheter.
2. Conscious sedation with midazolam (1 mg bolus doses on demand) was maintained until patients started feeling uncomfortable.
3. Patients underwent general anaesthesia only when uncomfortable. Propofol 1.5–2 mg/kg and fentanyl 1 μg/kg were used for induction. After tracheal intubation, anaesthesia was maintained using sevoflurane in O_2 and air; the end-tidal concentration was monitored and adjusted in order to keep patients normotensive and with a normal heart rate. Muscle relaxant (rocuronium) was used to adapt patients to mechanical ventilation. Volume-controlled mechanical ventilation was adjusted in order to maintain normocapnia.
4. Preoperative normovolemic haemodilution was performed in all adult patients, with a target haematocrit (Ht) value ranging from 32% to 35%.
5. Antibiotic prophylaxis was instituted preoperatively with cefazolin 2 g i.v. repeated every 6 h until completion of surgery. Antibiotic therapy was started thereafter with cefazolin 1 g i.v. every 8 h for 8 days.
6. Haemodynamic management: a Foley catheter, a central venous line and an arterial catheter were placed, and central venous pressure, invasive blood pressure and diuresis were continuously monitored. A normo-hypervolaemic status was obtained by crystalloid infusion, with a target central venous pressure (CVP) ranging from 6 to 8 mmHg (i.e., approximately 2–3 mmHg above normal values), along with normal urine output (≥1.0 ml/kg per hour). A target systemic blood pressure of >100 mmHg was obtained by modifying the depth of anaesthesia or by crystalloid infusion.
7. Prevention of microvascular thrombosis: dextran-40 (40,000 daltons molecular weight dextran 10% in normal saline) 250 ml i.v. was administered before arterial microvascular anastomoses were unclamped. Dextran-40 infusion was then continued at 20 ml/h for 7 postoperative days. Acetylsalicylate 300 mg was administered i.v. at the end of surgery, and it was subsequently continued p.o. for 15 postoperative days.
8. Inotropes: according to the surgeon's visual evaluation of the efficiency of arterial microvascular anastomoses, dopamine infusion was started at a dose range targeted for the inotropic but not vasoconstrictive effect of this drug (i.e. at 4–5 μg/kg per minute, which corresponds to dopamine β-stimulating effect). The goal of inotropic treatment was to increase driving pressure through microvessels. Inotropic infusion was maintained until end of surgery or until evidence of patient's failure to respond (no effect on blood pressure at the above dose range, onset of tachycardia).
9. Body temperature control was achieved by wrapping patients in warm-air-heated blankets and warming infusion lines. Internal body temperature was continuously monitored. In case of postoperative shivering, administration of meperidine 20–50 mg i.v. was planned.
10. Postoperative analgesic plan was based on continuous regional blocks: ropivacaine 0.2% was infused through axillary or peridural catheters at a rate of 6–10 ml/h for 7 days

after surgery. The first rescue analgesic was ketoprofen 100 mg i.v. slow bolus, repeatable every 8 h. In case of ketoprofen failure, as subjectively evaluated by the patient, subcutaneous morphine 10 mg was administered.

Results

Overall, this group of patients was healthy, and perioperative risks were positively evaluated in a risk/benefit preoperative interview with patients and/or relatives. Surgical results were good in all cases, and transplanted hands remained vital and functional all throughout hospital stay and at subsequent follow-up. Mean duration of surgical procedures was 12.5 h (range, 10–14).

Regional blocks: a brachial plexus axillary catheter was placed in 2 patients and an infraclavicular catheter was used in 1 patient for continuous regional anaesthesia and postoperative analgesia. *General anaesthesia*: surgery began under regional anaesthesia and conscious sedation. General anaesthesia was started after an average of 3.6 (1–5) h when patients started feeling uncomfortable on the operating table. Pain from the site of surgery was never reported before induction. During general anaesthesia, average opioid analgesic consumption was very low (remifentanil 0.05 μg/kg per minute for the whole duration). End-tidal concentration of sevoflurane necessary to ensure adequate depth of anaesthesia was also low (mean value 1.2±0.05%). Both observations were interpreted as signs of optimal pain control via continuous regional blocks. Tourniquet pain may occur during various phases of surgery. Even under general anaesthesia, it may be manifested as hypertension resistant to treatment [8]. In this series of patients, tourniquet pain was never detected.

Preoperative normovolemic haemodilution with 1:1 colloid reinfusion to target Ht was 780 (450–1,000) ml. Self-donated whole blood was reinfused at the end of the procedure. Estimated blood loss was 1,350 (850–1,700) ml. Normovolemia, as defined above, required a mean crystalloid infusion of 10.6 (10–12) ml/kg per hour. Dopamine infusion was started after microanastomoses declamping in 3 patients. Systolic blood pressure increased from 110±10 mmHg to 140±15 mmHg without tachycardia at a 4.6 (4–6) μg/kg per minute dose. The effect on flap perfusion was considered adequate by the surgeon, and infusion was maintained until closure. No clinically relevant side-effects were observed.

No thrombosis was detected intraoperatively, nor was clinical evidence of altered regional circulation observed postoperatively. No signs of fluid overload were observed, nor did anaphylactic reactions occur with the use of dextran. Perioperative urine output was adequate in all patients, and no clinically relevant signs of renal hypoperfusion due to the use of acetylsalicylate were observed.

Recovery from general anaesthesia and resumption of spontaneous breathing was uneventful in all cases. Mean time from end of surgery to a condition of eupneic normosaturated spontaneous ventilation, responsiveness and ability to cough and sustain lifted head was 6.5±3 min. No shivering occurred. Patients were pain free, and they did not report anxiety.

In the postoperative period, patients required 1 dose of ketoprofen per day from day 1 to day 5. Two patients required 1 dose of morphine at day 1 and one patient did not require opioid analgesia. No systemic or local complications were observed.

Discussion

Although success or failure of microsurgery depends mainly on surgeon skill, we worked on the hypothesis that anaesthesia may play a role. Therefore, we chose, among the many and constantly improving anaesthesiological techniques, methods based on the nature of the surgical object, i.e. anastomosed microvessels susceptible to closure due to intraluminal (thrombosis) and extraluminal (compression, opening pressure, sympathetic tone) factors. It should be also noted that hand transplant is an elective procedure, and, although it may substantially improve patient health status and quality of life, it is not

life saving. Therefore, every effort should be made to minimize perioperative risks and increase patient safety. Careful preoperative evaluation is necessary to assess whether the chosen methods may be applied to individual cases.

All our patients received intraoperative and postoperative continuous sympathetic block through locoregional techniques. Recent human studies demonstrated that both upper- and lower-limb regional blocks increase arterial distensibility with subsequent increases in blood flow [9, 10]. Although all tissues in the transplanted hand are completely denervated, with sympathectomy of all vessels, the feeding artery and the draining vein on which the flap vessels are anastomosed have intact innervation [3]. Arterial vasodilation [4] and/or increased distensibility [10] seem to be desirable conditions because they may improve blood flow through flap tissues. Dilation of the draining vein is essential also because there is no lymphatic drainage from the free flap, which makes it particularly exposed to engorgement and extravasation of fluids [l]. Regional vasodilation may exert beneficial effects on blood flow only if it is not associated with a decrease in total systemic vascular resistance, as demonstrated in an animal model [3] in which vasoactive drug infusion caused a severe reduction in free flap blood flow only when associated to a decrease in systemic vascular resistance and arterial pressure despite maintenance of cardiac output. In our patients, arterial pressure stability and, presumably, systemic vascular resistance, were not altered by brachial plexus blocks. In microsurgery, local anaesthetics may decrease vascular resistance more in normally innervated tissue than in the denervated free flap, causing a "steal effect", which results in reduced flap flow. Recent studies showed, however, that this effect is relevant only when sympathetic block is instituted after the free flap is transferred, i.e. postoperatively [12]. In all our patients, regional blocks were started before the beginning of surgery and continued as maintenance continuous infusion after transplant was completed.

Regional blocks extended to the postoperative period also provided excellent analgesia to our patients. Both pain and anxiety induce increase in sympathetic tone in the postoperative period. Severe acute pain results in sympathetic overactivity, which increases in heart rate and peripheral resistance [13]. It has been demonstrated that in the peripheral circulation, acute pain is associated with decreased limb blood flow, and this can be particularly deleterious in patients undergoing microsurgery. Pain relief with epidural blockade resulted in a reversal of reduction in blood flow associated with surgical trauma and acute pain [14] and in improved outcome [15]. Severe postoperative pain and high levels of sympathetic activity may be associated with reduced arterial inflow and decreased venous emptying [16]. In association with patient immobility, this may lead to venous thrombosis [17]. Pain prevention and treatment, therefore, may have a substantial role in maintaining adequate blood flow to the transferred tissues.

Average duration of surgical procedures was 12.5 h. Prolonged exposure to anaesthetic agents may induce potential problems. Inhaled agents, such as halothane and enflurane, have a significant negative inotropic effect. Sevoflurane has minimal effects on peripheral vasodilation and cardiac output, and therefore, it was chosen for maintenance in our patients [18, 19]. Nevertheless, anaesthetic gases decrease systemic vascular resistance and reflex tachycardia may result. A study conducted in an animal model showed that isoflurane provides stable haemodynamic conditions in flap surgery as long as hypovolemia is avoided [2]. We did not add nitrous oxide because it can lead to decreased cardiac function in patients with severe cardiac diseases. In healthy patients, it increases sympathetic nervous system tone and causes an increase in systemic vascular resistance [20]. In addition, a megaloblastic reaction [21], which occurs after a >24-h exposure to nitrous oxide, seems to be more precocious in trauma patients. A potential risk of prolonged exposure to inhalational agents is the creation of an anaesthetic reservoir due to the fact that various tissues with different vasculature equilibrate with the alveolar and arterial anaesthetic agent at varying rates. After a prolonged anaes-

thetic, saturated fat and muscle can release subanaesthetic doses of the agent for a sustained period after the anaesthetic has dissipated, and a slow emergence may be expected.

For all the above reasons, we chose a combined regional-general anaesthesia technique, which allowed a decrease in the total time of exposure to inhalational agents. Moreover, during the general anaesthesia phase, this combined technique made it possible to maintain a low alveolar concentration of sevoflurane, as shown by the low-end tidal concentrations, which were able to prevent haemodynamic responses to surgical stimuli. Maintaining a low concentration of inhalational agent contributed, together with maintaining a normovolemic or euvolemic status, to minimize cardiovascular effects of inhalational agents.

The 0.6 Inspired Faction of O_2 (FiO_2) was chosen because it has been shown that the composition of inspiratory gases influences the occurrence of atelectasis: reoccurrence of collapse of previously reexpanded atelectatic lung tissue during general anaesthesia in patients with healthy lungs is faster when patients inhale 100% oxygen [22]. Atelectasis is an important cause of impaired gas exchange during general anaesthesia and in the postoperative period, and maintaining adequate oxygenation to the transferred tissue is crucial since terminal arterioles constrict in response to metabolic factors such as hypoxia and acidosis [23].

Altogether, this combined technique allowed a quick recovery. No episodes of hypoxia or dyspnoea complicated the postoperative period. Patients were pain free and did not report anxiety. No rescue analgesics were needed during ICU stay. Predeposited blood units were reinfused in all cases during this period.

To prevent thrombosis, 40,000 daltons of dextran was used [24]. Apparently, its action enhances platelet surface negative electrical charges, thus decreasing their adhesion potential. Dextran also exerts an osmotic effect: by increasing intravascular water, expanding plasma volume and contributing to haemodilution, it may improve blood flow [4]. Low-dose acetylsalicylate, on the other hand, inhibits platelet cyclooxygenase, blocking thromboxane-mediated vasoconstriction. Combined use of the two agents efficiently prevented clinically relevant thrombosis in our patients without exposing them to haemorrhagic risks. No side-effects of dextran, anaphylaxis [25] or fluid overload occurred in our patients.

Intraoperative use of dopamine was aimed at acutely increasing perfusion pressure through the anastomosed vessels in two patients. A maximally dilated vessel requires a lower perfusion pressure in order to maintain adequate blood flow. If the flow is not ideal despite vasodilation due to sympathetic block, as occurred in these two patients according to the surgeon's evaluation, it is possible to act on the driving pressure, which depends on systemic arterial pressure. Dopamine acts on α-, β-, and dopaminergic receptors. With an infusion rate of 3–10 μg/kg per minute, β_1-receptor stimulation is seen with resultant increases in cardiac contractility and output. Rates above 5 μg/kg per minute stimulate release of endogenous norepinephrine, which contributes to cardiac stimulation but also has a mixed α_1- and α_2-agonist effect. Terminal arterioles respond to α_2-receptor agonists, and it has been shown that norepinephrine significantly reduces blood flow through microcirculation [26]. At dopamine infusion rates larger than 10 μg/kg per minute, the α-vasoconstrictive effect predominantes. For all the above reasons we did not exceed a dopamine infusion rate of 5 μg/kg per minute. In both our patients, arterial pressure increased with minimal tachycardia. Improvement of blood flow was noted by the surgeon, but further evaluation based on objective measurements should be required before routine use of dopamine in this setting. It should also be noted that dopamine was used in normovolemic conditions. During hypovolemia, the effects might be different. Intraoperative temperature control and antibiotic prophylaxis are part of perioperative management of any kind of surgical procedures; during microsurgery, prevention of hypothermia, acidosis and local infection-induced vasospasm assumes a particularly relevant role. In fact, cold produces vasoconstriction, increased Ht, increased aggregation of red cells and increased blood viscosity; this vasoconstrictive effect occurs even in denervated tissue [4].

In conclusion, we used an anaesthesia protocol

derived from elective microsurgical procedures based on combined regional/general anaesthesia. We used continuous infraclavicular or axillary blocks to prevent and treat intraoperative and postoperative pain, to maximally dilate the feeding artery and the draining vein of the transferred flap and also to decrease exposure to inhaled anaesthetic agents. Haemodynamic management was based on maintaining normovolemia. Inotropes (dopamine) were occasionally used in order to increase the driving pressure through already dilated microvessels. Dextran and acute normovolemic haemodilution were used to decrease blood viscosity. Dextran-40 was used mainly to prevent thrombosis in association with low-dose acetylsalicylate. Anaesthesiological management of hand transplant surgery required choices targeted to the physiological variables that regulate microcirculatory flow: vessel diameter, driving pressure, blood viscosity and blood volume.

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Section 5-c

Harvesting the Hand

Marco Lanzetta, Roberta Nolli, Lorenzo Bettella

Introduction

Harvesting a hand from a brain-dead donor means first of all that for some time, a team of surgeons have been on call waiting for the right donor. When finally there is a possible donor, especially if he or she is located far away from the hand transplantation unit, time is extremely important. No delays in preparing the necessary instruments and materials are acceptable. In our unit, we have a ready backpack containing what we need to face any possible scenario once we reach the intensive care unit where the donor is located. By having its own materials and instruments, the team is self-sufficient, and no request for materials or instruments are necessary once at the donor's place. Due to the characteristics of our country and the heavy traffic that can be expected on the major highways, we have a helicopter at our disposal.

Materials

Materials used for harvesting a hand are:

Five litres of Belzer [University of Wisconsin (UW)] solution for irrigation of the hand. They are kept in the fridge until the very last moment. Once on the ambulance or helicopter, they are stored in the portable fridge, which is the same one used for transporting the harvested limb back to base.

Contents of the backpack ing are:

- The basic hand set (marking pen; scalpel with no. 15 blade; Beaver blade handle with no. 64 blade; two Adson tissue forceps, 12 cm with 1–2 teeth; Adson dressing forceps, 12 cm plain; two Gillies skin hooks 17.5 cm; two Kilner skin retractors, double ended; sutures scissors, sharp/sharp, 12 cm; dressing scissors, sharp/blunt 15 cm; Stevens tenotomy dissecting scissors, 13 cm; five towel clips; five mosquito forceps, curved 12.5 cm; curette; soluble-ended small needle-holder with fine smooth jaws; Kleinert-Kutz periosteal elevator; bone/synovium rongeur single-action 15 cm; self-retaining retractor; blunt tendon hook; metal ruler; bipolar small coagulation forceps; small bowl for irrigating saline; 10-ml syringe for irrigation).
- One extra set of medium-size Kilner skin retractors and one extra set of large-size Kilner skin retractors, both double ended
- Two Gigli bone saws
- Two large Kocher clamps
- One scalpel with no. 21 blade
- One hand saw
- One extra medium-size periosteal elevator
- One Hegar needle holder
- One Esmarch elastic bandage to exsanguinate the arm
- A well-padded pneumatic tourniquet with 8 cm width cuff
- A camera for intraoperative photos
- The aesthetic prosthesis to restore body integrity.

Surgical procedure

Once at the intensive care unit, a detailed analysis of the donor's hand is made by the hand team. Size, skin texture and colour are compared with those of the recipient recorded on his or her chart. An X-ray of the hand is assessed to exclude previous injuries, arthritis or other skeletal conditions. If the right hand is to be harvested, it is expected that the donor has an arterial line in the left radial artery, as agreed by a protocol circulated among all participating hospitals.

A preliminary agreement between the hand team and the other teams converging on the site of the organ/tissue harvesting needs to be worked out in advance. In our case, it has been agreed that we would start harvesting the hand before the other teams start harvesting internal organs (i.e. kidneys, liver, heart, etc.).

We start by prepping the limb in the standard method used for a normal hand operation (Fig. 1). The limb is exsanguinated and an incision placed at the elbow level to identify the brachial artery and major veins. They are ligated, and the median, radial and ulnar nerves are identified and tagged. The muscles are dissected and cut with a unipolar coagulator and finally, the radius and ulna bones are cut with a hand or a Gigli saw. One team member closes tissues and skin to obtain a stump suitable to accept the prosthesis. The stump is bandaged firmly to avoid unnecessary bleeding and the tourniquet released. The cosmetic prosthesis is then fitted, and the field is left to the abdominal and thoracic surgeons (Fig. 2). During hand harvesting, drugs or anticoagulants are not used, neither systemically or locally, to avoid altering the body's conditions. The entire procedure should take around 15–20 min.

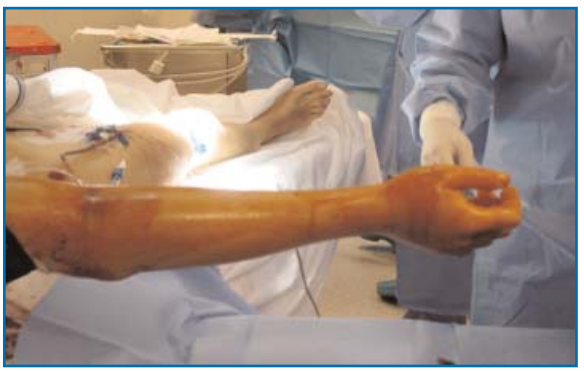

Fig. 1. The donor's limb is prepped and prepared for the harvesting procedure

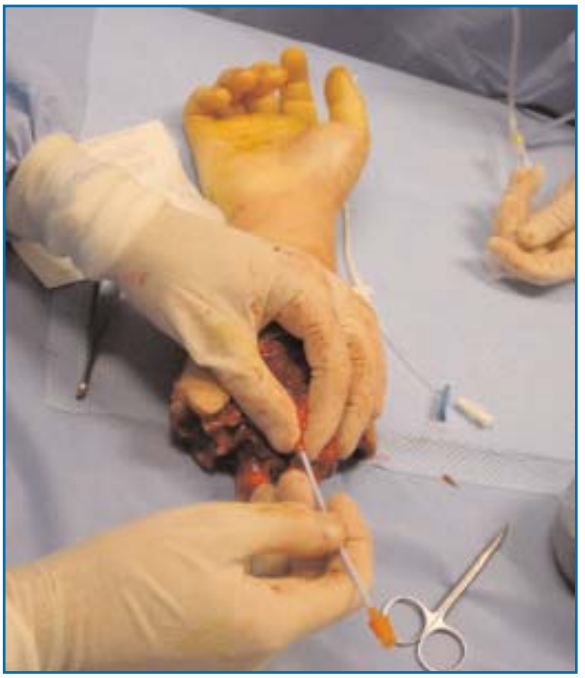

Fig. 2. Canulation of the brachial artery for perfusion with belzer (UW) solution

On a side table, the other member of the team cannulates the brachial artery so that the limb can be irrigated with Belzer (UW) solution at 4°C by placing the solution bags on a stand at a height of 2 m without additional pressure (Figs. 3, 4). Normally, 2–3 l of solution are needed to drain the blood from the limb or until a clear liquid is seen coming from the veins. The limb is wrapped in two sterile towels and placed in three different sterile bags and then in the portable fridge, avoiding direct contact with the ice. A member of the team is left behind so that a portion of the donor's spleen and thymus can be harvested for later analysis and use. Intraoperative pictures are taken throughout the entire procedure.

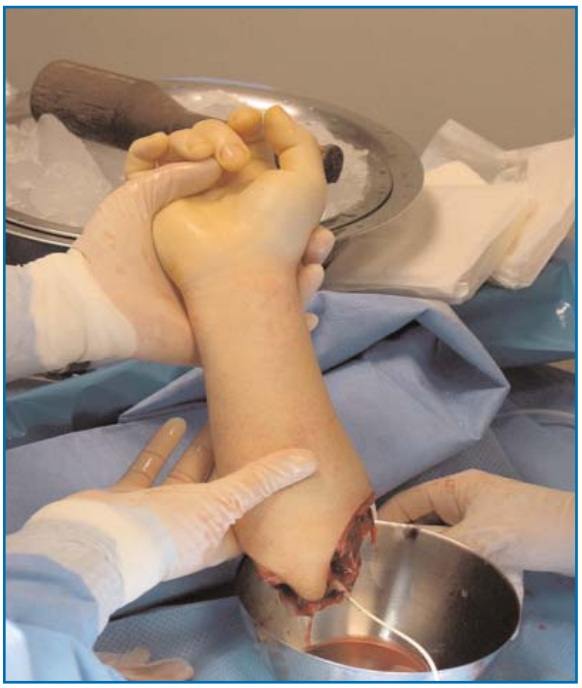

Fig. 3. Perfusion of the limb. Note venous drainage at the transected site

Fig. 4. Aesthetic prosthesis fitted at the end of the procedure

Section 5-d

Preparing the Recipient

Aram Gazarian

"Measure seven times, cut once".
Armenian proverb

Introduction

Hand transplantation (HT) has to be considered an experimental surgery in process of evaluation. It does not really represent any significant advance in surgical technique and has been possible thanks to the techniques developed in replantation microsurgery over the last 40 years combined with advances in the knowledge of transplantation immunology [1–4]. Although technically similar to replantation, worldwide experience accumulated since the first successful HT in 1998 [5] has revealed certain peculiarities important to consider while planning and performing this surgical technique, where any quantity and quality requirement of different anatomical structures can be harvested from the donor [1]. This fact has some specific impact on the preparation stage in the recipient.

The goal of recipient preparation is to create an *ideal ground for planting the donor limb* according to precise reconstruction planning. In fact, at the time of surgery, the course of the operative procedure has already been written during numerous meetings between the donor and recipient surgeons' teams and anatomical laboratory repetitions. Though it is during the HT that the recipient tissues are prepared, sometimes it is necessary to perform some preparative surgery beforehand. Therefore, this chapter is divided into two subchapters: preparing the recipient before hand transplantation and preparing the recipient during hand transplantation.

Preparing the Recipient Before Hand Transplantation

Any recipient tissue may theoretically be replaced with corresponding donor tissue at the time of HT. However, infection eradication and skin and vessel availability has to be ensured prior to HT. Adequate preparation may convert a nonoperable patient to an operable one.

Infection

In the context of immunosuppression, infection may be a disaster for the transplanted graft as well as for the patient. This is why there must be screening for latent infection, particularly in bones. The patient often sustains open trauma, multiple surgery, external fixator pin-track infection, etc. Only a detailed history in combination with clinical and instrumental investigations can evaluate the diagnosis. Blood tests, X-ray, bone scintigraphy and bone biopsy may be required. Any infection must be treated before HT using both surgical (bone resection, osteoplasty) and drug (systemic antibiotic therapy) treatment methods.

Transplant Amputation

For various reasons, the allograft may have to be amputated. A surgeon has to ensure conditions for possible prosthesis, with an adequate arm

length (≥7 cm, preferably at the junction between forearm middle and distal thirds) [6]. Adequate recipient skin has to be available to avoid excessive shortening at the time of possible graft amputation. Preliminary skin expansion or flap transfer may be necessary if the skin does not fit with mentioned requirements.

Vascular Planning

In particular, it is important to locate adequate veins, which must be connected, and decide by what method to connect them. Correct venous evaluation necessitates accurate Doppler or even phlebography procedures. In the case of poor cutaneous coverage and superficial venous network (Fig. 1), vascular surgical solutions should be found prior to HT, such as skin expansion for preparing adequate vein channelling.

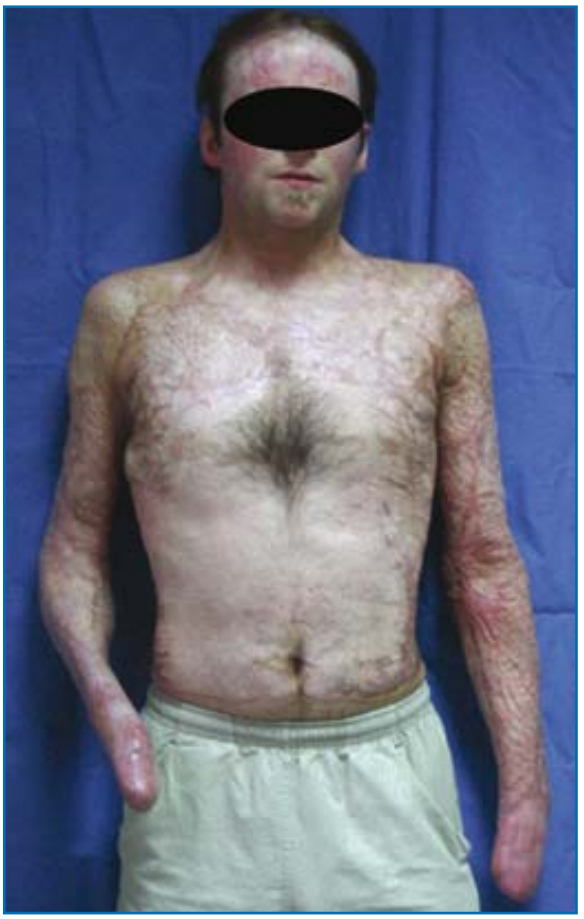

Fig. 1. Potential recipient. Poor cutaneous coverage and superficial venous network because of extensive burn injury

Preparing the Recipient During Hand Transplantation

We base our technical description on a clinically documented example. Our example concerns the left side of the last Lyon patient who underwent bilateral HT (30 April 2003; Pavillon M, Hôpital E. Herriot, Prof. G. Herzberg Department, under the direction of Prof. J.-M. Dubernard). This 22-year-old man sustained a bilateral crush-cut amputation on September 2000. The left hand was amputated through the radiocarpal joint. We planned to transplant through the forearm distal quarter in order to obtain wrist motion and because this level seems to be the most favourable for gaining optimal functional outcome, as we have learned from replantation experience [7–11].

Two surgical teams simultaneously prepare the recipient stumps while two others prepare the donor limbs. The patient is in decubitus dorsalis under general anaesthesia. The recipient's leg may be prepared also (in the event of autologous tissue grafts being required) (Fig. 2). Firstly, sterile pneumatic tourniquets are applied to the arms without exsanguination so the venous network becomes more visible. After mapping subcutaneous veins, the tourniquet is reapplied after gentle exsanguination of the limb. Chronologically, operators progress with tissue preparation in the following order:

1. Preparing soft tissue:
   a. Skin incision
   b. Palmar aspect (subcutaneous veins, ulnar and radial arteries, median and ulnar nerves, wrist and finger flexor tendons)
   c. Dorsal aspect (subcutaneous veins, sensory branch of the radial nerve, wrist and finger extensor tendons)
2. Preparing the bones (ulna and radius)

Preparing Soft Tissue

Skin

Skin incisions must be made as distal as possible to provide stump coverage with recipient skin only (without bone shortening), as a future

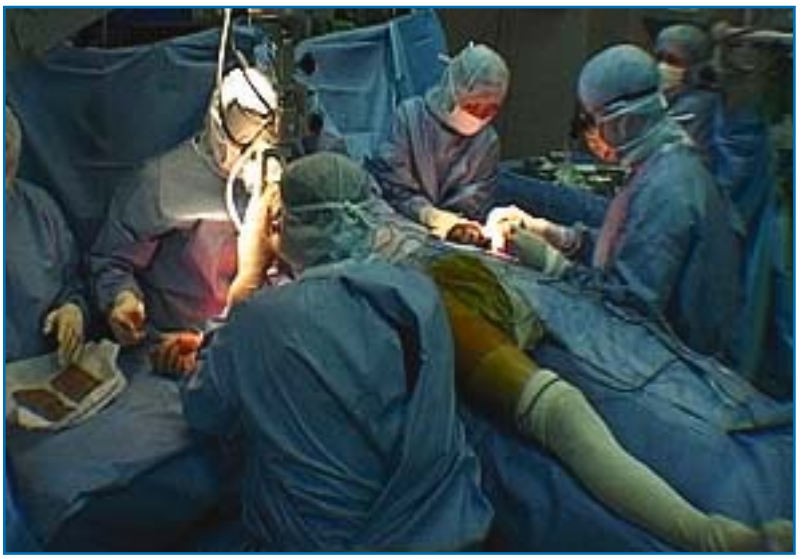

Fig. 2. Recipient preparation during hand transplantation

amputation may be necessary due to transplant failure or rejection. The dorsal skin is incised along the perimeter of the stump from the lateral side of the radius to the medial side of the ulna. The palmar skin is incised in the form of a "V", with its tip located proximally and with an angle between branches of 90°. Distally, these branches are connected in a curve shape with dorsal incision, resulting in an incision in the form of "collar V". The resultant drop-shaped skin portion containing the scar of the stump is left in place. This approach is enlarged on the palmar aspect through a linear extension of the proximal tip of the V-shaped incision, resulting in a Y-shaped incision and raising the radial and ulnar fasciocutaneous flaps vascularised by their arteries and dorsal continuity (Fig. 3). The triangular space obtained on the anterior-median aspect of the forearm (space between 2 branches of V-Y incision) will serve as the host bed for the donor limb palmar skin flap (Fig. 4). Figure 5 demonstrates the view of skin repair after the HT.

Hereafter, we present the sequence of recipient preparation not chronologically but tissue-by-tissue to avoid unnecessary repetition concerning management of tendons, veins and nerves, which are present both dorsally and palmarly. The surgeon works on posterior and anterior aspects while the assistant maintains the forearm in pronation and supination, correspondingly, with the aid of a Backhaus bone forceps applied to the radius stump. Dissection and

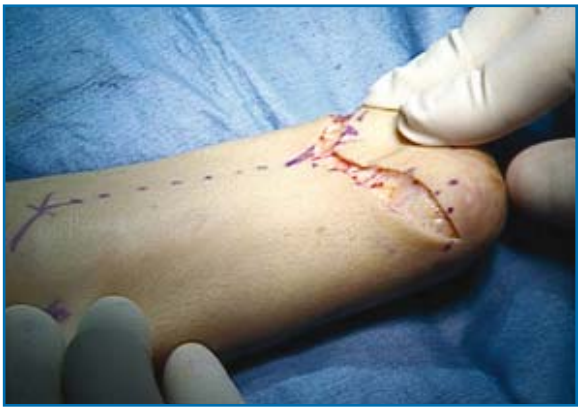

Fig. 3. Preparing the skin. Y-shaped incision

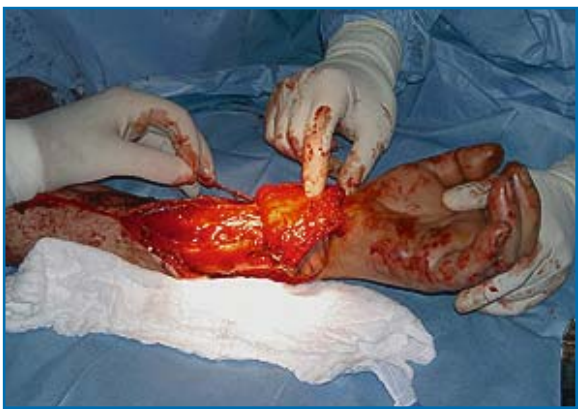

Fig. 4. Donor palmar skin flap inserted into the Y-shaped incision

essential minimal *débridement* must be performed up to the level of macroscopically healthy tissues.

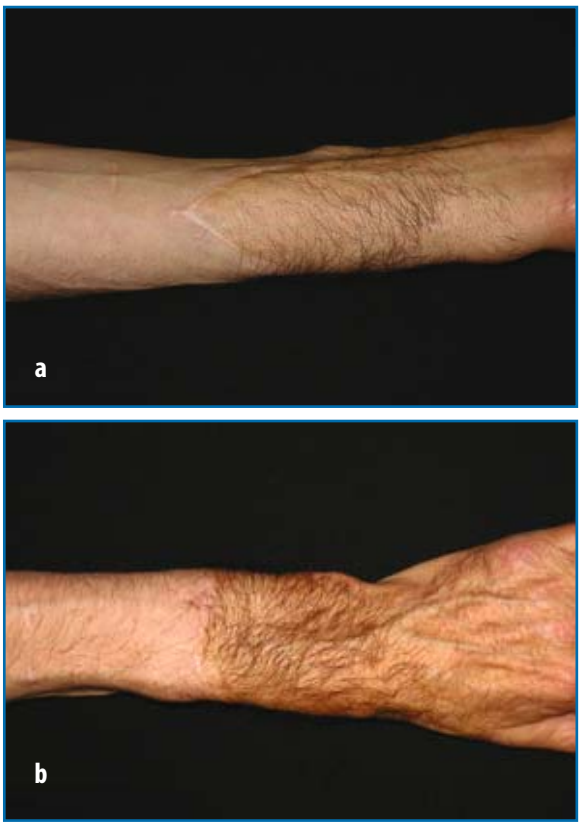

Fig. 5 a, b. The appearance of the left limb of the bilateral transplanted after hand transplantation (33 months follow-up). **a** Palmar view. **b** Dorsal view

Veins

Three to four superficial veins (marked previously) of sufficient diameter (up to 3 mm) are dissected, isolated and clipped during mobilisation of stump skin. Where there is an absence of such veins at proximity to the stump, they may be identified (preliminary marking is necessary) and isolated proximally up to the elbow or even above. The "donor limb team" is notified about the necessity of harvesting long veins, which will be conducted up to the recipient ones through subcutaneous channels (Fig. 6). We do not recommend relying on forearm artery satellite veins as major outflow pathways. Nevertheless, they may be used as a complementary option.

Arteries

The surgeon exposes ulnar and radial arteries and cuts them at a macroscopically healthy level, respecting the satellite veins (Fig. 7). The use of microinstrumentation is needed only for anastomosis.

Fig. 6. Skin forage for donor vein passage. A donor vein has already been channelled. Péan's forceps is inserted in another subcutaneous channel

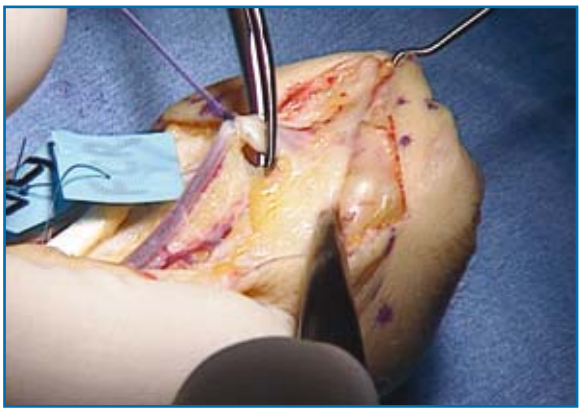

Fig. 7. Preparing the radial artery respecting the satellite veins

Nerves

Median and ulnar nerve stumps are freshened using Victor Meyer's guillotine up to the level of microscopically normal fascicles (Fig. 8). The sensory branch of the radial nerve is identified dorsally.

Tendons

We recommend tagging each tendon as one goes along the dissection during donor limb preparation in order to facilitate recognition at the time of tendon repair. The tendons are tagged using pieces of sterile plastic and surgical ink marker (a method we have found to be the best so far although time consuming) (Fig. 9).

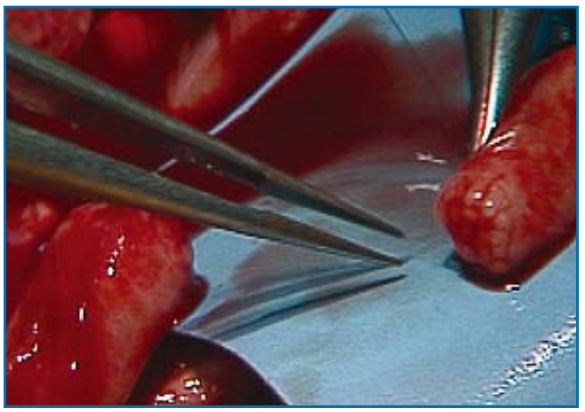

Fig. 8. Median nerve stump after refreshing by Meyer's guillotine

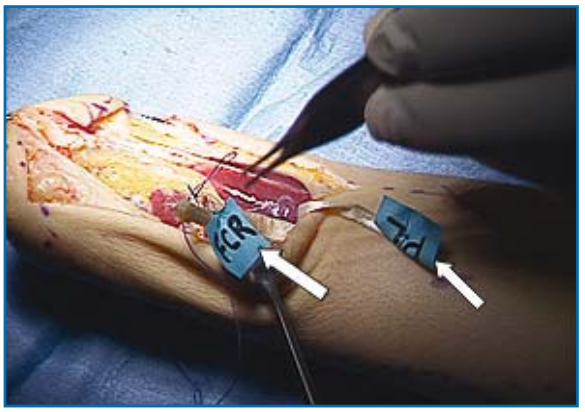

Fig. 9. Tagging the tendons. Arrows point to the plastic tags: *FCR*, flexor carpi radialis; *PL*, palmaris longus

1. Flexor tendons**:** The use of Victor Meyer's guillotine is also advised for refreshing tendon ends in case of end-to-end suture. However, one can omit this procedure if the more desirable Pulvertaft weave is to be used for tendon repair because it increases tendon juncture strength [12]. Shortening the neighbouring tendons in different amounts allows "multilevel" tenorrhaphies, which decrease the bulk, improve differential gliding and facilitate an early active-motion rehabilitation program.

2. Extensor tendons: The extensor tendons with circular cross-section should be managed as flexor tendons. The flat ones may be prepared for subsequent overlapped tenorrhaphy.

Preparing the Bones

The optimal level for HT is the one that allows for both the best-expected results (*see previous pages*) and the security of an adequate stump length for the prosthesis in case of failure. In this particular case, the necessity of at least three screws in the distal transplanted ulna and radius dictates the level of bone resection. The pronator quadratus muscle is resected, and the lower end of the ulna is exposed in a manner for performing dorsal or medial osteosynthesis (depending on where the plate fits best) using a DCP 3.5 plate with six holes. If the recipient ulna is full length, one may avoid any bone measurement during HT. The technique is to supply with the allograft all bone resected in the recipient. The precise length is half of the six-hole plate to be used (Fig. 10). In both recipient and donor, the plate has to be presented in the same manner, as distal as possible, i.e. the plate is conducted from proximal to distal unless its distal end bumps against the metaphyseal-epiphyseal slope without being separated from the diaphysis (Fig. 11). The slight difference of this landmark location, which may exist between the donor and the recipient, may be ignored.

The anterior surface of the radius is exposed in order to admit a 7-hole DCP plate or T plate (4 holes proximally and 3 distally to the osteotomy level). The distal end of the radius is not resected unless the ulnar osteosynthesis is completed. The plane of the donor radius cut will indicate the level of radius resection automatically. In this way, the amount of resected radius will be

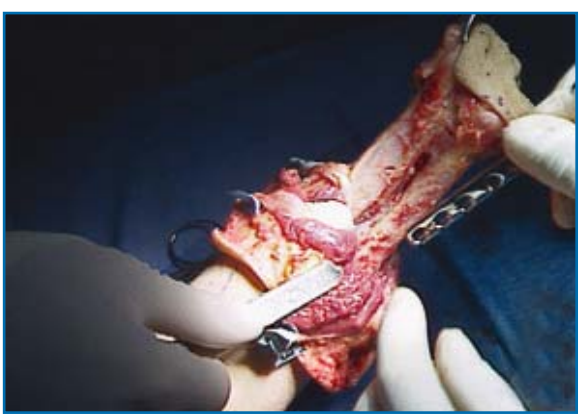

Fig. 10. Application of the plate to the ulna

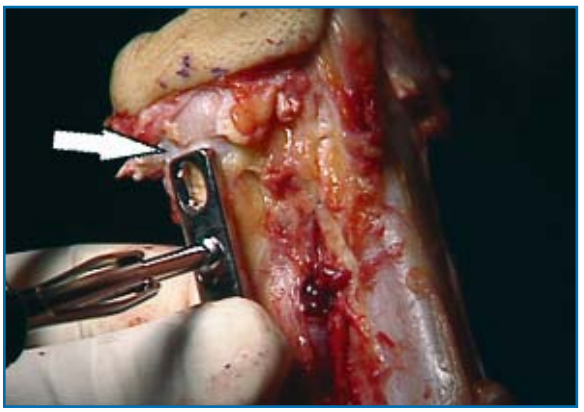

Fig. 11. The plate is conducted until the ulnar metaphyseal-epiphyseal slope (*arrow*)

the same as that brought with the allograft.

In the case of bilateral shortened forearm, the question of the previous exact forearm length in the recipient must be considered. Measurement of the arm parameters may help to predict the forearm length exploiting the following formula: RPL(cm) = 0,87HL+1,76 (r= 0,95), where RPL is radius predicted length and HL is humerus length (unpublished data, in collaboration with Dr. P. Braillon, Imaging Department, Hôpital Debrousse, Lyon, France). The radius length is measured after osteotomy [radius actual length (RAL)]. The resultant difference of RPL and RAL is the required radius donor length (RDL=RPL–RAL), which is recorded and notified to the donor team. In this case, bone procedure could begin with radius preparation and osteosynthesis.

The entire procedure of recipient preparation lasts 2–2.5 h. The recipient is "ready to admit" the donor hand(s).

Acknowledgements

This work would have been impossible without the help and collaboration of Dr Davit O. Abrahamyan, (Charcot Research Fellow, Yerevan, Armenia), Michael Hughes (photographer, Lyon, France), Dr Susan Higgins, (revision of English, Lyon, France), and each member of the hand transplant team

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Section 5-e

Technical and Surgical Details of Hand Transplantation

Milomir Ninkovic

Introduction

Achievement of survival and useful function in allotransplantation of the upper extremity is the main goal in the treatment of amputees. Experience in replantation surgery was prerequisite for successful application of this technique in the new field of allotransplantation. The first successful replantation of an amputated arm was reported in 1962 by Malt and McKhann [1], and Komatsu and Tamai [2] performed the first successful digital replantation in 1968. Since then, a large number of replantations have been undertaken all over the world with an increased number of parts surviving. With more experience, surgeons have rightly turned their attention to function, the foremost consideration in all surgery of the hand [3, 4]. It is known that initial survival of the transplanted part depends on patent microvascular anastomosis and immediate postoperative care, but ultimate function and acceptability is dependent on patient selection and performance of bone, muscle, tendon, nerve and soft tissue repair. Therefore, the allotransplantation surgeon must be first, a thoroughly trained and accomplished hand surgeon and second, a competent microsurgeon having the necessary experience for a predictable outcome of the part selected for allotransplantation as well as knowledge of immunosuppressive therapy. It is very difficult for a surgeon functioning independently to achieve high success rates in allotransplantations. A well-integrated team (hand plastic surgeon, orthopaedic trauma and transplantation surgeon, as well as anaesthetist and specialist for physical medicine and rehabilitation) seems to be essential if a high degree of viability and ultimate function is to be realised.

Preparation for Surgery

The selected patient comes to allotransplantation only after full preoperative evaluation. History taken from the patient and relatives includes not only details of the mechanism of injury but also information about other injuries and preexisting illnesses.

Mechanism of Injury and Level of Amputation

Certainly, patients with guillotine-type amputations at the level of the wrist joint and distal forearm are ideal candidates. However, this type and level of amputation is uncommon. Amputation proximal to this level is more frequent. Most hands are amputated by explosion, crush, electric or avulsing injury, which makes surgical reconstruction more difficult and lowers the percentage of functioning viability (Fig. 1). The problems are that very proximal muscle injuries do not leave any rest function, and very proximal nerve injuries make nerve recovery extremely questionable. In these cases, tendon

transfer and very proximal nerve repair have to be taken into consideration with the aim of achieving the best possible functioning result.

Surgical Technique

Allotransplantation has to be undertaken with at least two teams working simultaneously in adjacent operating rooms. One team is performing the operation on the donor and the second one on the recipient [5–7]. To keep ischaemia time as short as possible, donor and recipient operations have to be perfectly coordinated according to the time of transplantation and the amount of tissue taken and required for optimal functional and aesthetic results. It is better to have more than enough length of all donor structures so they can be joined with recipient tissue without any tension. This is especially important for neurovascular structures. Both teams must use a tourniquet to operate in a bloodless field for optimal identification of all available neurovascular structures, tendons and muscles [5–9].

The number of teams working simultaneously depends on the type of surgical procedure. In bilateral hand transplantation, the optimal number of available teams is four, as it could guarantee very successful results [5–7].

Skin Incision in Recipient Stump and Donor Part (Hands)

Depending on the recipient's amputation level, extensive incisions in the forearm must be made to expose the neurovascular structures for dissection. Incisions are not placed directly above nerves and vessels, as swelling may prevent direct closure, and the flaps must be prepared for Z-plasty transposition during closure. Skin incisions must be planned according to stump scaring and possibility of creating a zigzag skin-flap closure.

Incisions in the recipient stump can be placed over midpalmar and middorsal compartments to give access to the bone and main neurovascular structures, keeping optimal blood supply from the main vessel to the skin flaps (Fig. 1). One skin flap has blood supply from the radial artery and the second from the ulnar vessels. The second option of exposure is radioulnar skin incisions, raising one dorsal and one palmar flap (Fig. 2). At the same time, incisions of the transplanted hand have to be planned in a fashion that allows incorporation of skin flaps as a Z-plasty in closure around the extremities. This means that if the surgeon chooses a radioulnar incision on the stump, the incisions of the transplanted hand have to be planned in midpalmar and middorsal fashion in order to make transposition as a Z-plasty possible. Lengthening the

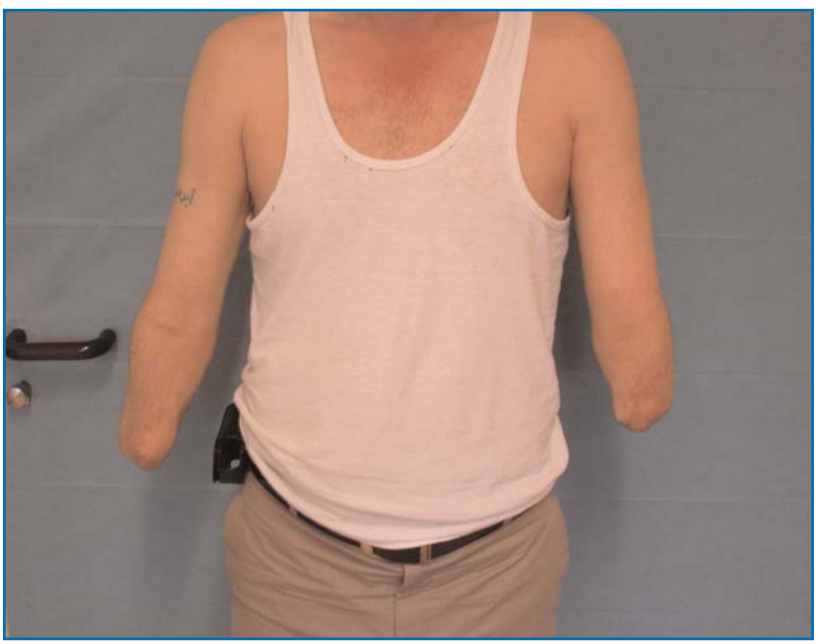

Fig. 1. Bilateral forearm amputation by bomb explosion. The problems are that very proximal muscle injuries do not leave enough function, and very proximal nerve injuries make nerve recovery very questionable. In these cases, complete forearm transplantation and very proximal nerve repair have to be taken into consideration in an attempt to achieve the best possible functioning result

circumferential scar is very important to avoid constrictive circular scaring. Postoperative oedema can cause a remarkable reduction of available skin. Therefore, loose zigzag closure with Z-plasty is extremely important. In addition, there is no skin flap oedema during the entire recovery period.

Dissection and Identification of All Available Neurovascular Structures and Tendon-Muscle Units in the Recipient Stump and Donor Hand

Dissection and identification start at the subcutaneous level and continue down to the bone surface. All available structures have to be tagged using different sutures or plates for tendon–muscle units. Vessels and nerves can be easily seen at this stage. Therefore, it is my practice to tag each vessel with two suture ends of equal length whereas the nerve is tagged with only one long suture end. Microvascular clamps are not used for identification because they can slip off in the course of subsequent manipulation. Furthermore, they can cause significant intimal damage, and they may be forgotten over the course of a long case. Tagging vessels and nerves in donor and recipient, as well as muscles in the recipient, prove very helpful and timesaving when working later in a bloody operating field. When the hand has been transplanted and the tissues are blood stained, neurovascular structures can retract into an amorphous mass of soft tissue, causing the surgeon extreme frustration. There is no need to label donor tendons because they are easily identified during the transplantation procedure. Depending on muscle–tendon injuries and scaring on the stump, all available and functioning muscle–tendon units must be identified and prepared for tendon transfer. Tendon transfer must be planned according to the principles of tendon transfer in the upper extremity.

Hemostasis in the Recipient Stump and Donor Hand

After identification of all available structures, the tourniquet is released to achieve haemostasis. It is important to perform exact haemostasis in the stump as well as the donor hand. The tourniquet is left off for at least 20 min and the flow observed – in the stump at the distal flap levels and in the donor hand at the fingertips. At the end of this observation time, the tourniquet can be reapplied for bone cutting and complete amputation at the donor site. The donor stumps must be sutured to fit custom-made aesthetic hand prostheses.

Transplantation Technique and Sequence

The operative sequence of hand transplantation varies slightly with amputation level (wrist versus proximal to the wrist) and injury type (clean cut, explosion, crush, avulsion). When preparation of all structures and haemostasis of the donor hand is complete, it must be perfused with 500 ml of chilled University of Wisconsin (UW) solution through the brachial artery, after which it is removed and the transplantation commenced. Structures are repaired in the following sequence:

- Osteosynthesis (bone preparation and fixation)
- Vessel repair (anastomosis of the one main artery and two veins)
- Muscle–tendon repair/transfer
- Definitive vessel repair
- Nerve repair
- Skin closure and dressing.

Bone Fixation

Firm skeletal fixation by internal methods is desirable because it promotes early union. Joints are not immobilised in order to permit early mobilisation. As with all osteosynthesis, attention must be paid to extremity length, alignment, rotation and angulation because subsequent correctional osteotomy is a tiresome and unnecessary procedure. During preparation and shortening of the bone, the periosteum must be carefully preserved. After bone fixation with

plates, the periosteum can be repaired with the benefit of better bone healing, which also helps to prevent tendon adhesions. A 5-0 absorbable suture is usually used.

Vessel Repair

Arterial repair follows bone fixation in order to reestablish circulation at the earliest possible moment. The main arteries and ulnar and radial arteries are prepared under the microscope, and one of them is anastomosed end to end. I prefer to anastomose the ulnar artery first and open the clamps to allow some bleeding from the transplanted hand. In between, the cephalic vein will be anastomosed. This provides the advantages of early revascularisation and very short ischaemia time and allows easier location of the most functional superficial veins. It is useful to anastomose a temporary second superficial vein (basilic vein) to have less bleeding from the transplanted hand. There is also considerable danger of uncontrolled acidosis if venous drainage is permitted to freely enter the circulation from the bulk of muscle. Locally, perfusion has been shown to be reduced by persistent systemic acidosis. We have found it beneficial to give intravenous sodium bicarbonate to the patients prior to the opening of the venous anastomosis. This early revascularisation allows complete tendon-muscle repair without any pressure as far as time is concerned.

Muscle-Tendon Repair/Transfer

After bone fixation and main vessel repair, the extensor tendon should be joined for further stabilisation. Depending upon amputation level, different suture techniques can be employed. Usually, a series of interrupted figure-of-eight synthetic 4-0 nonabsorbable sutures are sufficient. In some cases of severe avulsion injury of

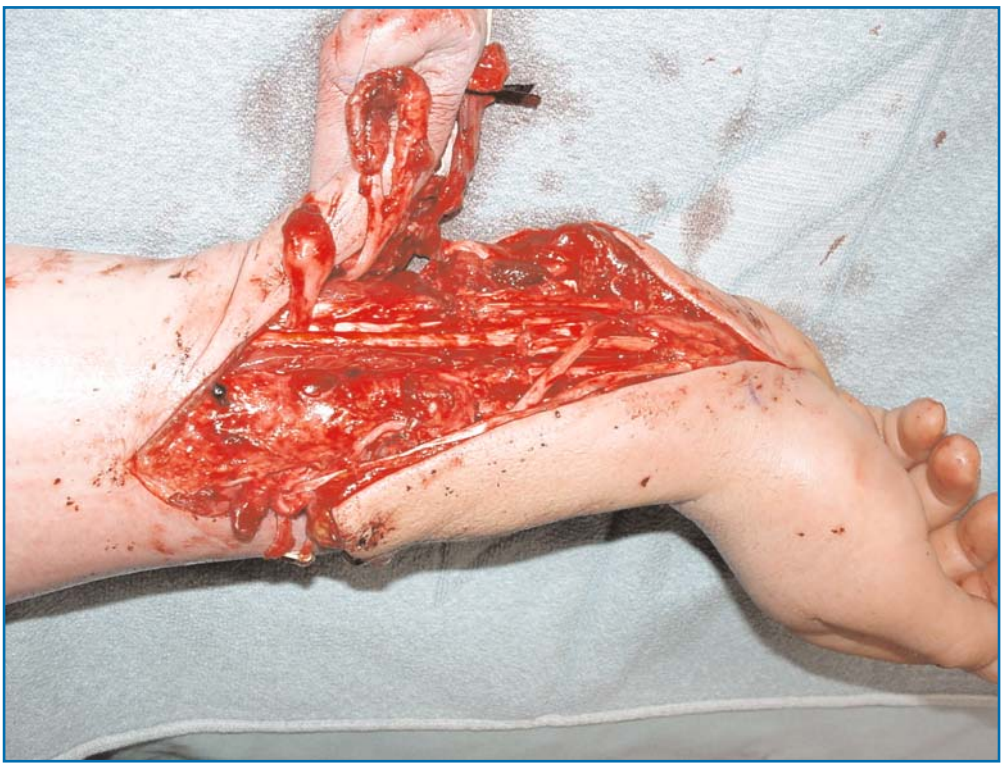

Fig. 2. Intraoperative view. Notice the radioulnar incision in the stump and corresponding midpalmar incision on the transplanted hand. Lengthening the circumferential scar is very easy to achieve using the palmar flap in the stump and radial skin flap on the transplanted hand as Z-plasty flaps. Therefore, loose zigzag circumferential closure is possible. Notice flexor tendon suture lines at different distal–proximal levels to avoid scaring and adhesions at the same surface. Notice complete dissection of the median and ulnar nerve neuromas

the stump with missing extensor muscles, tendon transfer using standard techniques such as pronator muscle for wrist-joint extensor or flexor carpi radialis or flexor carpi ulnaris for digit extensor are available for functional improvement. It is always important to put the wrist-joint extensor under appropriate tension, at least 45°, to achieve adequate balance at the end of surgery between extensor and flexor tendons.

All flexor tendons are repaired by the different suture techniques according to amputation level. Tendons are sutured with as much care as possible to allow the best chance of good functional results. Committed as we now are to early active motion following transplantation, particular care is taken to ensure the strength and hold of the core suture. If flexor muscles are missing, there are many possibilities for performing tendon transfer. These options must be taken into consideration in each case of explosion or muscle-avulsion injuries.

To avoid tendon adhesions, all adjoining tendons must be sutured on different levels, producing less scaring and a better gliding surface. This means that all flexor and extensor tendon suture lines must be placed in a zigzag fashion, with at least 1.5- to 2-cm distal-proximal distance between all neighbouring sutures. This can be achieved by cutting the tendons to the appropriate lengths.

At the end of tendon repair, it is important to test balance and tension between extensor and flexor tendons. I prefer to restore a natural finger cascade from index finger to small finger. The tension can be tested by passively moving the wrist to demonstrate the balance between extension and flexion. With the wrist in dorsiflexion, it should be possible to easily flex the fingers completely into the palm. With the wrist in volar flexion, the metacarpophalangeal (MP) joints should pull into full extension but not hyperextension.

Definitive Vessel Repair

After achieving optimal balance between flexor and extensor tendons, the definitive vessel repair can be obtained. First of all, correction of vessel lengths must ensure appropriate tension between vessels, avoiding kinking or too much tension. For this purpose, I use a very simple test: Vessel ends that cannot be approximated by double microvascular clamps or by the use of a 9-0 nylon suture are under excessive tension. If we try to repair them despite such tension, either by traction on the vessels or the use of large sutures, the results will be unsatisfactory. These difficult leaking anastomoses, left uncorrected, will lead to a failed transplant. Therefore, appropriate vessel length is prerequisite for a functioning anastomosis. First, the second main artery, the radial artery, must be anastomosed. Next, at least one of two comitantes veins must be anastomosed. Then the excess length of the first repaired (ulnar) artery can be resected, and under adequate tension, reanastomosis can be performed. One or two deep veins must be anastomosed to provide optimal drainage. All other superficial veins of suitable calibre must be repaired. The more veins repaired, the greater the chance of graft survival. If the two main arteries are repaired, at least 4–6 veins must be anastomosed. It is important anastomose not only superficial veins but also deep comitantes veins. This is the guarantee for optimal runoff.

Nerve Repair

All three main forearm/hand nerves (median, ulnar and radial or superficial radial, depending on amputation level) must be prepared for coaptation under the microscope. The normal nerve is identified proximal to the neuroma, after which the neuroma, including some of the surrounding scar tissue, is isolated by sharp dissection. After complete dissection of the neuroma, a no. 11 scalpel blade is used to trim the nerve to normal fascicular tissue. This must be done very far proximal from the neuroma, especially in avulsion injuries. The use of 10-0 monofilament nylon is recommended for nerve suture, but a few large suture sizes may be utilised if more strength is needed to bring the nerve ends together. All peripheral nerves are coapted without any tension at the suture line. The fascicles are aligned without distortion, strangulation or

excess. Which technique of nerve repair is used depends upon the surgeon's preferences. I repair groups of fascicles on forearm major nerves and perform either fascicular or epineural repair on smaller nerves. Attention is therefore directed less at the choice of technique (fascicular or epineural) that at its execution with respect to the fascicles on completion of the repair. Fascicular sutures require at least one stitch per fascicle and, in some cases, two or three. Judgment regarding the number of sutures depends on how each fascicle lies after the placement of each suture. Fibrous tissue is provoked by each nerve suture, obstructing axonal sprouting and maturation; therefore, a minimal number of stitches are recommended for nerve repair.

Skin Closure and Dressing

Meticulous haemostasis is obtained after all the structures have been repaired. The skin is loosely approximated using the skin flaps in a Z-plasty manner. Incorporation of Z-plasty is prerequisite for avoiding primarily oedema or compression and later circumferential scar contracture (Fig. 3). In all cases, however, a critical eye must be kept on the colour of digital pulps. Any duskiness or increase in speed of refill after blanching is corrected by releasing appropriate skin sutures.

The wounds are covered with small strips of paraffin gauze. Care must be taken in the placement of these strips so that they are always placed longitudinally and never in a circumferential manner. A second layer is fluffed dry gauze applied in abundance, again longitudinally. Then, a plastic sponge 1- to 3-cm thick is wrapped once around the entire limb, leaving a gap down one side; it is held in place by a stretch gauze bandage applied circumferentially. The sponge extends out as far as the tips of the fingers. Finally, a plaster splint is applied over the customary paper felt bandage. The plaster is again laid longitudinally; it must pass around the flexed elbow and incorporate a plaster sling beyond the fingertips, from which the limb is suspended. The fingers are checked on one final occasion for circulation and absence of constrictive dressings. Finally, the arm is elevated above the elbow using the plaster sling.

Postoperative Care

Observation: Circulation Checks

Initially, circulation checks are performed at hourly intervals. Colour, pulp turgor, capillary refill and warmth are very useful aids in monitoring the transplanted graft. The colour of the nail bed and return of blood after blanching must be controlled continuously. The perfect response exists when the colour is pink, with return comparable to that in an adjacent non-transplanted area. Digital pulse monitoring, oxygen probe and temperature monitoring using a thermocouple are very useful guides.

Medication

Heparin is given to maintain prothrombin time at approximately 50 s; and it can be replaced after 1 week with oral acetylsalicylic acid (100 mg/day), which can be maintained for a long time. The protocol of immunosuppressive thera-

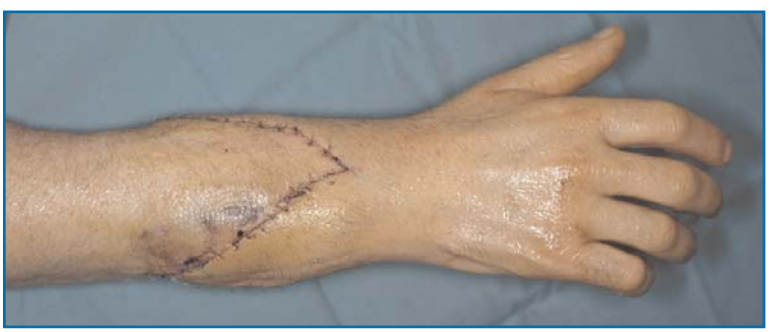

Fig. 3. Postoperative view. The skin is loosely approximated using the skin flaps in a Z-plasty manner. Ten days after transplantation, there is no sign of primarily oedema or compression. Incorporation of the Z-plasty is a prerequisite for avoiding later circumferential scar contracture

py and infection prophylaxis is prepared by transplant surgeons [7, 10].

Acknowledgements
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Section 5-f

Hand Replantation and Transplantation: More Differences than Similarities

Marco Lanzetta, Roberta Nolli

From a surgical point of view, hand transplantation is technically not very different from a hand replantation. However, the procedure presents unique requirements, especially from an organization point of view. In our experience, there is no doubt that there are more differences than similarities between hand transplantation and replantation, so that the two operations must be considered quite different and a completely different approach must be followed [1]. The main differences of hand transplantation, when compared to hand replantation, can be summarized as follows:

1. Complex organisation:
   a Large number of staff required
   b On-call period may be long
   c Multidisciplinary competence required

Probably two or more independent teams for harvesting and transplant

2. Very difficult matching
3. Perfusion of harvested limb
4. Different planning of surgical procedures
   a No shortening of the bone
   b Degree of fibrosis
   c Degree of muscles atrophy
   d Tendon group repair
   e Possible tendon transfer
5. Relatively long time of cold ischaemia
6. Complex postoperative management.

Hand transplantation presents definite advantages and disadvantages when compared to autologous hand replantation. One of the major advantages is that the allograft is harvested according to specific needs. This is done in a bloodless field, and the different tissues can be dissected and tagged carefully and atraumatically, including some extra-length if needed. These advantages are somehow balanced by the fact that the forearm stump presents a degree of scarring, normally due to either the previous trauma of amputation or the surgical procedure(s) undertaken to regularize it. The muscles show some degree of atrophy and contracture, and nerves always need to be transected more proximally due to neuroma formation and degeneration. Similarly artery or veins may need to be resected very proximally in search of better quality. However, microsurgical repair of nerves and vessels can be performed in an ideal situation, without tension or need for nerve, artery or vein grafts. The two ends of the nerves/artery/veins are perfectly healthy and can be joined in a well vascularized area. Tendons can be joined at the same level or at different levels according to preoperative planning, minimizing adhesions that can arise due to the contiguity of multiple healing tendons. Skin incisions and closures can be planned to achieve a cosmetically acceptable scar thus improving appearance. Limb's length is always restored to normal, as shortening of the different tissues, which is advisable in most hand or arm replantation cases, is not needed.

If obviously in case of replantation the amputated part does not present problems in terms of size, color or specular matching of the various tissues to be reattached and/or repaired, this is completely different in transplantation. The

hand will have to come from a donor of the same sex, but color, size, age will never be exactly the same. A certain degree of compromise must be expected. However it should be pointed out that as hand transplantation is a non-life-saving procedure, we can afford the luxury of waiting for the most ideal donor for aesthetic reasons.

Another substantial difference lies in the perfusion of the harvested hand. In routine replantation, this procedure has never been widely accepted, as it is considered either unnecessary or not advantageous. As in any other transplantation procedure, blood from the donor limb is cleared completely. The initial clinical experience support the conclusion that hands should be perfused with the same modalities as in solid internal organs and that this procedure does not negatively affect the revascularization of the different tissues.

A definite disadvantage of transplantation compared to replantation is that while the amputated limb is normally transported to the hospital with the patient, harvesting a hand to be transplanted requires members of the team to reach a different location, and includes completely different organization issues. Also, the ischaemia time in our experience is two to three time longer that what is normally achieved in routine hand replantation.

The hand transplanted patient follows an articulated immunosuppressive therapy to prevent rejection of all tissues: skin, subcutaneous tissue, nerve, vessels, muscle and bone [3]. His/her rehabilitation period must be supervised closely, and the immunosuppressive regimen and drug levels checked frequently, therefore there is the need to take up temporary residency close to the hospital. While for a replanted hand the patient is rarely directed to a psychologist, in hand transplantation a psychological support after the operation is mandatory.

We believe that the complex organization required to activate a successful hand transplantation program, coupled with the need for additional knowledge in complementary medical areas (i.e. immunology, psychology, etc.) is currently the main factor in preventing many of the existing hand surgery and microsurgery unit from engaging in such activities.

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6. Immunology of Hand Transplantation

Section 6

Current Concepts

Olivier Thaunat, Emmanuel Morelon

Introduction

The concept of limb transplantation depicted by Fra Angelico in one of his most famous retable, the healing miracle of Cosmas and Damian, as early as the fifteenth century, is one of the oldest models of transplantation in humans. This artistic testimony demonstrates that a long time ago, the prodigious potential that composite tissue allotransplantation might bring to tissue reconstructive surgery had been anticipated. At present, limb transplantation cannot be regarded as a surgical challenge because advances in microsurgical techniques have allowed since the early 1960s routine replantations of whole limbs to be performed after traumatic amputations [1]. Thus, hand transplantation can neither be regarded as a new concept nor surgically unattainable. Nevertheless, clinical achievement of hand transplantation is fairly recent, suggesting that the real hurdle preventing the development of hand transplantation is, rather, immunologic. Indeed, unlike solid organ allografts, hands are histologically heterogeneous and are composed of tissues that express varying degrees of immunogenicity. Among these tissues are skin and lymphoid organs, which are highly immunogenic and elicit a strong immune response. Transplantation of a hand graft was thus believed to require a very high level of immunosuppression outweighing the anticipated benefits in a non-life-threatening situation, thus precluding regular clinical practice.

Surprisingly, experimental data available from several studies conducted during the last two decades on animal models [2] and clinical experience from the first hand transplants performed in humans since 1998 [3] have proved that the immunological obstacle can be overcome without major difficulties and that the graft is subjected to bone consolidation and tissue healing with a standard level of immunosuppression. Together, the conceptual hurdles anticipated by transplant immunologists were confirmed neither by experimental models nor by clinical observations, as if grafted forearms themselves extend a helping hand to the immunologist to understand further the immune response directed against composite tissue allograft.

Composite tissue allografts, such as the hand, behave in many ways like other allografts regarding immunological mechanisms leading to their rejection. The purpose of this chapter is not to recapitulate all the data accumulated by immunologists in the field of transplantation but, rather, focus on two characteristics of hand allografts that set them apart from other solid organ allografts: (1) they contain skin tissue that is highly immunogenic and therefore elicit a strong immune response from the recipient's immune system, and (2) they contain lymphoid tissue (such as bone marrow and lymph nodes), which have the potential both to attack the recipient [(graft-versus-host disease (GVHD)], and also to down-modulate the host immune response and induce tolerance.

Immunogenicity of Parts versus Immunogenicity of the Whole: Lessons from Hand Transplantation

As early as the late 1960s, experimental data suggested that the level of immunogenicity was variable from one tissue to another. Because it was possible to induce tolerance to most organs but not skin grafts, skin was thought to be the tissue carrying the higher immunogenic potential [3]. This theory was further developed a few years later by Murray et al. [4], who proposed a relative scale of immunogenicity of tissues and organs, ranking the skin first, far above all the other tissues tested. At the time, transplant immunologists anticipated that the global immunogenicity of a composite allograft would be the sum of the immunogenicity of each tissue constituting it and thus predicted that hand transplantation would require a high level of immunosuppression, incompatible with further clinical application. This concept prevailed until the 1980s when several groups demonstrated that long-term survival of limb transplantation was achievable in animal model using low-dose cyclosporine [2, 5, 6]. These unexpected results suggested that immunogenicity of the whole limb was lower than that of the skin alone and raised new questions regarding the interaction of recipients' immune system with vascularized limb allograft.

Modulation of Immunogenicity

The "passenger leukocyte" theory described by Snell almost 50 years ago proposed that leukocytes within the allograft act as the critical stimulus for sensitization of recipient alloreactive T-lymphocytes [7]. Among leukocytes, dendritic cells have been identified as the most potent stimulator of the recipient's immune system during the afferent phase of rejection [8]. Immunogenic potential of an allograft was thus thought to be mainly related to the quantity of dendritic cells present within the tissue. Relying on this concept, transplant immunologists believed that the elimination of dendritic cells from the graft was the only approach to decrease its immunogenicity [9, 10]. Because it was demonstrated that skin is an immune organ that contains a high number of professional antigen-presenting cells – Langerhans cells – Russell [11] proposed to perform "selective transplantation", in which the more immunogenic portion of the composite limb allograft (the skin) would be removed prior transplantation. Although interesting and efficient in an experimental model [12], this technique reached a dead-end from a clinical point of view.

One of the most interesting immunological lessons from limb transplantation is that it is possible to down-modulate the immunogenicity of a tissue by grafting it along with others. The first experimental demonstration of this concept was provided by Lee et al. in early 1990s [13]. Using an experimental model of transplantation across a strong histocompatibility barrier in rats, they compared cell-mediated and humoral immune responses generated against individual limb tissues (skin, muscle, bone and blood vessels) and a whole limb. They showed that the various tissue components interacted with the host immune system in patterns differing in timing and intensity and, more strikingly, that the whole limb allograft was rejected more slowly and generated lower immune response than allografts of its individual components. Although several hypotheses have been put forward to explain this phenomenon, a definitive immunological explanation is still lacking. Some have proposed that the decrease in skin immunogenicity observed in the whole-limb transplantation model is explained by the fact that vascularization of the skin arises from the donor in the whole-limb model versus the recipient in the conventional skin graft. However, a number of studies found no difference when comparing survival of conventional skin allografts with primarily vascularized skin allografts [13–15]. Other possible explanations include the occurrence of a "consumption phenomenon" as the immune system of the host is overwhelmed with the tremendous antigen load, antigen competition and/or generation of regulatory cells.

Split Tolerance

Despite a relative decrease in immunogenicity when transplanted within a vascularized limb allograft, skin remains the principal target of the recipient's immune system. This was first reported by Goldwyn et al. [16] in a canine limb transplantation model. This was further experimentally documented by the group of the Massachusetts General Hospital, who showed that tolerance was achievable in a swine model of musculoskeletal allograft without skin [12, 17] and demonstrated that despite indefinite survival of the musculoskeletal portion of their allografts, animals were still able to reject a vascularized skin paddle from the same donor [18]. Confirmation in the human setting was obtained after the first human hand allograft was removed during month 29 posttransplantation for uncontrolled rejection due to noncompliance with the immunosuppressive regimen. Pathologic examination of the allograft showed, indeed, that the most severe changes were found in the skin while only mild inflammation was found in muscles and tendons, and bone and joints were spared [19]. The phenomenon of simultaneous tolerance to one tissue and rejection of another coming from the same donor was first described by Billingham et al. in 1959 [20], who coined the term "split tolerance". Loss of the epidermal component of the limb allograft, in the context of long-term survival of the remaining tissues, is, however, a unique example validating this concept within one single graft.

Hand Transplantation: A Model of Vascularized Bone Marrow Transplantation

With the first successful hand transplantation performed in Lyon in 1998 [21], composite tissue transplantation has moved from the field of research into clinical practice. However, the success of these transplants is currently dependent on chronic immunosuppression, which subjects patient to infections, malignancies and drug toxicity [22] that may outweigh the benefits for correcting a non-life-threatening condition. A protocol for tolerance induction to composite tissue allografts that would eliminate the need for immunosuppressive therapy is therefore required to turn this technique into a widespread treatment modality for reconstructing large tissue defects.

The presence of haematopoietic tissue within the hand allograft could potentially increase the possibility of inducing donor-specific tolerance to the transplant limb but also, conversely, increase the risk of GVHD. These two issues are discussed in this chapter.

Role of Chimerism in Induction of Tolerance and/or GVHD

Mixed chimerism is one of the oldest and best-studied approaches for establishing tolerance in organ transplantation. Two different types of chimerisms have been described: macrochimerism and microchimerism. Macrochimerism occurs when bone marrow is transplanted in a conditioned recipient. Conditioning ablates the recipient's bone marrow to make space for allogeneic bone marrow and immunosuppresses the recipient, thereby preventing rejection of the transplanted bone marrow. The pluripotent haematopoietic stem cell engrafts in the recipient bone marrow and produces all its lineages. A new immune system establishes in the recipient, and newly developing T lymphocytes that recognize the donor antigens are clonally deleted in the thymus. It has been shown experimentally that as low as 1% of haematopoietic cells of donor origin are sufficient to induce a robust state of tolerance to donor-specific tissues [23].

Microchimerism arises as a result of migration of passenger leukocytes from a transplanted allograft into a nonconditioned recipient. Donor pluripotent haematopoietic stem cells do not engraft in the host. Consequently, only very low levels of donor cells are found in the host. In the early 1990s, Starzl et al. [24] observed that some patients who had ceased taking their immunosuppressive medication maintained their organ transplants. Furthermore, all of them had donor

cells present in their peripheral blood. The Authors proposed that the failure of the host immune system to reject the donor organ was due to the peripheral microchimerism. However, because organ allograft rejections have also been reported despite the presence of microchimerism [25, 26], it is still debated whether peripheral microchimerism can induce tolerance or whether it is only one of its side-effects. Of note, a recipient's cells can also migrate into the graft and thus turn the graft itself (and not the recipient) into a chimera. Recent investigation indicates that this process is involved in the regeneration of grafted solid organs. It is conceivable that besides reparative compensation of cell loss, chimerism of endothelial cells might also alter immunologic properties of the graft, thus favouring adaptation and graft survival. However, data indicate, rather, that this process is associated with the development of chronic rejection [27].

GVHD, which remains a major and devastating complication of bone marrow transplantation, occurs when donor mature immunocompetent cells present within the graft, attacking tissues of the patient receiving the transplant. Wick et al. [28] have suggested that there are three necessary factors in the development of this disease: (1) a sufficient number of immunocompetent cells within the graft, (2) major immunogenic differences between host and recipient, and (3) the inability of the host immune system to mount an effective response against the graft. Because recipients of hand allograft fulfill these three criteria, they were considered in principle to display high risk for GVHD.

Composite Tissue Allografts, Chimerism and Tolerance

Immunologists anticipated that hand allograft would induce chimerism even if the recipient would not receive myeloablative drug prior to transplantation because hand allograft, which contains viable bone marrow compartment, was regarded as a vascularized bone marrow transplant. In this case, donor bone marrow cells are transplanted within their own stromal microenvironment. They are thus expected to function immediately upon transfer and to provide a continuous supply of donor bone marrow cells [29, 30].

Experimental studies using the rat limb transplantation model have confirmed the establishment of macrochimerism in the recipient. Hewitt et al. [31] transplanted vascularized limb allografts from Lewis to Lewis X Brown-Norway F1 and reported long-term survival of eight recipients treated with cyclosporine. In 2/8 animals, immunosuppression was discontinued, resulting in no histologic evidence of rejection. These interesting results have, however, never been replicated in a large-animal model. Indeed, in a model of induction of tolerance to musculoskeletal allografts across minor antigen mismatch in swine, Bourget et al. [17] have shown that peripheral chimerism was present only in the immediate postoperative period and that it was not necessary for maintenance of such tolerance. In a subsequent study, they examined the fate of donor bone marrow after transplantation and found that despite tolerance to musculoskeletal components, there was no evidence of persistence of donor bone marrow cells either in the graft marrow compartment (that had been repopulated by host bone marrow cells) or in the recipient's lymphohaematopoietic tissues (bone marrow, thymus, spleen and mesenteric lymph nodes). In the clinical setting, only few data are available. Granger et al. [32] performed kinetic studies on peripheral blood of two subjects after hand transplantation and evaluated donor-specific reactivity and chimerism. They reported that donor-specific hyporesponsiveness did not develop clinically or in mixed lymphocyte reaction and that donor macrochimerism was not detectable. Peripheral microchimerism was observed in some of the early posttransplantation specimens and was undetectable thereafter.

Kanitakis et al. [33] investigated the development of intragraft chimerism by performing immunohistochemical analysis on sequential skin biopsies from a hand allograft. The Authors reported that the dermis was transiently infiltrated by mononuclear cells of the recipient's origin whereas the recipient's bone-marrow-

derived antigen-presenting cells (i.e. Langerhans cells) progressively replaced the donor's cells in the epidermis.

It is not surprising that peripheral microchimerism was not detected in recipients of a hand allograft since the mass of bone marrow engrafted with the composite allograft was far less important than the recipient's own bone marrow; therefore, the recipient's blood-borne cells were more likely to be present in the allograft than were the donor's cells to be retrieved in the recipient's blood. However, rapid replacement of allograft Langerhans cells by the recipient's cells is interesting, and because this process turns the allograft into a real chimera of the resident skin cells, it could possibly be advantageous for the survival of the allograft.

Composite Tissue Allografts and GVHD

In composite tissue allograft, GVHD was first studied in an unmodified hosts across a semiallogeneic barrier [34] in the rat hind-limb transplantation model. Interestingly, only 37.5% of recipients developed lethal GVHD whereas the remainder of the animals recovered from a self-limiting course of GVHD and developed long-term tolerance. Additional study showed that removal of the popliteal lymph nodes without graft irradiation eliminated GVHD [35], suggesting that vascularized bone marrow transplant was not the component that caused GVHD, which was, rather, the result of mature lymphocytes in the graft. The mechanism for the lack of GVHD in vascularized bone marrow transplantation is yet undetermined although the stromal microenvironment with its rich resources of signalling mechanisms may be responsible. Together these experimental data are encouraging and suggest that it is possible to achieve tolerance induction while avoiding GVHD in recipients of composite tissue allografts. Clinical data are sparse, but Granger et al. [32] report no histopathologic evidence of GVHD in skin and colon biopsy samples of two recipients of a hand allograft. It must, however, be underlined that neither of these recipients developed detectable chimerism or tolerance.

Conclusion

Composite tissue allotransplantation took its first steps in the clinical arena with the successful hand transplant performed in Lyon in 1998. The development of composite tissue transplantation gives hope to broadening the realm of reconstructive surgery to physical handicaps with no current solution. The use of vascularized tissues harvested from a different subject could theoretically be extended to all reconstructive procedures currently using autologous tissues to obtain better functional and esthetic results and to reduce morbidity from tissue harvesting. Despite these advantages, the current application of composite tissue transplantation is limited by immunologic hurdles such as the side-effects of chronic immunosuppression and the uncertainty of long-term outcome due to chronic rejection.

From an immunological point of view, composite transplantation is particularly challenging because it adds the difficulties of solid organ transplantation to those of bone marrow transplantation while enabling the new concepts come to light. More efforts from the immunological community are therefore warranted to extend a helping hand to clinicians by providing definite answers regarding how the recipient's immune system deals with composite allograft.

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7. Immunosuppressive Therapy

Section 7-a

Induction and Maintenance Therapy

Palmina Petruzzo

Introduction

The optimal immunosuppressive regimen for initial, maintenance and rejection therapy after hand transplantation has not yet been identified. Composite tissue allograft (CTA), such as hand transplantation, has been performed only experimentally for many years because of the skin's high degree of immunogenicity. Indeed, it was assumed that the dosage of immunosuppressive drugs required to prevent rejection were too high to be used safely in the clinical setting. Advances in immunopharmacology and the encouraging results achieved in animal models [1–3] in this last decade allowed the realization of different human CTAs, including larynx, knee, hand and part of a face. These positive outcomes have been made possible through the use of the latest induction and maintenance regimens.

Induction Therapy

At present, all successful treatments of human disease by transplantation (other than between monozygotic siblings) require the use of general immunosuppressive agents [4]. Induction therapy consists of administration of a brief course of high-dose immunosuppression in the early post-transplant period, and it precedes and overlaps with less intense long-term maintenance immunosuppression. The primary objective of induction therapy is to decrease the incidence of acute cellular rejection as well as delay the onset of the first episode and, when possible, delay the introduction of calcineurin inhibitors. Induction therapy generally refers to the use of polyclonal or monoclonal antibodies.

Polyclonal Antibodies

Polyclonal antibodies are directed against lymphocyte antigens, but instead of the single specificity of the monoclonal antibodies, these antilymphocyte antibodies are directed against multiple epitopes. Antithymocyte globulin [5] is a polyclonal antibody derived from either horses (Atgam) or rabbits (Thymoglobulin). The agents contain antibodies specific for many common T-cell antigens, including CD2, CD3, CD4, CD8, CD11a and CD18. The antithymocyte globulin binds lymphocytes that display the surface antigens previously listed. This effectively depletes T-cell concentration in the body through complement-dependent cytolysis and cell-mediated opsonization followed by T-cell clearance from circulation by the reticuloendothelial system [6].

Monoclonal Antibodies

Monoclonal antibodies are antigen-specific immunosuppressants that reduce immune response to alloantigens of the graft while preserving the response to alloantigens to unrelated

antigens. These agents are specific to the blocking of T-cell activation, resulting in rapid depletion of T cells from circulation by binding antibody-coated T cells to Fc receptors on phagocytic cells. Muromonab-CD3 is the first type of murine monoclonal antibody directed against the epsilon chain of the CD3 molecule (an integral part of the T cell receptor complex). It modulates the receptor and inactivates T-cell function, blocking both naïve T cells and cytotoxic T lymphocytes (CTLs). This results in rapid depletion of T cells from circulation and cytokine release [7]. Basiliximab (Simulect) is a chimeric (70% human, 30% murine) monoclonal antibody utilized in the prevention of acute organ rejection. This monoclonal antibody has a specificity and high affinity for the "a subunit" of the interleukin (IL)-2 receptor (IL-2Ra, also known as CD25 or Tac), preventing IL-2 from binding to the receptor on the surface of activated T cells. By acting as an IL-2Ra antagonist, basiliximab inhibits IL-2-mediated activation and proliferation of T cells, the critical step in the cascade of cellular immune response of allograft rejection. Daclizumab [8] is a similar agent to basiliximab but is a more humanized IgG monoclonal antibody (90% human, 10% murine). It also binds to and inhibits the "a subunit" of IL-2 receptor.

Maintenance Therapy

Maintenance immunosuppression refers to the classic combination therapy to which transplant recipients usually adhere for the rest of their lives. The combination includes a corticosteroid, a calcineurin inhibitor and an antiproliferative. Concurrent administration of these three drugs has distinct combined effects on each individual. The balance of dosages can be altered to enhance efficacy of immunosuppression, but the most effective combination of prescriptions is unique for each individual. As with inductive therapy, the goal of maintenance immunotherapy is to find a balance between "underimmunosuppression" (which results in graft rejection) and "overimmunosuppression" (which exposes the patient to high risks of infection and other potentially fatal side-effects). The various side-effects of each drug must be considered, as well as potential interactions between drugs, especially those that cumulatively present significant risk factors to certain patients.

Corticosteroids

Corticosteroids are an important part of maintenance therapy because of their anti-inflammatory and immunosuppressive effects [9]. They inhibit cytokine production, circulation of lymphocytes, acid metabolites and microvascular permeability. They also block T-cell activation and proliferation and thus the clonal response. The major elements blocked are IL-1 and IL-6. Secondary effects of corticosteroids include the blocking of IL-2, IFN-γ, and TNF-α. Prednisone and methylprednisolone are two of the most commonly prescribed corticosteroids for organ transplant recipients. These drugs are nonspecific and suppress the immune system in a global manner.

Calcineurin Inhibitors

In order to combat activated T cells (which play a pivotal role in graft rejection), immunologists employ calcineurin inhibitors, which have come to be the integral cornerstone of triple therapy for transplant recipients. Calcineurin inhibitors block clonal expansion of T cells and therefore significantly reduce acute rejection and improve graft survival. This inhibition ultimately inhibits the production and secretion of IL-2. The interaction between IL-2 and the IL-2 receptor is crucial in the activation and differentiation of B and T cells. Cyclosporine (CSA) and tacrolimus are the two most prominent drugs in this category; they have comparable immunosuppressive efficacy and nephrotoxicity, which is their most common serious side effect. CSA [10, 11] is a fungal metabolite extracted from *Tolypocladium inflatum Gams*, which works by binding a protein – cyclophilin – found in the cytosol, and this complex inhibits calcineurin. Tacrolimus is a metabolite of an actinomycete [12, 13], *Streptomyces tsukubaensis*, which works in a mechanism similar to that of CSA, bonding the

cytosolic protein FKPB-121. This complex inhibits calcineurin in a manner parallel to CSA.

Antiproliferatives

The final part of triple therapy is antiproliferatives, such as mycophenolate mofetil (MMF), azathioprine and sirolimus. These antimitotic drugs inhibit DNA synthesis and thus the division of T cells. Before the advent of calcineurin inhibitors, antiproliferatives were the primary form of maintenance immunotherapy.

MMF is absorbed and rapidly hydrolyzed in the blood to its active form, MPA, which inhibits the key enzyme in the de novo pathway of purine biosynthesis, IMPDH1. Rapidly dividing cells, such as activated lymphocytes, depend on the de novo pathway for production of purines necessary for RNA and DNA synthesis. In this way, activated lymphocytes are selectively inhibited since they are not allowed to proliferate once activated [14–16].

Sirolimus [16, 17] is a macrocyclic lactone produced by *S. hygroscopicus* and resembles tacrolimus and binds to the same intracellular binding protein or immunophilin known as FKBP-12. However, sirolimus has a novel mechanism of action: it inhibits activation and proliferation of T lymphocyte in response to stimulation by antigens and cytokines (IL-2, IL-4 and IL-15). This inhibition is believed to be mediated by a mechanism distinct from that of tacrolimus, CSA or other immunosuppressants. It binds to the immunophilin FK binding protein-12 (FKBP-12). The sirolimus FKBP-12 complex, which has no effect on calcineurin activity, binds to and inhibits activation of the mammalian target of rapamycin (mTOR), a key regulatory kinase. This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting cell-cycle progression from the G1 to the S phase.

Induction and Maintenance Therapy in Hand Transplantation

Induction and maintenance regimens used in hand transplantation were similar to those employed in solid organ transplantation. Clinical experience of CTA showed that it does not need a particular immunosuppressive strategy. The majority of teams involved in hand transplantation performed induction therapy using antithymocyte globulins (ATG) while the others used basiliximab [18]. The results in terms of efficacy for basiliximab are on par with ATG in recipients with low risk of acute rejection, but it has less success with higher-risk patients. However, it elicits less adverse events than ATG. Since the question of the effectiveness of monoclonal or polyclonal antibody therapy remains highly controversial in organ transplantation, ischemia-reperfusion injury determined by a long cold ischemia time has to be considered in hand transplantation. It is likely that in limb transplantation this injury may be greater than in whole-organ transplantation because of operation length and because of the mass of different transplanted tissues. Recent studies showed that ATG might contribute to decrease graft cellular infiltration during acute rejection and possibly after postischemic reperfusion [19]. Moreover, there is an advantage to using polyclonal antithymocyte globulins, as they are a mixture of antibodies against lymphocyte receptors and adhesion molecules with consequent lymphocyte depletion but also significant down-regulation or binding of other receptors and new mechanisms of immunosuppression, such as apoptosis of activated lymphocytes, which could be important in tolerance induction [20].

Maintenance therapy used in hand transplantation by the large majority of teams has been a combination regimen including glucocorticoids, tacrolimus and MMF. The overall effect of combining several drugs that act by different mechanism is to achieve a powerful immunosuppressive effect with low doses of each drug, reducing drug-related toxicity. Glucocorticoids were used in all CTAs, and we have learned that it would be better to use high doses only in the initial period posttransplantation and decrease them slowly at a level of 5 mg. Although chronic use of glucocorticoids does not seem to have particular implications in CTA, as no alteration of wound nor bone healing were reported, the toxicity of chronic use of steroids is well known [21, 22], and it has been confirmed by a case of hip necrosis in a hand-grafted patient.

In solid organ transplantation, a number of studies have demonstrated that acute rejection is the primary determinant for the later development of chronic rejection, and treatment of this occurrence increases risks and costs of transplantation. For this reason, in kidney transplantation, the combination of prednisone, tacrolimus and MMF was used to provide an effective immunosuppression resulting in less rejection [23]. The same regimen was used in hand transplantation showing its efficacy and safety in experimental and clinical studies. Use of these three drugs allowed a decrease in dosage of glucocorticoids and tacrolimus, thus influencing the incidence of side-effects such as diabetes mellitus and nephrotoxicity. In addition, tacrolimus not only is known to decrease the number of acute rejection episodes compared with cyclosporine in renal allograft recipients, but it seems to accelerate axonal regeneration, increasing the synthesis of axotomy-induced growth-associated protein (GAP-43) [24] and enhance osteoblastic differentiations induced by bone morphogenic protein-4 [25]. It is very interesting to note that the association of glucocorticoids, tacrolimus and MMF has no adverse effect on vascular ingrowth; in fact, early callus formation and revascularization are normal compared with forearm replantation as well as the next phase of bone maturation to chondral and ossified callus [25].

Although sirolimus was used only in two hand-grafted patients, it may be used as rescue therapy for refractory allograft rejection or when calcineurin inhibitors determined major adverse effects. It is a powerful immunosuppressive drug with antiproliferative effect and may have a potentially important role on prevention of chronic allograft rejection in the future of CTA.

Although immunosuppressive treatments used by various hand transplantation teams achieved results that were previously considered a "miracle", careful management of the recipient is still indispensable and requires drug combination to strengthen rejection prophylaxis and reduce doses of individual drugs in order to avoid toxic effects.

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Section 7-b

Side-Effects and Potential Complications

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Introduction

Since the first hand transplantation carried out in September 1998, there has been a great deal of discussion concerning the potential risks inducted by the immunosuppressive therapy for the patients transplanted, as they need lifelong immunosuppression. This discussion has been particularly emotional, as composite allograft transplantations (CAT) are not considered as life saving whereas immunosuppressive therapy can expose the recipients to serious side-effects and life-threatening complications. Transplantation is routinely indicated and accepted for non-life-threatening situations, such as dialysis-dependent renal failure and poorly controlled diabetes, because it allows a significant improvement in patient's quality of life. Consequently, the above rationale might be applied to hand transplantation when functional recovery and improvement in quality of life are demonstrated. At this point, we must analyse whether the adverse effects of immunosuppression outweigh the benefits of reconstruction of the upper extremity. The question is then: does the improvement of function and quality of life justify the risk for the patient? To answer this question, we will try to objectively discuss the side-effects of long-term immunosuppression.

As the total number of CAT done is too small and because long-term follow-up is required to report side-effects objectively, the only way to analyse the risk is to consider data available for other organ transplantation. Considering the immunosuppressive regimen used across the world for hand transplantation [FK506: 5–10 ng/ml trough level; mycophenolate mofetil (MMF): 2–3 g/day], which is comparable to that used in kidney transplantation, we can assume that the risk of immunosuppression associated with hand transplantation is similar to that of kidney transplantation. We will therefore analyse published data from kidney transplantation to determine the risk incurred. However, we must keep in mind that recipients for human hand transplantation are not equivalent to recipients for kidney transplant, who have a higher preexisting morbidity due to renal insufficiency. Consequently, the risk of posttransplant morbidity and mortality is undoubtedly higher after kidney transplantation. Risk analysis of allogeneic hand transplantation has already been done in a very contributive paper published by Baumeister et al. [1]. The following risks are classically identified:

- Development of posttransplant malignancies;
- Posttransplant diabetes mellitus (PTDM);
- Steroid sides effects;
- Opportunistic infection;
- Pharmacological toxicity.

Development of Posttransplant Malignancies

An increased incidence of de novo malignancies due to immunosuppression in patients after organ transplantation is commonly reported [2–6]. The Collaborative Transplant Study (CTS)

[1] reported that the risk of developing cancer is approximately 3 % within the first 5 years after transplantation, which represents a 6.6-fold risk increase compared with the risk of the normal population.

Skin Cancer

Most of posttransplant malignancies (one third) are skin tumors. This risk is increased 21.3 fold at 5 years posttransplantation if compared with the nontransplanted population, and its yearly incidence can be estimated between 0.1 and 0.3%. About 50% of these skin tumors are squamous cell carcinoma.

Non-Hodgkin's Lymphoma

Lymphomas are the most serious form of early malignancies. The risk of developing lymphomas within the first 5 years after transplantation is estimated at 0.58 % and represents a 25.2-fold increase compared with the nontransplanted population. Most lymphomas occur within the first year. Survival if patients who experience lymphomas is 82% at 1 year and 69% at 5 years. Mortality is mainly due to lymphoma refractory to chemotherapy. The incidence of lymphomas in patients treated with FK506 would be no higher than reported in patients treated with cyclosporine.

Posttransplant Diabetes Mellitus (PTDM)

More often, PTDM is defined as insulin requirement for more than 30 days after transplantation or insulin requirement 6 and 12 months posttransplant. The incidence of PTDM has been reported to range from 9.7% to 19.9% [7–10], is higher for patients treated with FK506 than those treated with cyclosporine, and depends on the dose used and the blood trough level of FK506 [11]. Most patients can be weaned off insulin when FK506 and corticoids are tapered. To date, transient hyperglycemia reported by the International Hand Transplant Registry (IHTR) occurred in 50% of cases (n=9) and disappeared after FK506 was tapered [12].

Steroid Sides Effects

Cataracts, glaucoma, eye swelling, and retinitis have been attributed to steroid medication [13]. The CTS reported that the incidence of cataract in transplanted patients between ages 15 and 40 is 6% and 10% at 5 and 10 years, respectively [1]. This risk is lower than previously reported, as doses of corticoids are actually lower than a couple of years ago. Arterial hypertension and osteoporosis are common complications in kidney-transplanted patients and can be linked to corticoids but also to end-stage renal disease itself. Consequently, it seems useless to extrapolate such results from kidney transplantation to patients who experienced hand transplantation, as the latter do not suffer from any form of renal complication. The IHTR reported Cushing's syndrome in 5.5 % of cases (n=1).

Opportunistic Infection

Transplanted patients are clearly exposed to bacterial, viral, and fungal infection due to the immunosuppressive regimen, but the exact incidence of infection is not clearly reported in the literature. It is said that 25–75% of kidney-transplanted patients experience infection within the first year after transplantation. The most common infections described are caused by herpes virus, cytomegalovirus (CMV) or Epstein-Barr virus (EBV), *Candida*, *Aspergillus* and *Pneumocystis jiroveci*. Prophylaxis against CMV and *Pneumocystis* is commonly used to prevent and limit the severity of the disease when it occurs. However, in some cases, deaths caused by these opportunistic infections have been reported. IHTR reported 44% CMV incidence, including reactivation, one case of intestinal *Clostridium difficilie* and 20% cutaneous mycosis [7, 9, 14].

Pharmacological Toxicity

Nephrological Toxicity

Calcineurin inhibitors (cyclosporine and FK506) are very well known to be nephrotoxic. In kidney transplantation, nephrotoxicity is difficult to dis-

tinguish from chronic allograft rejection. However, in recipients of liver transplantation, the nephrotoxicity of calcineurin inhibitors is widely reported [15–18], with an incidence of approximately one third of patients, of whom 10% required dialysis in long-term follow-up. Nevertheless, it must be kept in mind that hand-transplanted patients are always healthy patients and that the risk of developing nephrotoxicity is probably overestimated by analysing the liver transplant population. To date, an increase in creatinine level has been reported in 11% of cases (n=2) in the IHTR.

Neurological Toxicity

Most neurological side-effects have been attributed to FK506 but are reversible in most cases by temporary discontinuation or by decreasing the dose [19]. Tremor seems to be the most frequent complication [9], occurring in more than 50% of cases, followed by headache (44%), insomnia, and paresthesia. Once more, end-stage renal disease also predisposes patients to neurological comorbidity, and neurotoxicity is probably overestimated in the hand-transplanted patients if compared with renal-transplanted patients.

Gastrointestinal Toxicity

FK506, corticoids, and MMF can cause gastrointestinal disturbance [8, 9, 13, 14, 20] ranging from nausea and diarrhoea to gastritis, ulcers, and gastrointestinal bleeding. The most frequent cause of gastrointestinal toxicity is the use of MMF. The incidence of side-effects is highly dose dependent and this is the reason why a 3 g/day regimen is usually abandoned by most transplant teams.

Conclusion

To summarise, the risk of secondary malignancies is likely to be very similar in renal and hand transplantation, with an approximately 3% incidence within the first 5 years after transplantation. Concerning the risk of posttransplant morbidity (gastrointestinal, neurological and nephrological toxicity) and mortality, it is likely to be lower in hand transplantation than that reported in renal transplantation, as recipients of a human hand are usually young and healthy.

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Section 7-c

CMV Infection and Reactivation

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Introduction

Herpes virus infections are common causes of morbidity and mortality in solid organ and haematopoietic stem cell transplant recipients. Recent innovations in diagnosis, prophylaxis and treatment have reduced the incidence of herpes virus infections during the early post-transplant period, but they continue to significantly influence the outcome after transplantation [1].

The cytomegalovirus (CMV) is a large DNA virus and member of the herpes virus family. CMV represents the most common systemic virus complicating solid organ transplantation. The virus has high species specificity and has developed together with humans, which resulted in a unique interaction of the virus with the hosts immune system. Frequently, transmission of the virus between humans occurs in early childhood. Lifestyle in Western civilizations, however, has led to a dramatic reduction in prevalence of the virus [2]. In solid organ transplantation, CMV infection and disease have been associated with acute and chronic rejection, increased risk for subsequent infections and reduced patient and graft survival [2–5]. The highest risk for CMV infection and disease has been reported for transplantation in a CMV-mismatched combination (donor positive, recipient negative for CMV) and/or for those individuals who require high-level immunosuppression [6, 7]. Moreover, the viral burden of a CMV-positive graft differs with size as well as tissue [7]. CMV disease in the immunocompromised host is characterized by fever, leukocytopenia, malaise and organ involvement such as pneumonitis, gastroenteritis or retinitis, the latter most frequently seen in HIV-infected individuals. In more severe cases, central nervous system involvement may occur. Indirect effects of CMV on the immune system result in a higher rate of opportunistic infections and trigger acute and chronic rejection [2–5]. In addition, cotransfection with Epstein-Barr virus (EBV), human herpes virus (HHV) 6 and 7, and some rarer complications including arterial thrombosis or association with malignancy have been discussed [8, 9].

Composite tissue allotransplantation (CTA) has emerged as a therapeutic option after loss of a hand. Good results have been achieved with hand transplantation with regard to sensibility and motor function; however, a level of immunosuppression comparable with pancreas, heart or kidney transplantation is required to prevent rejection [10–16]. Among all opportunistic infections, CMV infection was found to be the most common disease complicating the clinical course after hand transplantation [17]. For infectious complications other than CMV, five cases of cutaneous mycosis, one herpes simplex infection, one osteomyelitis caused by *Staphylococcus aureus* and one *Clostridium difficile* enteritis have been reported [13]. In addition, various infections were responsible for a large number of graft losses following femur/knee transplantation [18].

The prophylactic use of antiviral agents in CMV risk constellations has been strongly recommended [3]. Currently, licensed anti-CMV agents include ganciclovir, foscarnet and cidofovir. These agents together with specific hyperimmunoglobulin and new diagnostic tools have positively influenced patient morbidity and mortality through rapid diagnosis, improved treatment and introduction of novel strategies for prophylaxis. The nature of CMV infection/disease has therefore changed significantly during the past decade.

CMV: Mechanism of Action

Between 50% and 90% of healthy adult individuals are infected with CMV [2]. Once the virus has established infection, it causes a strong immunoreaction rendering it to lifelong latency within the host. CMV-induced disease occurs when the immune system has not fully developed (as in the foetus) or when it is compromised, as in allograft recipients, suggesting that the balance between virus escape and host control plays a central role in the pathogenesis [19]. CMV has evolved complex molecular mechanisms to avoid host immune detection and destruction. Collectively, these mechanisms have been termed "immunoevasion" or "escapology". The most essential mechanism for virus survival within the host is latency, a form of reversible, nonproductive infection of host cells by replication-competent virus [20].

The complex defence mechanisms against CMV include immunoglobulins, which limit viremia; however, T-cell response is the most important mechanism to destroy the replicating virus. CD8+ T lymphocytes can eliminate viral infection by induction of host-cell lysis or apoptosis [21]. The preferred targets of cytotoxic T cells are human leukocyte antigen (HLA) A2-restricted epitopes of CMV phosphoprotein pp65 with A1, A11, B7 and B35, amongst others, functioning as alternative regions [22, 23]. Little is known about the role of HLA class II alleles in this context. Intracellular viral control without destruction of host cells by secretion of cytokines, such as interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α), has been described [24]. Such a reaction is triggered by the encounter of CD8+ T lymphocytes with their cognate antigen, even in the absence of detectable CMV replication. When a cytotoxic T-cell reaction is compromised, the virus can change from latent or persistent status to lytic replication [20]. It is important in this context that intracellular pathogens, namely, herpes family viruses, are preferably targeted by major histocompatibility complex (MHC)-restricted cytotoxic T-cell reaction [25–27]. As donor and recipient are rarely HLA-identical in organ transplantation, donor-derived antigen-presenting cells cannot be recognized by recipient-specific CD8+ lymphocytes. Therefore, either donor-specific cytotoxic T cells must function as counterparts, or alternative targets for an immunologic response must be found. It is unknown whether alternative defence strategies such as the innate immune system can sufficiently control donor-derived infections. For the endothelium, it is well established that recipient-derived cells replace donor endothelium within days to weeks and that, as a consequence, antigen-presenting cells and cytotoxic T cells are of donor origin [28].

CMV: Immunosuppression and Rejection

It is well established that the type and intensity of immunosuppression are important cofactors for CMV infection. Antithymocyte antigen (ATG) is a potent TNF releaser and can therefore cause reactivation of the latent virus [29]. Furthermore, high-dose steroids as well as high levels of calcineurin inhibitors and mycophenolate mofetil (MMF) can cause an increase in virus replication by paralyzing the immune system [29]. In addition, poor pretransplant condition, retransplantation and mass transfusions are associated with an increased risk for CMV infection and disease.

The risk of developing CMV-associated complications varies profoundly depending on the

transplanted organ. The amount of tissue and its composition determine the viral load, and therefore, a renal graft contains a lower virus load when compared with a multivisceral graft including liver, pancreas and intestine. Increased incidence of acute rejection associated with CMV disease, either caused by upregulation of cytokines or by direct alteration of the graft followed by enhanced immunogenicity, has been discussed [3]. Whether the long-term effects of CMV are caused by immunological phenomena, chronic nonlytic latent infection, recurrent subclinical exacerbations of the acute disease or coinfection with a so far not classified pathogen is yet unknown.

Composite tissue allografts are believed to contain a particularly high viral load due to the large amounts of endothelial cells, white blood cells and stem cells within the graft. The required immunosuppression following composite tissue transplantation is high, and patients seem to be as vulnerable to CMV infection as recipients of a lung or small bowel [17].

Monitoring and Treatment

For decades, testing for anti-CMV-specific IgG and IgM antibodies was the only diagnostic tool available. Today, these methods are still used for pretransplant risk assessment but not for surveillance. Assays presently available and frequently used include conventional and shell-vial culture, CMV antigenaemia assay, polymerase chain reaction (PCR) for CMV DNA, hybrid capture assay for CMV DNA and detection of CMV RNA by nucleic acid sequence-based amplification [3, 26, 30]. The low sensitivity and limited reproducibility of conventional cell culture and shell-vial assays limit their role in the management of CMV infection. Diagnostic assays, such as the pp65 antigenaemia and other molecular assays, have improved the ability to detect CMV infection quickly and accurately. Early after transplantation, tests should be carried out once a week and CMV infection and disease judged according to previously proposed criteria [31]. In brief, CMV infection can be assumed when a specific assay for antigen or antibody detection is positive in the absence of clinical symptoms and CMV disease when any CMV test is positive and clinical symptoms specific for CMV disease are detected that can otherwise not be explained. Resistance to ganciclovir (GCV) can be assumed when CMV replication is detected despite GCV prophylaxis [31].

As continuous CMV monitoring has become routine in transplantation, strategies for CMV prophylaxis and treatment have been developed. Whereas for patients at normal risk administration of prophylactic versus preemptive therapy based on CMV PCR or pp65-antigenaemia assay is a matter of debate, patients undergoing CMV-mismatched transplantation or patients receiving high-level immunosuppression should receive CMV prophylaxis routinely [32, 33]. GCV is an effective, narrow-spectrum antiviral agent acting via DNA polymerase inhibition, which proved to be effective in preventing CMV infection and disease in solid organ transplant recipients and thus represents the current standard of care for prevention of CMV disease. However, GCV given i.v. is inconvenient for longer-term use, thus, oral GCV was introduced, offering an alternative to long-term intravenous application. Oral GCV proved to reduce CMV infection and disease and posttransplant CMV morbidity and mortality [34]. Nevertheless, its low bioavailability (6–10%) may be a limiting factor and predisposes for the development of GCV-resistant CMV strains [35]. Recently, valganciclovir (valGCV), the valine-ester prodrug of GCV, was developed. It delivers GCV with a bioavailability of approximately 60%, which is 6- to 10-fold higher than that of oral GCV [36]. GCV plasma concentrations similar to those with i.v. application are achieved [37]. However, despite GCV and valGCV prophylaxis breakthrough infections and diseases have been reported in up to 50% of CMV-mismatched transplantations [38]. Therefore, a combination of these drugs with anti-CMV hyperimmunoglobulin has been advocated for patients at excessive risk [39].

Neutropenia is the most common side effect of GCV. In addition, GCV-resistant CMV strains, in particular the UL97 mutant, as well as breakthrough infections caused by non-GCV-resistant

strains occur in up to 50% of patients following CMV-mismatched transplantation. Foscarnet or cidofovir, both DNA polymerase inhibitors, are valuable alternatives in this situation [40, 41]. Foscarnet has severe side-effects, such as nephrotoxicity and mucosal necrosis. Cidofovir is a broad spectrum antiviral agent active against CMV, herpes simplex virus (HSV), varicella-zoster virus (VZV), EBV and HHV6, 7 and 8 but also against the pox virus, BK and human papilloma viruses (HPV). Importantly, it is also active against GCV-resistant CMV strains such as the UL97 mutation. The major side effect again, is nephrotoxicity.

As for hand transplantation, CMV prophylaxis with GCV or valGCV for 6 months in combination with anti-CMV hyperimmunoglobulin is now considered mandatory after transplantation in a CMV-mismatch combination, which is difficult to avoid considering all factors that have to be matched between donor and recipient [17]. For treatment of CMV infection, immunosuppression should be reduced in addition to GCV/valGCV treatment. Two more toxic drugs, foscarnet and cidofovir, should be considered third-line intervention.

CMV Infection in Hand Transplantation

CMV infection and disease represent severe complications negatively affecting the long-term outcome in solid organ transplantation [3, 4, 42–44]. As for hand transplantation, a graft from a CMV-positive donor may contain a larger viral load when compared with solid organs, such as the kidney or the liver, as the amount of endothelial cells is high and haematopoietic precursor cells from the bone marrow may host latent CMV. In addition, severe immunosuppression and usually poor HLA match limit antiviral T-cell response required for virus control [45, 46]. This may explain in part why antiviral prophylaxis and/or treatment are insufficient to eliminate the virus in some of these patients. It may be assumed that CMV is the most relevant infectious complication following hand transplantation.

In a previous study, we retrospectively analyzed CMV match, infection, incidence and treatment of all hands, forearms and digits transplanted worldwide and provided a comprehensive description of patients experiencing CMV infection or disease after CTA transplantation [17].

Pertinent Data

Since the new era of human hand transplantation began in 1998, 23 hand and 1 thumb transplantations in 18 male patients have been reported to the International Registry on Hand and Composite Tissue Transplantation (IRHCTT) [13]. Recipients'mean age was 32 years, and time between hand loss and transplantation was 5.4 years. Mean donor age was 33 years. In 17 cases, donors and recipients were tested for CMV prior to transplantation. However, as the relevance of CMV infection in these patients was not known previously, CMV status was not used as a criterion for donor selection. Donors and recipients were matched for blood group, size, texture and cosmetic appearance. Mean HLA mismatch was 4.75, and lymphocytotoxic crossmatch was negative in all cases. Ischaemia time varied between 150 min and 720 min.

As for immunosuppression, induction with ATG or an IL-2 receptor antagonist was given in 15 patients. MMF and steroid bolus was given in all patients together with a calcineurin inhibitor. Maintenance immunosuppression included calcineurin inhibitors (CyA, tacrolimus), MMF and steroids in 15 patients. One patient was treated with tacrolimus and steroids only, in one patient rapamycin and MMF were administered and one patient received rapamycin only. Steroids and tacrolimus were used topically in some centres. For the treatment of rejection, tacrolimus or corticosteroid ointment was used in all cases. In addition, steroids, ATG or Campath-1H were used, depending on rejection severity and treatment response.

All patients received wide-spectrum antibiotics early after transplantation, prophylaxis for *Candida* and/or *P. carinii* infection was given to 50% of patients. All patients receiving a graft

from a CMV-positive donor or being positive for CMV themselves (or both) received prophylaxis for CMV.

Prior to transplantation, 35.3% of donors and 35.3% of the recipients were positive for CMV (17 donors/recipients tested). CMV match was negative/negative in 8 patients, negative/positive in 3, positive/positive in 3 and positive/negative in 3. Seven patients received GCV i.v. at 10 mg/kg BW (Body Weight) followed by oral GCV (3 g/day) or valGCV (900 mg/day) for prophylaxis. After transplantation, CMV replication was found in 5 of 11 patients tested, and 5 of 9 cases where either donor or recipient were positive for CMV prior to transplantation. Among the 3 CMV-negative patients receiving a CMV-positive graft, 2 patients developed CMV disease.

Between 0 and 5 (mean: 1.6) rejection episodes were encountered, all of which were completely reversible in compliant patients. High-dose steroids were administered systemically as first-line treatment in addition to steroid and/or tacrolimus ointment in all patients. Treatment of progressive or repeated rejection varied between centres and patients [10–16].

For a cohort of six hand transplant recipients, detailed clinical courses were analyzed. Three of the 6 recipients and 4 of 6 donors were CMV positive. Donor/recipient CMV match was negative/negative (n=1), positive/positive (n=2), negative/positive (n=1) and high-risk positive/negative mismatch was given in two cases. Antiviral prophylaxis consisted of GCV (10 mg/kg) i.v. for 1 week and 3/1,000 mg GCV (n=3) or 1/900 mg valGCV (n=1) orally thereafter. Foscarnet and cidofovir were used for second-line treatment of CMV infection/disease.

Clinical Courses

The first patient in this series was transplanted in the high risk positive/negative mismatch combination. Prophylaxis for CMV was applied according to the protocol described above. The patient experienced a biopsy-proven tissue-invasive CMV disease with abdominal pain and diarrhoea at 15 weeks after transplantation. GCV i.v. was given, followed by oral GCV for 9 months. Clinical symptoms disappeared soon; however, CMV DNA assays remained positive for 9 months. The first rejection episode in this patient was encountered on week 6; second and third rejection episodes were observed following CMV disease (week 20 and 27). All episodes resolved completely upon treatment with i.v. methylprednisolone or oral prednisone together with topical tacrolimus and clobetasol [10].

The second patient was transplanted in a high-risk CMV-mismatch combination. Despite GCV prophylaxis, he developed a CMV infection with excessive virus replication on day 34. Foscarnet was given, and virus replication was effectively abolished. However, nausea and diarrhoea prompted discontinuation of foscarnet treatment, and oral GCV was thus restarted. A second breakthrough infection was observed on day 78 and virus replication associated with fever and malaise. A GCV-resistant UL97 mutation was suspected, but PCR amplification and sequencing proved sensitivity to GCV. GCV given i.v. prevented CMV activity during the time of application; however, virus replication recurred whenever the drug was administered orally. After two treatment courses with cidofovir, the patient finally became negative for CMV and has remained CMV negative since then. Interestingly, an acute rejection episode was observed early after the first and second CMV infection.

In the third case, the donor was negative but the recipient positive for CMV prior to transplantation. CMV infection was first detected on day 53. At that time, the patient was still on oral prophylaxis. GCV application was switched to i.v. for 10 days. Subsequently, GCV was again given orally together with anti-CMV hyperimmunoglobulin but failed to eliminate the virus. Treatment with foscarnet was started and continued until antigenaemia became negative 3 weeks later. On day 149, CMV replication was again demonstrated by antigenaemia assay and cidofovir was given for 3 months. Again, three rejection episodes occurred at the same time as CMV replication was noted, and the virus was suspected to have triggered graft rejection. After CMV had become negative, no more episodes of rejection were observed.

Both donor and recipient were CMV positive in the fourth patient, and CMV prophylaxis with GCV was initiated immediately after transplantation. At 5 weeks, CMV infection became apparent. Foscarnet was given for 14 days but had no effect on virus replication. Also, cidofovir failed to overcome CMV; instead, CMV antigenaemia assay further increased to 1,200/200,000 leukocytes. At this point, anti-CMV hyperimmunoglobulin was given together with foscarnet, which led to reduction of CMV replication. However, complicating side-effects, such as an increased creatinine, nausea and vomiting, forced placing the patient back on GCV. Subsequently, CMV antigenaemia levels increased again, and foscarnet was readministered for 2 weeks followed by GCV i.v. at a dose adjusted to creatinine clearance. On day 152, MMF and steroids were discontinued and tacrolimus reduced (blood trough levels 5–7 ng/ml). In response, CMV dropped to very low levels. When treatment with valGCV was initiated and continued for 12 weeks, the patient finally became CMV negative. In this patient, one steroid-resistant rejection had occurred prior to CMV replication.

Donor/recipient CMV status was positive/positive in patient number 5, and prophylaxis with GCV i.v. was started immediately after transplantation and switched to valGCV according to a previously designed protocol. On day 137, the patient developed neutropenia. Granulocyte colony-stimulating factor was ineffective for restitution of leukocyte count. Myelotoxicity of valGCV was assumed and the drug withdrawn. In response, leukocyte count returned to normal. Five weeks later, however, the patient developed CMV infection, and cidofovir was commenced together with anti-CMV hyperimmunoglobulin. This treatment resulted in a sustained response but was accompanied by oedema of both transplanted hands thought to be related to the hydration protocol accompanying the drug. This oedema resolved spontaneously a few weeks later. Three rejection episodes were observed on days 10, 46 and 95. The third was severe and progressed rapidly under steroid as well as ATG treatment. Hence, Campath-1H was given, and lesions disappeared completely within 2 weeks [16].

In a sixth patient, no antiviral prophylaxis was given, as donor and recipient were CMV negative. One mild rejection episode occurred on postoperative day 76 and responded promptly to systemic methylprednisolone. No more episodes of rejection and no CMV infection were seen throughout the entire observation period of more than 5 years.

Conclusions and Recommendations

As for CTA, tissue-invasive CMV disease in a hand transplant recipient reported by the Louisville group provided first evidence for the clinical relevance of CMV in this field [10]. Among all 18 recipients of a hand, 11 were tested for CMV after transplantation. Of these patients, 45.5% tested positive for CMV at more than one time point. Both patients transplanted in the high-risk CMV-mismatch combination developed CMV disease. In the patients outlined here in more detail, CMV infection or disease complicated the postoperative course after CTA transplantation in all but the CMV negative/negative patient. In two patients receiving a graft from a CMV-positive donor, viral loads were high when compared with recipients of, for example, a kidney.

For CMV testing, PCR has been introduced to clinical application during the past 3 years. PCR testing was not routinely performed after hand transplantation; however, for future protocols, PCR testing should be done in CTA recipients, as CMV seems to be particularly relevant in this setting. In these patients, low-level replication within the graft without systemic infection and therefore a low level of pp65-positive cells in the peripheral blood must be considered. Hence, serum or plasma PCR might be superior to any other assay in CTA recipients.

Drug toxicity became clinically relevant in particular following the switch from GCV to either foscarnet or cidofovir that became necessary when CMV replication was unaffected by GCV treatment. When the CMV strain was isolated and tested for the GCV-resistant UL97 or UL58 mutation in two patients, no such mutation was found. Treatment failure despite GCV susceptibility was assumed. High-level immunosuppression is sus-

pected to have contributed to treatment failure. In such a situation, GCV alone may not be sufficient to control the virus. In some patients, GCV prophylaxis or maintenance therapy was associated with neutropenia and in one case valGCV had to be discontinued. Neutropenia, however, might also be caused or aggravated by CMV disease itself, and it remains unknown whether CMV and/or GCV are responsible for the low leukocyte count. In one patient, cidofovir had to be withdrawn because of a significant increase in serum creatinine.

Despite the limited number of patients transplanted, CMV and its close correlation with acute rejection represents an important issue in hand transplantation. At least one acute rejection occurred in all five patients with CMV infection/disease, and a close time correlation between virus replication and rejection was observed. In some patients, CMV infection/disease preceded acute rejection. In the Louisville hand transplant recipient, biopsy-proven CMV enteritis was seen at week 15 after transplantation, and a first rejection episode followed 2 weeks later [10]. In other patients, CMV infection was followed by repeated rejection episodes although immunosuppression was maintained at the same level, which led to the assumption that the virus may have triggered the immune response against the graft. An association between CMV and a higher incidence of acute rejections after solid organ transplantation has been discussed frequently, but no causal relationship has been conclusively proven [3].

In contrast to the lung or the small bowel, a hand has not primarily been affected by CMV disease so far, and no skin disease following organ or stem cell transplantation has been attributed to CMV. In the immunocompetent host, however, acute CMV disease might initially present with skin lesions. Therefore, differentiation between CMV disease of the skin and acute rejection should at least be taken into consideration. The role of CMV in altering other structures of CTA, such as tendons, bone, muscle, nerves and vascular structures, is completely unknown and warrants further investigation. In addition, CMV has been shown to accelerate development of chronic rejection, e.g. in the heart, lung, liver or kidney [47, 48]. This effect might also be relevant for CTAs [4, 11].

The treatment modalities applied were effective in controlling the virus. However, in hand transplantation, CMV infection turned out to be a major problem, and patients who either received a graft from a CMV-positive donor or experienced CMV infection in the past are at high risk for developing CMV infection or disease. High-level immunosuppression and the high viral load of CTAs are considered particularly relevant in this context. Since the performance of prospective randomized clinical trials for this kind of transplantation might not be realistic in the near future, retrospective data need to be collected to serve as the basis for developing future clinical protocols. Based on the observations, made it was concluded that despite availability of effective antiviral agents, in hand transplantation, CMV and in particular a CMV-mismatch combination (positive donor, negative recipient), represent important risk factors. Given the severity of immunosuppression in these patients, additional protection against EBV-associated complications might be desirable. Adequate reduction of EBV viral loads has been shown for GCV. Further, immunoglobulins have been demonstrated to reduce the incidence of posttransplant lymphoproliferative disease (PTLD) following liver transplantation. As dose reduction of GCV for neutropenia was necessary in patients receiving GCV for a prolonged period, the addition of immunoglobulins seems to be justified.

It was proposed that CMV-mismatched hand transplantation should be avoided whenever possible [17]. Waiting for a CMV-negative donor could spare cost-intensive, long-term CMV prophylaxis and treatment. Such a concept seems plausible, as only one third of all hand donors were CMV positive. In addition, anti-CMV prophylaxis with valGCV for 6 months in combination with anti-CMV hyperimmunoglobulin should be mandatory after transplantation of a hand from a CMV-positive donor. For treatment of CMV infection, immunosuppression should be reduced in addition to GCV/valGCV treatment. Application of the two more toxic drugs, foscarnet and cidofovir, should serve as third-line therapy.

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Section 7-d

Specific T-Cell Response to HCMV Infection

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Introduction

The first isolation of human cytomegalovirus (HCMV) in a renal transplanted patient dates back to 1965. Since then, this infection has been one of the most challenging for immunosuppressed patients. In spite of the danger represented by this herpes virus, the specific cellular response induced by it in affected humans has been detected and investigated in its deep mechanisms only recently.

Evidence for the role of T cells in the immune control of CMV infection is proved by uncontrolled viral replication in immunocompromised patients with impaired T-cell function [1]. The importance of both CD4 and CD8 HCMV-specific T cells in this immunity process has been remarkably demonstrated, assessing the absence of both cell populations in seronegative healthy patients [2]. The cellular-mediated immunological answer was first analysed in immunocompetent patients. During the primary CMV infection, naïve T cells follow a proliferation and differentiation pattern until they become effector and then memory cells. According to Van Lier et al. [3], the differentiation model for specific CMV CD8 T cells is achieved through an early (CD27+CD28+), an intermediate (CD27–CD28+) and a late stage. This last stage is represented by fully differentiated effector T cells with high perforin content, ability to secrete interferon gamma (IFNγ) and ability to mediate cytotoxicity. According to this model and to a general consensus, it is believed that most fully developed HCMV-specific CD8+ T cells are CD27– CD28–CD57+CCR7–, and this phenotype is believed to induce immune control [4–6].

The role of CD4 HCMV-specific T cells has also been studied, first of all in healthy patients. IFNα-secreting CD4 T cells are an active pool of cells rising both during the primary infection – in contrast to what happens in other virus primary infections (i.e. HIV) – and in the chronic phase of the CMV infection. Almost 90% of these CD4 T cells are CCR7– and therefore belong to the effector memory cell population [7].

Recent studies have helped in understanding the specific T-cell response during primary infection in symptomatic and asymptomatic transplant recipients under immunosuppressive treatment [8, 9]. In asymptomatic patients, CD4 IFNγ T cells (CCR7–) appear and rise at a maximum of 10 days after detection of positive CMV DNA in blood. Seven days after that, immunoglobulin (Ig) M and IgG are detected in blood whereas 14 days posterior to that detection, specific CD8 cells appear. CD45RA–CD27+CCR7– are the predominant CD8 effector population in the acute phase of protective immune reactions to CMV and appear to be functionally competent.

In symptomatic individuals the CMV-specific effector-memory CD4+ T-cell response is delayed and may be present only after antiviral therapy. Clinical overt disease in these patients suggests that functional CD8+ T cells are not enough to control viral replication and that the rise of effec-

tor-memory CD4+ T cells is needed to solve the infection [10]. This last affirmation has clearly been confirmed by other studies [2, 8].

In case of chronic virus reactivation, Dunn et al. showed how, in immunocompetent patients, modulation of both HCMV-specific CD4 and CD8 is involved in smouldering chronic infection [11]. Details of CD8+ action in immunocompetent patients have demonstrated how chronic infection can be controlled: it seems possible to create small clones of efficient CMV-specific cytotoxic T lymphocytes or to compensate less functional cells by upregulating the size of the T-cell clones [12]. Discordant opinions have been reported upon behaviour and balance of specific CD4 and CD8 repertoire in chronic infection and reactivation in immunosuppressed patients. Engstrand et al. report the hypothesis of a present but less functioning CD8+ cytotoxic T-cell repertoire [13]. There are studies demonstrating CD8 prevalence among the specific HCMV immune population in chronic disease, which could be due to CD4 reduced proliferative capacity or their greater susceptibility to apoptosis [14, 15], but this thesis is not consistent with other groups' findings [16].

Investigations may, as a matter of fact, be undermined by technical difficulties. Whereas general T-helper activity is traced with standard and assessed techniques [17, 18], analysis of specific HCMV leukocyte response is more difficult. Many techniques have been experimented with for this purpose. Chromium release assays and limiting dilution were very common until recent years, but both are time consuming and not as sensitive as was hoped [19]. Proliferation assay requires few cells but does not allow precise quantification of frequencies of helper CD4 T cells and is difficult to standardise; the same happens with the possibility of intracellular cytokine staining that needs flow cytometry analyses [20]. HLA-peptide tetrameter technology and epitope peptide stimulation allows direct staining of circulating CD8+ T cells, but the technique does not show complete response against the virus and requires that both patient HLA type and viral epitopes be known. Limited availability of defined peptide epitopes (class II tetramers especially) and lack of functional correlation prevents wide-spread use of the technique [21]. The use of a carboxy fluorescein succinimidyl ester (CFSE) has been developed to detect mononuclear cell evolution after antigen-specific stimulation and has great use in characterising the type of cells using multiple markers [20]. The enzyme-linked immunospot assay (ELISPOT) allows delineation of the functional properties of T cells and their ability to secrete cytokines after antigen stimulation. It has a high degree of sensitivity and is good for quantitative but not for qualitative evaluation of antigen-specific responses [19].

In 2000 and 2001, a new method made it possible to analyse both CD4 and CD8 T cells using a whole protein spanning peptide pools regardless of HLA type and known epitopes. Lacking in this method is the opportunity to enable simultaneous quantification of HCMV-specific CD4 and CD8 T-cell responses to multiple viral antigens [22, 23]. Recently, the properties of dendritic cells (DC) as antigen-presenting cells have been used to detect specific T-cell response to HCMV. Immature DCs are infected with an endotheliotropic and leukotropic HCMV strain and then used as a stimulus to determine functional HCMV-specific CD4 and CD8 cells. Infected DCs are cocultured with autologous peripheral blood mononuclear cells, and both arms of T-cell activation are determined by intracellular flow cytometry analysis of IFNγ production [24].

With this last method, T-cell response to HCMV has been analysed in an Italian transplanted patient. The patient is an HCMV D+/R– recipient and was transplanted in 2002 at the age of 33, 10 years after a car crash that amputated his dominant right hand at wrist level. Being a high-risk patient for HCMV infection, standard valganciclovir prophylaxis was applied perioperatively. In spite of this, the patient experienced repeated recurrences of disseminated HCMV infection for over 3 years posttransplantation. Careful monitoring and preemptive treatment with valganciclovir avoided overt HCMV disease, but virologic parameters (DNAaemia, viraemia and antigenaemia) in blood were consistently positive during the follow-up period. The prolonged and repeated episodes of HCMV disseminated infec-

tion suggested an impairment of HCMV-specific T-cell-mediated immune response.

HCMV-specific CD4+ and CD8+ T-cell response has been evaluated by cytokine flow cytometry following incubation of patients' peripheral blood mononuclear cells with autologous dendritic cells infected with a dendritic cell tropic HCMV strain. The frequency of CD4+ and CD8+ bright T cells producing IFNγ in response to HCMV stimuli was calculated by subtracting the value of the sample incubated with mock-infected culture medium or control antigen (consistently <0.05%) from the test value. To determine the total number of HCMV-specific CD4+ and CD8+ T cells, the percentages of HCMV-specific T cells positive for IFNγ were multiplied by the relevant absolute CD4+ and CD8+. The patient showed absolute CD4+ T-cell counts consistently <100 for over 36 months following transplantation. HCMV-specific CD8+ T-cell response was below the cutoff value until 19 months posttransplantation. HCMV-specific CD4+ T-cell response was impaired until 34 months posttransplantation. Coincidental with the lack of HCMV-specific CD4+ and CD8+ T-cell response was the consistent positivity of viral parameters in blood.

In conclusion, as stated from previous analysis of a solid-organ transplant recipient, HCMV-specific immune response might be impaired in individual D+/R– hand-transplant recipients due to the aggressive immune suppressive regimen required to control rejection episodes. In these cases, antiviral treatment alone appears unable to control repeated recurrences of HCMV infection. Careful monitoring of HCMV-specific T-cell-mediated response should parallel the monitoring of HCMV load in transplant recipients and should be included in protocols for preemptive treatment of HCMV infections.

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Section 7-e

Ocular Complications after Hand Transplantation

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Introduction

To our knowledge, there are no publications regarding ocular complications following hand transplantation. The reasons are that this is a recently developed practice, cases are numerically limited and follow-up is necessarily short. However, one can presume that these complications can be considered comparable with the wider cases available for liver, kidney and heart transplant patients undergoing similar immunosuppressive therapy.

Theoretically, drug dosages used post hand transplantation should be higher compared with solid-organ transplantation due to the composite tissue and hence the greater immunogenic nature of the transplanted hand. However, this is not the case because, as opposed to heart, lung, liver and kidney, the hand is visible and easily inspected so that any rejection episode can be diagnosed in the initial phases. Therefore, lower drug dosage is permissible and thus comparable to solid-organ transplantation [1]. Immunosuppressive therapy is the direct cause of ocular complications in transplant patients. For this reason, those complications will be discussed relative to each drug (Table 1).

Table 1. Possible ocular complications due to immunosuppressive therapy

Drug or condition	Possible complications
Steroids	
	Posterior subcapsular cataract
	Central serous chorioretinopathy
Increased intraocular pressure	
Tacrolimus	
	Cortical blindness
	Optic neuropathy
Cyclosporine	
	Microvascular retinopathy
	Optic nerve head oedema
	Cortical blindness
Infectious complications	
	Cytomegalovirus retinitis
	Herpes zoster retinitis
	Candida endophthalmitis
	Aspergillus retinitis
Ocular surface complications	
	Herpes simplex keratitis
	Sterile and infectious corneal ulceration
	Keratoconjunctivitis sicca
	Bacterial and viral conjunctivitis
Pseudomembranous	conjunctivitis
Diabetes	
	Diabetic retinopathy
	Cataract
	Neovascular glaucoma
	Neuroophthalmic disorders

Immunosuppressive Drugs that May Cause Ocular Complications

Steroids

Prolonged use of glucocorticoids is a significant risk factor for the development of *posterior sub-*

capsular cataract (PSC). Its incidence after renal transplantation varies from 22% to 78% [2, 3]. It appears bilaterally and has some peculiar characteristics, as it occupies the polar region of the posterior cortex extending forward in an irregular fashion. Its borders are usually sharp. Black et al. observed that PSC developed only after a patient had been on high-dose steroid treatment for longer than 1 year [4]. However, early cataractous changes can be noticed within the first year [5]. Even though a link between systemic steroid use and PSC is evident and apparently dose dependent, the mechanism for opacification is unknown. Attempts have been made to adapt mechanisms proposed for other types of cataract, such as oxidation (glucocorticoids may affect the activities of mechanisms involved in protection of the lens from oxidative stress) osmotic change (steroids may inhibit the sodium–potassium (Na–K) ATPase pump, giving rise to localised water accumulation and refractive index fluctuations) or protein adduct formation (steroid–protein adducts may aggregate due to disulfide bonds and nonspecific hydrophobic interactions, producing light scatter). However, the specific clinical manifestation of steroid cataracts make it unlikely that any of these would be applicable, lacking convincing experimental evidence. Another interesting hypothesis concerns ocular growth factor imbalance, leading to aberrant cell behaviour [6].

Compelling evidence links glucocorticoids and *central serous chorioretinopathy (CSCR)*. In 2004, Haimovici et al. described a strong association between systemic steroid use and CSCR by use of multivariate analysis. The odds ratio was 37.1 [7].

CSCR is characterised by serous detachment of the neurosensory retina associated with retinal pigment epithelial (RPE) detachment at the posterior pole. Glucocorticoids may initiate a first attack of CSCR, exacerbate an ongoing episode or cause a recurrence. The occurrence of CSCR is described 1–6 months after systemic steroid use [8, 9]. Decreasing or discontinuing the drug usually leads to resolution of the detachment. The link between CSCR and corticosteroids is impressive, but the biological basis is not known. Corticosteroids may probably influence transcription and expression of adrenergic receptor genes. Choroidal hyperpermeability, involved in the development of CSCR, seems to be due to the interaction between catecholamines and adrenergic receptors within the vascular bed. CSCR is associated with type A personality, stress and hypertension. All these conditions are characterised by elevated levels of endogenous catecholamines [10]. Patients who experience CSCR show decreased visual acuity and may progress to the chronic stage of the disease, with irreversible lesion of the fundus. Moreover, CSCR may be associated with severe complications, such as choroidal neovascularisation, bullous retinal detachment, central RPE alteration and RPE tear.

Steroids may cause a dose-dependent *increase in intraocular pressure (IOP)* in about 10% of patients after renal allograft [1]. The mechanism is not known. However, it has been suggested that it is related to a biological effect mediated by activation of steroid receptors on the trabecular meshwork cells and the resulting deposition of extracellular material, including myocilin and collagen [11]. IOP usually occurs some weeks after steroid intake. However, individual variability is considerable. If therapy is received for several months, some patients continue to have high IOP, even if the steroid is withdrawn [11].

Glaucoma is more often associated with topical ocular or periocular steroids than with systemic steroids. However, it is mandatory that patients receiving steroids are evaluated and eventually treated to prevent development of glaucomatous optic nerve damage.

Tacrolimus (FK506)

Tacrolimus may have a neurotoxic effect through mechanisms that are still unclear. Some theories suggest direct neurotoxic effect, similar to cyclosporine (CsA), or vasoconstriction mechanism through alteration of prostacyclin–thromboxane interactions, or a combination of the two

Acute cortical blindness, associated with bilateral occipital white matter lesion, has been reported as a potential early complication of

tacrolimus therapy, generally reversible a few weeks after discontinuation of the drug [12–14].

Bilateral optic neuropathy has been recorded in a patient on tacrolimus therapy 3 months after liver transplantation [15]. The clinical features resembled ischaemic optic neuropathies. This complication is not reversible despite discontinuation of the drug [16].

Preclinical toxicity studies in rats showed that tacrolimus may cause *cataracts* due to accumulation of sorbitol into the lens secondary to the diabetogenic effect of the drug [17]. It is supposed that cataracts would not develop with tacrolimus if diabetic parameters are under control.

Cyclosporine

Although CsA is not routinely used following hand transplantation, possible severe ocular complications associated with its use should be carefully considered by physicians. CsA has been implicated in some ischaemic fundus lesions due to the induced capillary/arteriolar damage. In vitro evidence suggests direct endothelial injury by CsA [18].

The clinical manifestation of these ischaemic lesions is a marked *microvascular retinopathy* with multiple cotton-wool spots, retinal haemorrhages, macular stars and retinal oedema. Visual deterioration usually appears 10–29 weeks after starting CsA treatment [19].

CsA has been associated with the development of *optic nerve head oedema*, in some cases associated with pseudotumor cerebri.

CsA has also been shown to cause neurologic side-effects, such as *cortical blindness*. Occipital white matter seems to be susceptible to the neurotoxic effect of CsA (similarly to tacrolimus) [20]. Withdrawal of CsA is associated with resolution of these clinical entities: recovery of vision is almost immediate in the case of cortical blindness while retinal lesions resolve after several months

Transient unilateral or bilateral *sixth nerve palsies and ptosis* can be seen after the use of CsA [21]. Furthermore, the rate of steroid-induced *cataracts* increases with the use of CsA [22].

Other Complications Associated with Immunosuppressive Therapy

Posterior Segment Infectious Complications

The use of immunosuppressive agents, such as mycophenolate mofetil (MMF), CsA, methylprednisolone and tacrolimus, has been associated with an increased risk of *cytomegalovirus (CMV) retinitis*. Its incidence after solid-organ transplantation ranges between 2% and 15% [23, 24]. CMV retinitis is a potentially destructive retinal infection that typically responds to ganciclovir therapy [25, 26].

Another cause of viral retinitis is herpes zoster. *Herpes zoster retinitis* clinically assumes the features of acute retinal necrosis and responds to intravenous acyclovir therapy [20].

Fungal infection may occur: *Candida endophthalmitis* and *Aspergillus retinitis* have been reported after solid organ transplantation.

Toxoplasmic retinochoroiditis is also reported. This is often a reactivation of existing ocular toxoplasmosis [20].

Tuberculosis remains an important endemic infection in some countries, where the incidence of systemic tuberculosis following renal transplantation is high. Furthermore, the infection is commonly disseminated or is extrapulmonary. Though few cases have been reported, the risk of ocular involvement should be considered [27].

Ocular Surface Complications

Ocular surface complications are often described secondary to the use of immunosuppressive agents. They typically include: *herpes simplex keratitis activation*, *sterile* and *infectious corneal ulceration*, *keratoconjunctivitis sicca*, *bacterial* and *viral conjunctivitis* and *pseudomembranous conjunctivitis*. Ocular surface complications are common and often resolve with topical treatment. The application of artificial tears and/or local retinoic acid may alleviate dryness.

Immunosuppressants and Diabetes Complications

Posttransplant diabetes mellitus has emerged as a major adverse effect of immunosuppressants [28]. Subsequently, ocular microangiopathy may develop. CsA and tacrolimus may cause posttransplant diabetes mellitus by a number of mechanisms, including decreased insulin secretion, increased insulin resistance or a direct toxic effect on β cells. For corticosteroids, the induction of insulin resistance seems to be the predominant factor. However, few studies have examined the mechanism of diabetogenicity at the molecular level. Tacrolimus causes a high incidence of posttransplant diabetes mellitus in recipients of kidney transplants (up to 20% in some reports) [29], and the diabetogenicity of CsA-based regimens is comparable with that of tacrolimus-based regimens in recipients of liver transplants [30].

Ocular complications of diabetes mellitus are numerous and include diabetic retinopathy, cataract, neovascular glaucoma and neuroophthalmic disorders. The most frequent complication of diabetes and leading cause of vision loss is diabetic retinopathy. Diabetes duration is the major risk factor in the development of diabetic retinopathy. Considering the fact that hand transplant patients are usually young, the duration of immunosuppressant-inducted diabetes may be significant, and hence the possible development of diabetic retinopathy should be considered. Diabetic retinopathy involves vascular and neural damage in the retina. It is divided into two categories: nonproliferative and proliferative. *Nonproliferative diabetic retinopathy* is characterised by abnormalities of retinal circulation, including microaneurysms, intraretinal hemorrhages, cotton-wool spots, retinal oedema and exudates and intraretinal microvascular abnormalities. The most common cause of visual loss during nonproliferative diabetic retinopathy is macular oedema. Patients with macular oedema may be asymptomatic or may complain of blurred or distorted central vision. Ophthalmoscopic examination reveals retinal thickening that is often associated with lipid exudate, microaneurysms and intraretinal hemorrhages. *Proliferative diabetic retinopathy* is characterised by the proliferation of newly formed blood vessels from the optic disc, retina or iris as the result of widespread retinal ischaemia. The vitreous plays a critical role in the development and progression of proliferative diabetic retinopathy. As the posterior vitreous detaches, traction on the fibrovascular tissue increases. This may result in recurrent vitreous hemorrhage, traction retinal detachment, or both.

Severe and moderate vision loss from diabetes are essentially preventable with timely detection and treatments, careful long-term follow-up and comprehensive diabetes mellitus care firmly based on clinical evidence.

Preliminary Experience in Hand-Transplanted Patients

Three patients received unilateral hand transplantation at the Hand Surgery and Reconstructive Microsurgery Unit of the University of Milan-Bicocca (Monza, Italy) and were prospectively followed up for ocular complications. All patients were men aged 32, 33 and 35 years. Follow-up was 52, 36 and 61 months, respectively. Immunosuppressant therapy for each patient included: prednisolone (5 mg/day, 2.5 mg/day, 5 mg/day), tacrolimus (6 mg/day, 9 mg/day, 5 mg/day), MMF (1,500 mg/day, 750 mg/day, 1,000 mg/day). During the follow-up the patients received ocular examination at regular intervals.

Patient 1 did not show any relevant ocular involvement. In patient 2, a peripheral retinal hole was diagnosed 12 months after transplantation and promptly treated with laser photocoagulation. However, this condition was considered not to be related to immunosuppressive therapy. At the moment of hand transplantation, patient 3 suffered from ocular hypertension treated with a topical β-blocker (timolol 0.5%). Five years after surgery, IOP is controlled with the same topical monotherapy without any relevant change in the visual field, as evaluated with automated perimetry.

Conclusions

In summary, the incidence of ocular complications, such as cataract, increased IOP and minor corneal and conjunctival complications, after immunosuppressive treatment may be significant. Although occurrences of severe retinal complications or neurotoxic cases are relatively rare, they can potentially result in devastating visual loss. Understanding these clinical entities is an important initial stage in minimising potentially sight-impairing complications. Therefore, we suggest that patients receiving immunosuppressants after hand transplantation should undergo routine ophthalmologic evaluation, especially during the first year of therapy. Patients scheduled for hand transplantation and postoperative immunosuppressant therapy should receive visual acuity measurement, complete eye examination, automated perimetry and colour fundus photography before and every 3 months after transplantation for the first 2 years. Diabetic retinopathy should be investigated if diabetic parameters have not been kept under control for a long time. Ancillary examinations should be performed in selected cases when indicated.

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8. Limb Rejection and Monitoring

Section 8-a

Skin Rejection in Human Hand Allografts: Histological Findings and Grading System

Jean Kanitakis

Introduction

Composite tissue allotransplantation, i.e. allotransplantation of heterogeneous non-organ tissues containing skin, muscles, bones, tendons and vessels, has been experimentally performed in animals for several decades, with reports dating back to the beginning of the twentieth century [1]. With the advent of cyclosporine, limb allografts were tried again in primates in the 1980s but resulted invariably in more or less rapid immunological rejection, manifesting mainly on the skin [2, 3]. However, discovery of safer and more efficient immunosuppressive drugs, such as tacrolimus and mycophenolate mofetil (MMF), along with advances in (micro)surgical techniques, has made allotransplantation of composite tissues possible in humans, opening a new era for replacement of missing tissues due to traumatic or postoperative loss and congenital defects [4, 5]. Until now, allografts of vascularised tendon [6], nerve [7], veins [8], muscle [9], femur, knee [10, 11], larynx [12], intestine and abdominal wall [13], facial skin and ears [14] and tongue [15] have been performed in humans. Very recently, a partial allotransplantation of the face was performed in France.

Successful allografting of hands in humans was predicted to occur before the end of the twentieth century [16]. In 1963, a hand allograft was performed in Ecuador before the era of modern immunosuppression but, not surprisingly, it was rapidly rejected and amputated two weeks posttransplantation [17]. The first successful (single) human hand allograft (HHA) was performed in Lyon in 1998 by an international team headed by J.M. Dubernard [18, 19]. To date, 24 HHAs have been performed in eight medical centres worldwide (11 monolateral and four bilateral hand transplantations, two bilateral forearm transplantations and one thumb transplantation) [20, 21]. HHA, by virtue of its complex structure encompassing several tissues of variable antigenicity (skin, muscles, vessels, nerves, tendons, bones) can be considered the "gold standard" of composite tissue allografts (CTA).

The success of any CTA depends on adequate functional recovery and prevention of allograft rejection. The combined use of older immunosuppressants (such as steroids and azathioprine) and more recent ones (such as cyclosporine A, MMF, tacrolimus and rapamycin) can efficiently prevent rejection of human CTA although the balance between tolerance and rejection remains subtle and needs to be continuously evaluated. Experience obtained from limb allografts in animals suggests that each component of a CTA interacts with the host immune system with a special degree of antigenicity, with the skin behaving as the most antigenic [22]. This was subsequently confirmed by clinicopathological observations of human-skin-containing CTA (namely HHA), showing that skin is preferentially affected during periods of graft rejection [23].

Thus, the pathologic study of CTA is important for at least two reasons. The primary one is early detection of graft rejection; indeed, experience obtained so far strongly suggests that clinical and pathological monitoring of the skin is the most reliable way to detect allograft rejection and is more sensitive than clinical signs (inflammation, fever) or other biological tests [such as C-reactive protein (CRP) and anti-human leukocyte antigen (HLA) antibodies]. The second is that pathological study of the CTA may confirm its structural integrity, which is a prerequisite for good allograft function; it may also show whether allograft cells (including immunologically relevant ones) are in the mid- or long-term replaced by cells of recipient origin, therefore rendering the allograft less antigenic towards its host and allowing for tapering of immunosuppressive treatment.

We review here the main pathologic features of HHA, based mainly on our own experience obtained in six recipients allografted in Lyon and Milan [18, 19, 24, 25] and followed up for up to 5.5 years. Available data concern primarily the skin since this is the most accessible tissue for visual inspection and microscopic study. Furthermore, skin biopsies are easy to obtain and do not significantly impair the allograft since the resulting wounds heal rapidly and completely.

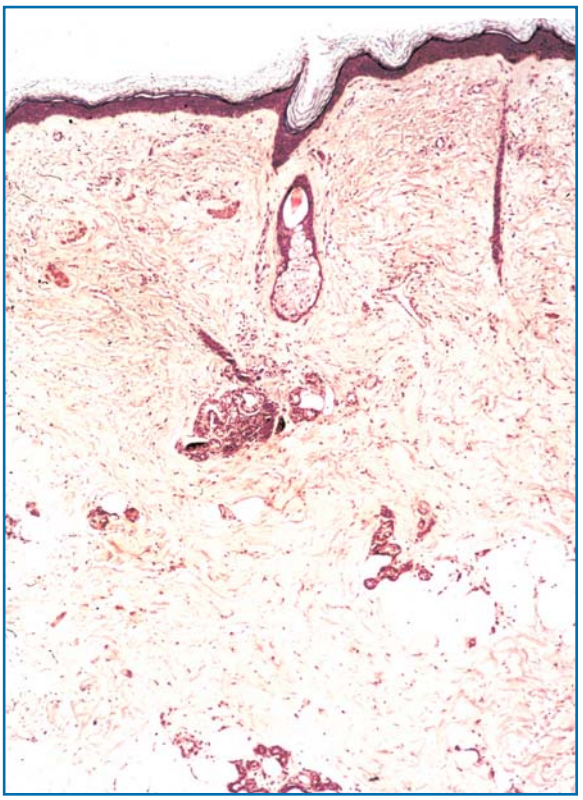

Fig. 1. Histological aspect of allografted skin in a human hand allograft: the three layers of the normal skin are visible (epidermis, dermis, hypodermis). The epidermis contains all its normal layers, and the dermis contains sweat glands, pilosebaceous follicles and vessels (haematoxylin-eosin)

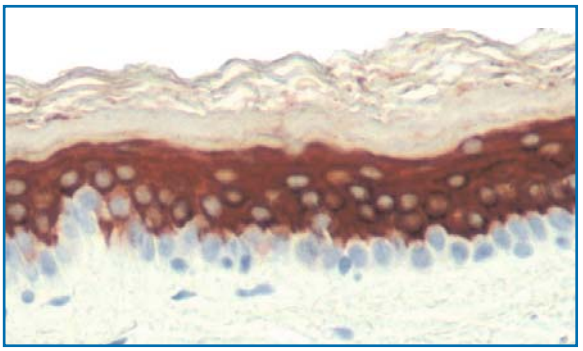

Fig. 2. Normal expression of high molecular weight keratins 1 & 10 in suprabasal epidermal keratinocytes of a human hand allograft (immunoperoxidase revealed with aminoethyl carbazole)

Nonrejection Conditions

Apart from periods of graft rejection (*see further*), skin contained in HHA maintains after allografting a normal histological structure, being composed of its three major layers (epidermis, dermis and hypodermis) (Fig. 1). The epidermis is organised in four characteristic cell layers (from bottom to top: basal, spinous, granular and horny) and contains all its normal cell types, i.e. keratinocytes (KCs), melanocytes, Langerhans (LC) and Merkel cells. KCs express their characteristic antigens, such as keratins (expressed in a characteristic pattern by all epidermal-layer KCs) (Fig. 2), involucrin (within the upper epidermal layers) and filaggrin (within the granular layer), reflecting a normal epidermal differentiation process. Basal-layer KCs express normally the proliferation-associated nuclear antigen Ki67, showing they are cycling and capable of regeneration (Fig. 3), and the nuclear p63 antigen involved in epidermal dif-

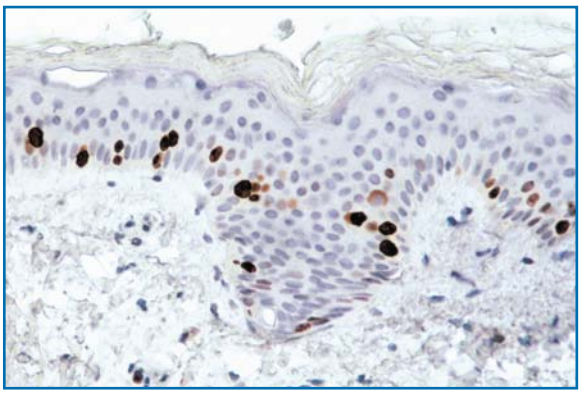

Fig. 3. Expression of the cell-cycle-associated nuclear antigen Ki67 in basal epidermal keratinocytes in a human hand allograft (immunoperoxidase revealed with aminoethyl carbazole)

ferentiation. Biopsies taken from the junction between donor and recipient skin show that epidermal KCs of donor and recipient origin blend smoothly to produce a normal-looking epithelium, the respective origin of which can be differentiated thanks only to the expression of donor- or recipient-specific antigens (such as HLA) (Fig. 4). Nonkeratinocytic cells, detected thanks to the expression of their specific antigens, are also normally present in the epidermis and its appendages. Melanocytes, expressing the melanoma antigen recognised by T cells (MART)-1 antigen, tyrosinase and S100 protein are present in normal numbers in the basal cell layer (Fig. 5). LCs, the antigen-presenting cells of the epidermis recognised thanks to the expression of CD207/Langerin and CD1a antigens, are found in normal numbers within the mid-stratum spinosum (Fig. 6). LCs are mobile cells originating from CD34-positive bone-marrow precursors; their replacement by cells of recipient origin could therefore be expected. This possibility was monitored immunohistochemically with an antibody recognising a recipient-specific HLA antigen. In the first HHA, a limited number of LCs (approximately 10%) of recipient origin was detected in the allografted epidermis during an episode of graft rejection [24]. However, long-term follow-up (5.5 years) of another HHA showed no epidermal LCs from the recipient, suggesting that under steady-state

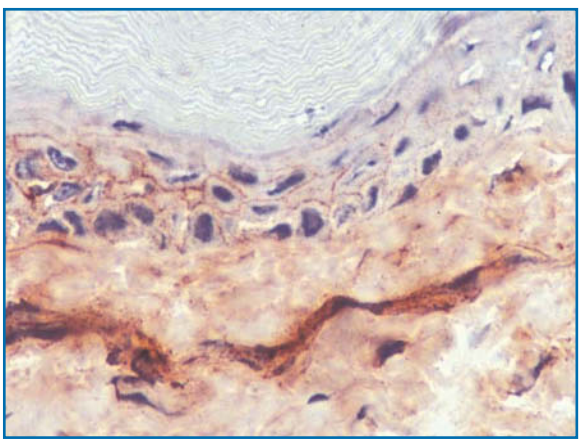

Fig. 4. Histological aspect of the skin of a human hand allograft taken at the junction between recipient (*left*) and donor (*right*). Recipient (but not donor) epidermal keratinocytes express the human leukocyte antigen (HLA)-A24 (immunoperoxidase revealed with aminoethyl carbazole)

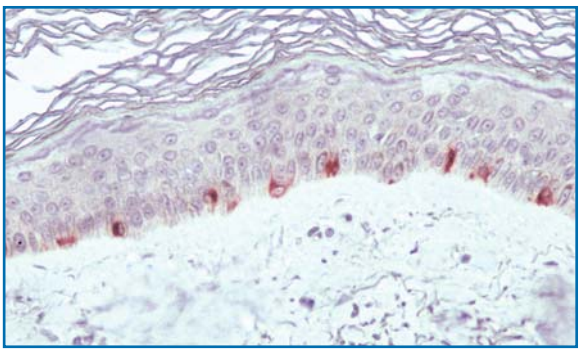

Fig. 5. The epidermis of a human hand allograft contains normal numbers of (MART)-1+ melanocytes located within the basal cell layer (immunoperoxidase revealed with aminoethyl carbazole

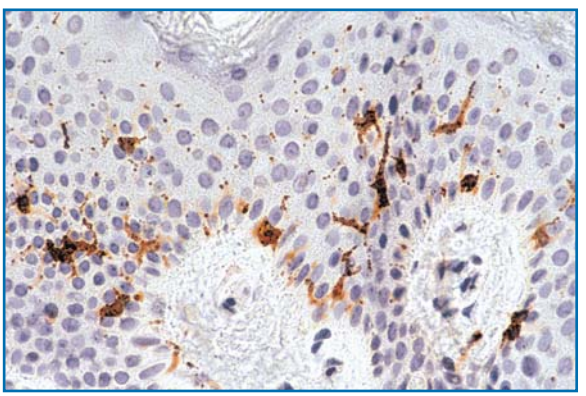

Fig. 6. The epidermis of a human hand allograft contains several dendritic CD1a+ Langerhans cells (immunoperoxidase revealed with aminoethyl carbazole)

conditions, the renewal of LCs in human epidermis is attributable to mitotic divisions of preexisting LCs or to local progenitors [26], in keeping with experimental data obtained in mice [27]. Merkel cells, expressing namely keratin 20, are also found in the basal epidermal layer. In the dermis, epidermal adnexae (pilosebaceous follicles and sweat glands) are present and show normal histological structure; they normally express their characteristic differentiation antigens, such as carcinoembryonic antigen (sweat glands) and epithelial membrane antigen (sweat and sebaceous glands) and contain basal cells expressing Ki67 and p63, suggesting normal growth. The dermis shows normal structure as to the presence of collagen and elastic fibres and contains all cell types found in normal conditions, such as perivascular factor XIIIa+ dermal dendrocytes (Fig. 7), CD34+ deep dermal dendrocytes, tryptase+ mast cells and fibroblasts. The dermal vasculature shows a normal structure, accounting for normal skin trophicity (colour, temperature and healing process). Endothelial cells express their characteristic antigens (von Willebrand factor, CD31 and CD34). Nerve bundles are also present in the dermis and are made of (donor) perineurial fibroblasts and Schwann cells, expressing their characteristic antigens (epithelial membrane antigen and S100 protein, respectively) (Fig. 8). In the early postgraft period, cutaneous nerves do not contain axons (due to their degeneration following amputation during graft procurement); however, axons (presumably of recipient origin), recognisable by their expression of neuronal markers [such as neurofilaments and protein gene product (PGP) 9.5] progressively reappear in dermal nerves [28] and also in the epidermis, vessel walls, arrector pili muscles and around sweat glands (Fig. 8). The progressive reinnervation of the skin completes its normal histological appearance and parallels sensory return. The hypodermis shows normal structure, consisting of adipocytes arranged in lobules separated by connective tissue septa; they normally express their characteristic antigens (vimentin and S100 protein) (Fig. 9).

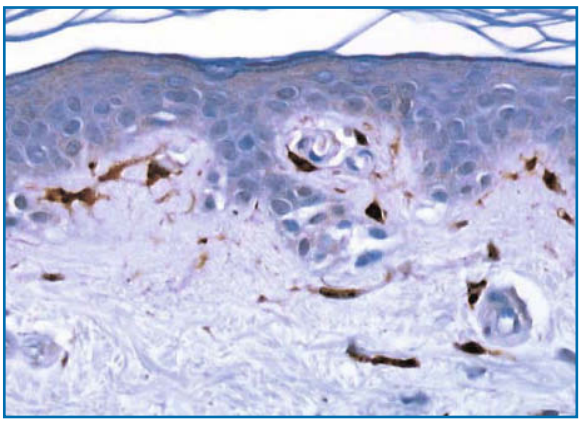

Fig. 7. The upper dermis in a human hand allograft contains several factor XIIIa+ dermal dendrocytes (immunoperoxidase revealed with aminoethyl carbazole)

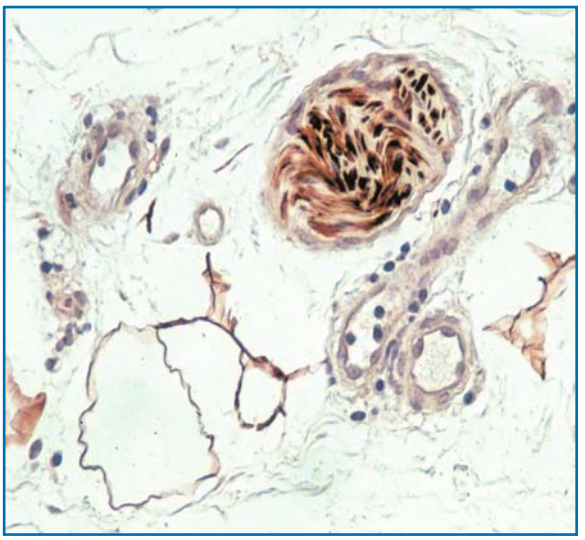

Fig. 8. A dermal nerve in a human hand allograft contains Schwann cells, labelled by an antibody to S100 protein. This antigen is also expressed by adjacent adipocytes (immunoperoxidase revealed with aminoethyl carbazole)

The deeper tissues (muscles, bones, tendons) have not been studied histologically in nonrejection conditions; however, it can be reasonably assumed that, similarly to the overlying skin, they do not show obvious pathological changes. Future studies are needed to show which, if any, of the cellular constituents of these tissues are replaced by host cells. This possibility does not seem very likely in view of the fact that (similarly to the skin) the allografted tissues contain their own stem cells, which are capable of dividing and maintaining tissue homeostasis, at least under steady-state conditions.

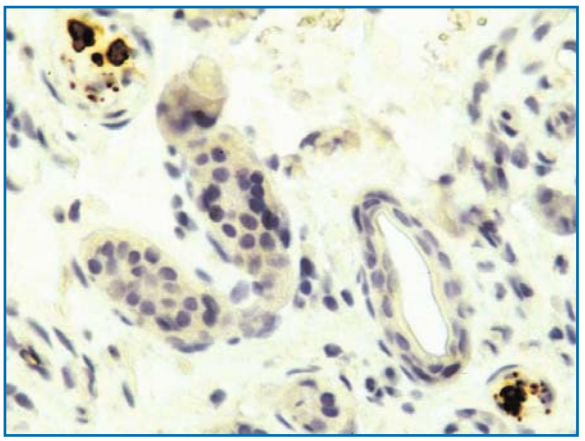

Fig. 9. Neurofilament immunoreactivity showing the presence of axons is seen within a dermal nerve in a human hand allograft at month 18 postgraft (immunoperoxidase revealed with aminoethyl carbazole)

Allograft Rejection

Pathological features of allograft rejection manifesting in the skin in the setting of forelimb allotransplantation have been studied in experimental animal (namely rat [29–31] and swine [32]) models, and scoring systems for assessing the severity of rejection have been proposed. In these models, rejection manifests clinically with redness, erosions, blisters and necrosis of the skin.

In the case of HHA (and intestine with abdominal-wall allografts), signs of allograft rejection appear rather regularly in the early posttransplant period, around the seventh to ninth week postgraft. Clinically, they manifest as erythematous asymptomatic macules that appear insidiously over the skin of the HHA [13, 33]. These signs of acute rejection can be reversed within 10–15 days with increased systemic immunosuppressive treatment and adjunction of local immunosuppressants (steroids and/or tacrolimus). If (as happened in the first HHA) immunosuppression is discontinued, cutaneous lesions progress slowly to scaly, erythematous or violaceous papules that coalesce to produce lichenoid or psoriasiform plaques over the allografted limb, affecting eventually the nails. These (chronic) changes occur several months postgraft (between months 16 and 28).

Pathologic changes of allograft rejection in the skin vary greatly according to severity of rejection and affect the dermis, epidermis and, in most severe episodes, hypodermis. Considering the spectrum of these changes, we recently proposed a scoring system of five degrees of severity of allograft rejection that can be used to monitor development of rejection and its regression upon adjustment of immunosuppressive treatment [34]. Changes seen in each grade are the following:

Grade 0: no rejection. The skin shows normal histological structure, as described above. Occasionally, a small number of lymphocytes may be present around blood dermal vessels, but the density of this infiltrate is not sufficient to raise suspicion of rejection (Fig. 10). This grade corresponds clinically to normal-looking skin.

Grade I: mild rejection. This is characterised by a mild dermal lymphocytic infiltrate forming small perivascular cuffs in the upper and occasionally mid dermis (Fig. 11). Lymphoid cells consist of both CD4+ and CD8+ T cells and are of recipient origin, as shown by their expression

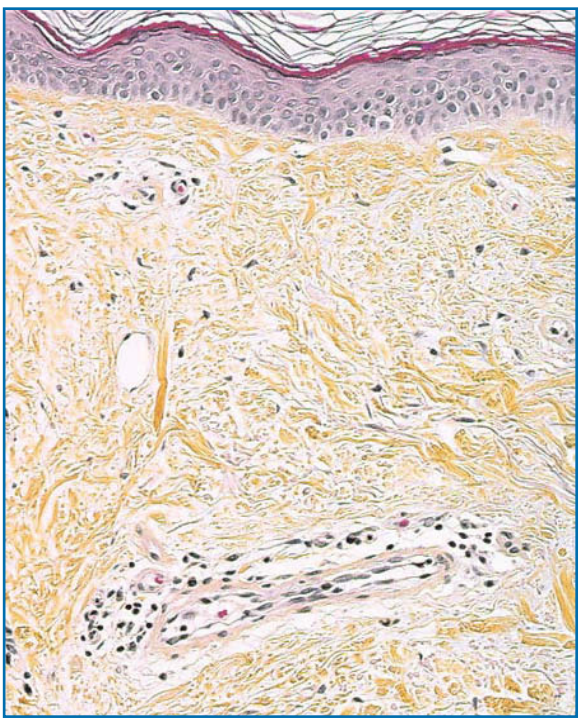

Fig. 10. Biopsy from normal-looking skin of a human hand allograft shows no signs of rejection (grade 0). Note the presence of a minute number of perivascular lymphocytes (haematoxylin-eosin)

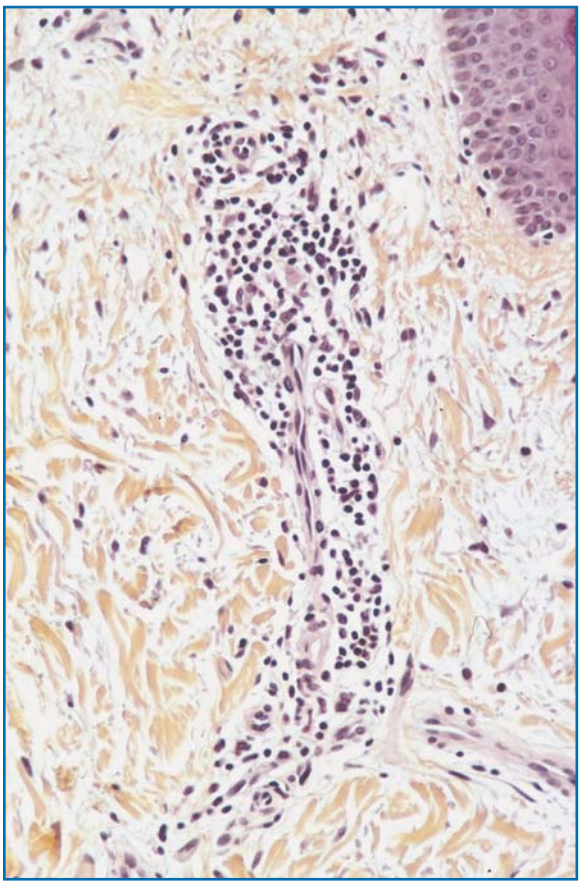

Fig. 11. Mild allograft rejection (grade I) in a human hand allograft: a mild perivascular lymphocytic infiltrate is seen in the dermis (haematoxylin-eosin)

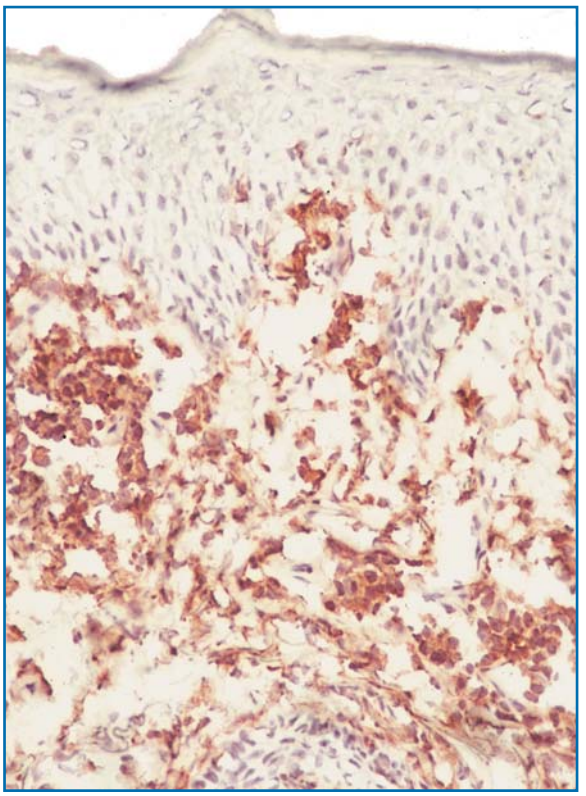

Fig. 12. The dermal lymphocytic infiltrate is of recipient origin, as shown by the expression of the recipient's specific human leukocyte antigen (HLA)-A24 antigen. The (donor) epidermis is HLA-A24-negative (rejection grade III) (immunoperoxidase revealed with aminoethyl carbazole)

of recipient-specific HLA antigens (Fig. 12). The epidermis is as a rule unaffected. This grade corresponds macroscopically to pink noninfiltrated macules developing within weeks posttransplantation; they may also be noted in clinically normal-looking skin, suggesting that starting (mild) rejection may not be visible clinically.

Grade II: moderate rejection. This is characterised by a moderately dense dermal infiltrate, forming perivascular aggregates and diffusing somewhat between collagen bundles. The infiltrate is predominantly lymphocytic but may contain occasional monocytic/histiocytic cells (Fig. 13). The epidermis may be unaffected or may show a mild degree of infiltration with inflammatory cells (exocytosis) and/or intercellular oedema (spongiosis), predominating within the lowermost cell layers. These changes are found in erythematous, noninfiltrated macular skin lesions.

Grade III: severe rejection. This is characterised by both epidermal and dermal changes. The most regular ones are seen in the dermis and consist of a dense, mainly lymphocytic, infiltrate forming nodules around capillaries of the upper dermis, larger blood vessels of the mid and lower dermis, and eccrine sweat glands (Fig. 14). The epidermis contains scattered necrotic KCs and shows focal vacuolar degeneration of the basal cell layer, which is invaded by lymphocytes (interface dermatitis). Occasionally, changes indistinguishable from those seen in cutaneous graft-versus-host disease (GVHD) are seen, such as epidermal hyperplasia (orthokeratotic hyperkeratosis, hypergranulosis, acanthosis and papillomatosis), with a dense subepidermal band-like lichenoid lymphocytic dermal infiltrate (Fig. 15). Scattered apoptotic/necrotic KCs may be seen in epidermal adnexae also (hair follicles, eccrine excretory ducts). This grade corre-

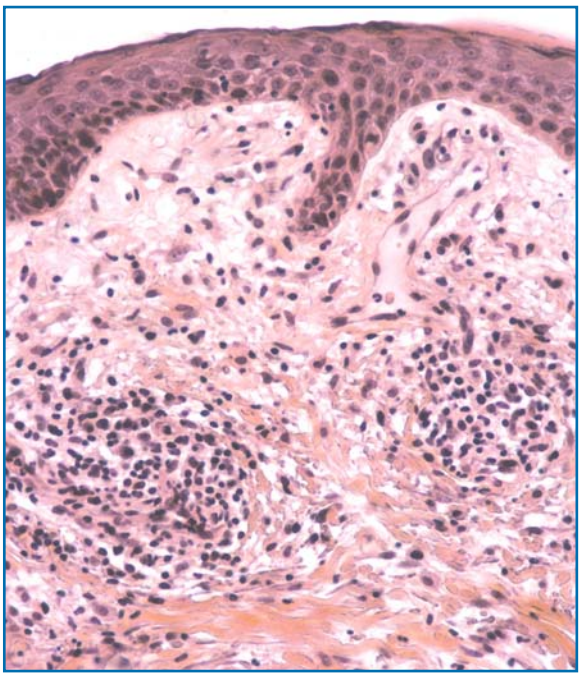

Fig. 13. Moderate allograft rejection (grade II) of the skin in a human hand allograft: a moderately dense lymphocytic infiltrate forming perivascular cuffs is seen in the dermis (haematoxylin-eosin)

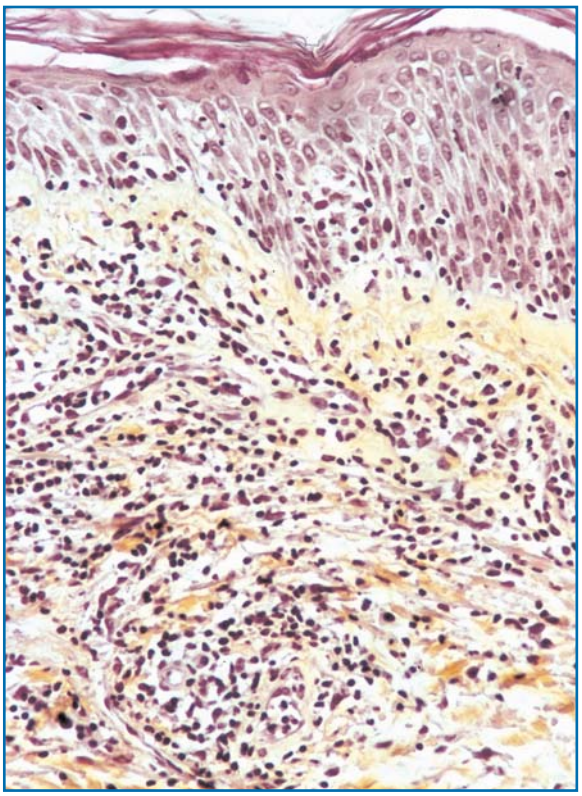

Fig. 14. Severe rejection (grade III) of the skin in a human hand allograft: a dense lymphocytic infiltrate is seen in the dermis. The overlying epidermis contains foci of spongiosis and lymphocytic exocytosis and shows some degree of basal-cell vacuolisation (haematoxylin-eosin)

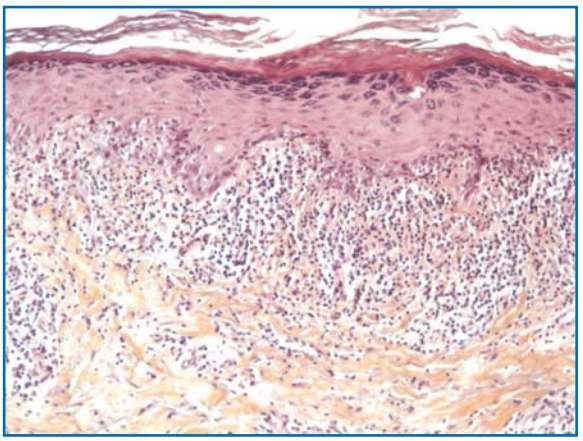

Fig. 15. Severe rejection (grade III) of the skin in a human hand allograft showing histologically an aspect of graft-versus-host disease (orthokeratotic hyperkeratosis, hypergranulosis, acanthosis, papillomatosis, dense dermal infiltrate forming a horizontal band in the papillary dermis) (haematoxylin-eosin)

sponds to papular erythematous, infiltrated, more or less scaly papules that are either isolated or coalescing in plaques, developing several months posttransplantation.

Grade IV: very severe rejection. This is characterised by an epidermis of variable thickness comprising both highly hyperplastic, lichenoid areas and zones of epidermal thinning and necrosis resulting from the confluence of necrotic KCs (Fig. 16). Intraepidermal lymphocytic exocytosis is seen, especially within areas of epidermal hyperplasia. Subepidermal clefts may

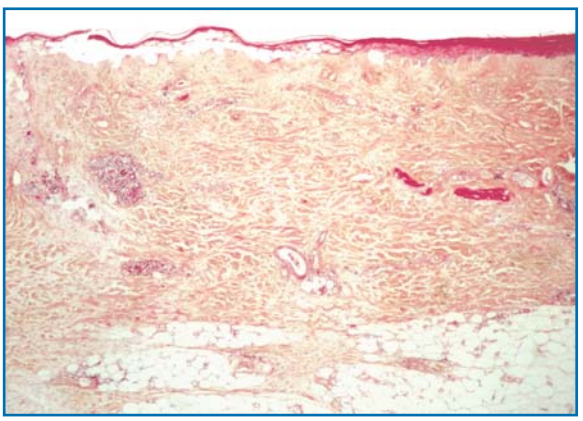

Fig. 16. Very severe rejection (grade IV) of the skin in a human hand allograft: the epidermis appears still hyperplastic on the *right*, but is thinned on the *left* where a subepidermal cleavage has developed. A dermal perivascular infiltrate is present (haematoxylin-eosin)

form as a result of KC necrosis and basal-cell-layer vacuolisation. The dermis contains an inflammatory infiltrate forming large aggregates around blood vessels, hair follicles and eccrine glands, and smaller ones around tactile corpuscles and nerves (Fig. 17); this extends focally to the hypodermis in the form of perivascular nodules. Eccrine secretory ducts show basal cell vacuolisation and infiltration by lymphocytes; they also often display malpighian metaplasia and contain apoptotic KCs (Fig. 18). The wall of some large vessels (venules) of the deep dermis may show heavy lymphocytic infiltration. The inflammatory infiltrate is polymorphous, made mainly of activated (HLA class II+) CD45RO+ memory T cells, with abundant eosinophils and lower numbers of CD20+ B cells, CD79a+ plasma cells, tryptase+ mast cells and histiocytic cells. Up until now, this grade has been found in the amputation specimen of the first HHA recipient (obtained during the 28th month postgraft) that showed macroscopically, along with changes observed in previous grades, superficial erosive and necrotic areas.

Almost identical cutaneous clinicopathologic findings have been reported during graft rejection in other patients with HHA [35, 36] and abdominal-wall and intestine allotransplantation [13], and pathological grading systems very similar to the one described above have been proposed [37, 38]. Since follow-up of the patients with CTA is relatively short, these grading systems will probably have to be refined in the future. Indeed, the possibility exists that additional pathologic changes (such as dermal fibrosis resulting in a sclerodermoid state) could develop in the long term. Furthermore, the role of lymphoid cells infiltrating the skin needs further evaluation. Indeed, we have recently observed that a small subset (usually around 10%) of skin-infiltrating lymphocytes both in normal-looking skin and during episodes of rejection expresses the FoxP3+ phenotype of CD4+/CD25+ T-regulator cells (Fig. 19). These cells could induce tolerance rather than rejection [39]. Therefore, the functional properties of the

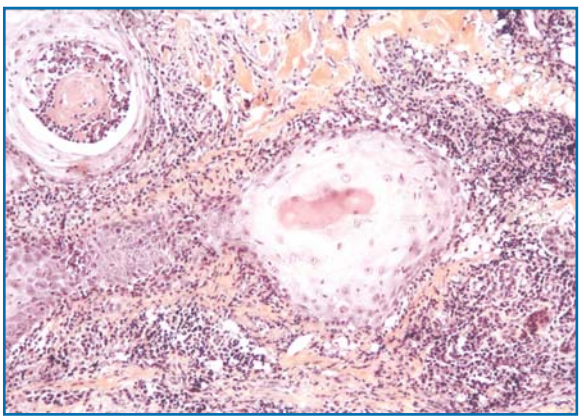

Fig. 17. Very severe rejection (grade IV) of the skin in a human hand allograft: the dermis contains a heavy lymphocytic infiltrate forming perivascular and perifollicular nodules (haematoxylin-eosin)

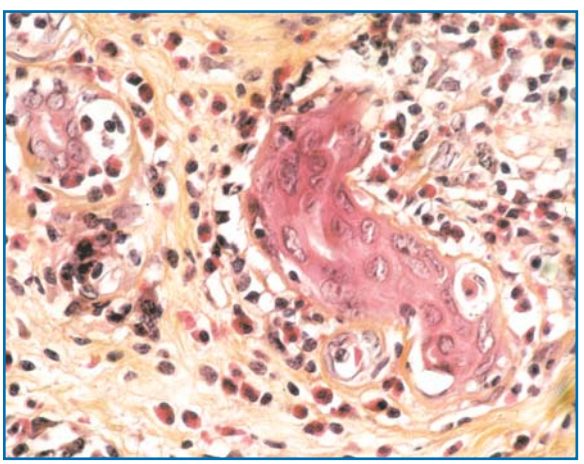

Fig. 18. Very severe rejection (grade IV) of the skin in a human hand allograft: the dermis contains a dense infiltrate made of lymphocytes and eosinophils. An eccrine sweat gland duct contains necrotic keratinocytes (haematoxylin-eosin)

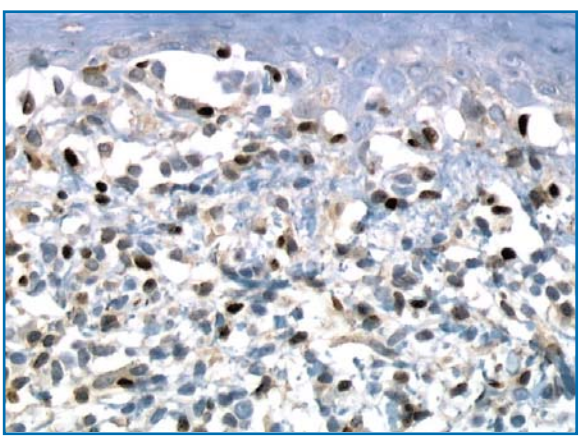

Fig. 19. FoxP3+ T-regulatory cells are present in the skin of a human hand allograft during the fifth year postgraft (immunoperoxidase revealed with aminoethyl carbazole)

lymphocytic infiltrate will probably need to be considered in the assessment of the severity of rejection.

Pathological data concerning underlying tissues (such as muscles or bones) during episodes of rejection of skin-containing CTA are sparse since these tissues are usually not subjected to pathological study as long as the allograft has not been removed. Such tissues were studied in the amputation specimen of the first HHA; they showed considerably less-severe changes compared with cutaneous ones, highlighting the higher degree of antigenicity of the skin. The main changes consisted in mild to moderate perivascular lymphoid cell infiltrate present within muscle fibres and tendons (Fig. 20). Some muscle fibres looked atrophic, probably reflecting lack of adequate re-education (rather than graft rejection). The cartilage and bones (including bone marrow) of small joints did not show obvious changes [23]. These results are similar to those observed during rejection of rat limb allografts, showing pathological changes mostly confined to the skin [31]. A preliminary study of an HHA from China reported stronger rejective pathologic changes in muscle and nerve compared with the skin [40]. The reasons for this discrepancy remain unclear.

In conclusion, pathological monitoring of the skin appears at this time to be the most reliable test allowing early detection of allograft rejection in the setting of HHA (and also of other CTAs containing skin, such as abdominal wall and intestine). Existing pathological grading systems of rejection allow assessment of the severity of allograft rejection and the effect of antirejection treatments. Future studies should aim at defining more precisely the functional role of skin-infiltrating host lymphocytes and the possible development of long-term changes.

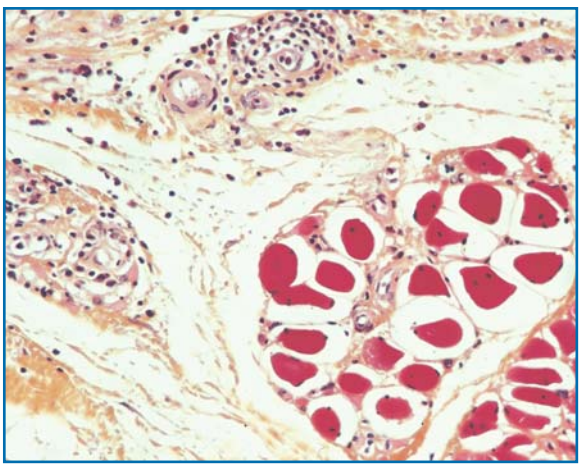

Fig. 20. Amputation specimen of the first human hand allograft showing in the skin very severe rejection (grade IV): a mild perivascular lymphocytic infiltrate is seen within a striated muscle (haematoxylin-eosin)

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Section 8-b

Pharmacological Treatment of Rejection

Palmina Petruzzo

Introduction

At present, rejection of transplanted organs remains the main obstacle in transplantation. It occurs as a result of humoral and cell-mediated responses by the recipient to specific antigens present in donor tissue. These antigens are known as major histocompability complex (MHC) molecules in humans, and this group of molecules is referred to as human leukocyte antigen (HLA) complex molecules. Once graft rejection has begun, it can be classified in hyperacute rejection, acute rejection or chronic rejection [1]. Symptoms of rejection vary depending on the transplanted organ or tissue; usually, the principal sign is improper function of the organ and, rarely, pain or swelling in the location of the transplanted organ. For example, patients who reject a kidney may have oliguria and increase in serum creatinine values, and patients with heart rejection may have symptoms of heart failure.

Acute Rejection in Hand Transplantation

Despite the combined actions of the triple maintenance therapy, most transplanted hand grafted patients (about 70%) experienced at least one episode of acute rejection [2]. It is important to note that the diagnosis of acute rejection in hand transplantation is easier than in organ transplantation as it is based on macroscopic observation of the skin and its biopsy [3], thus explaining the high rate of acute rejection episodes reported in this field of transplantation.

Hand transplantation is considered a composite tissue transplantation (CTA) since it contains several tissues showing various degrees of antigenicity and rejects through different mechanisms [4, 5]. Several Authors [4–7] considered that skin and bone marrow induce earlier and more severe rejection than muscles, tendons, bone and cartilage. Moreover, transplanted muscles elicit mainly a cell-mediated immune response while skin transplantation elicits both cellular and humoral responses [6]. However, it seems that the various components interact with the host immune system in a complex pattern, eliciting less immune response than an individual tissue allograft [4], and that the cell-mediated immunity plays a minor role in rejection of CTA compared with antibody-mediated response [5]. So far, only acute rejection episodes have occurred in human hand transplantation, and the main rejection process was the skin, as confirmed by pathological study of the amputation specimen of the first hand transplantation [8].

The risk of acute rejection is highest in the first 3 months posttransplantation after the first week because during this period, the T cells involved in rejection have to differentiate and the antibodies, in response to the allograft, have to be produced before rejection is initiated. After 6 months, the body adapts to the new organ or

tissue, and acute rejection is less likely. T cells cause graft cells to lyse, or produce cytokines that recruit other inflammatory cells, eventually causing necrosis of allograft tissue. In our bilateral hand transplantations, all rejection episodes occurred within the first 3 months posttransplantation while the vast majority of rejection episodes reported by the International Registry on Hand and Composite Tissue Transplantation (IRHCTT) are between weeks 7 and 14 [2]. In addition, in our hand-grafted patients [9] lymphoid infiltrates were present in the skin during episodes of rejection. These infiltrates consist of lymphocytes of recipient origin, which express mainly CD3+CD4+ or CD3+CD8+ phenotype. Recently, our studies showed that a subset express a phenotype of T regulatory (i.e. CD4/CD25/FoxP3), thus the functional properties have to be investigated more than the density of the lymphoid infiltrate.

It is interesting to note that the acute rejection episodes reported by different teams [2] are always characterised by cutaneous lesions, which may start with faint, hardly visible pink macules that may progress to red infiltrated lichenoid papules with or without oedema of the upper limb. It is also very important to remark that never reported, except for the first case of unilateral hand transplantation, was a functional impairment due to the acute rejection episode.

Treatment of Acute Rejection

In the vast majority of rejection episodes in solid organ transplantation, temporary treatment by high doses of corticosteroid is used to combat rejection by severely depressing the immune system. For those rejection episodes resistant to corticosteroid treatment, polyclonal and monoclonal antibodies are often employed as a rescue therapy [10, 11]. The more recently explored monoclonal antibodies, such as muromonab-CD3 and basiliximab, are more specific than their polyclonal counterparts; this is important because medications with higher specificity have fewer pathways by which to induce serious side-effects. Finally, antiproliferative drugs have also been found to be effective in treating rejection episodes. When utilised for rescue therapy, antiproliferatives are delivered at much higher dosages [12].

As reported by the IRHCTT [2], in most cases (78%) of hand transplantation, treatment of the first rejection episode included high-dose i.v. steroids followed by an increase in oral steroid dosage (44%). In cases where no steroids were administered intravenously (22%), oral steroid treatment was increased. Treatment of a second, third or fourth rejection episode varied considerably, from application of topical drugs only (steroid and/or tacrolimus creams: 22%), to i.v. steroids (44%), with or without topical creams (22% and 22%, respectively) to the use of antithymocyte globulins or basiliximab with oral steroids and topical drugs (34%). In one, case alemtuzumab, a humanised monoclonal antibody against the CD52 antigen (Campath-1H), was used to treat an acute rejection episode resistant to steroids and antithymocyte globulins [13]. In order to avoid some complications, it is also necessary to remember that patients should receive pneumocystic and cytomegalovirus (CMV) prophylaxis for 3 months after the last dose of steroids and/or monoclonal or polyclonal antibodies.

All acute rejection episodes treated were completely reversed no matter which therapy was employed. In one case, following the patient's non-compliance with immunosuppressive therapy, cutaneous lichenoid-like lesions occurred, and the grafted hand was amputated. It is interesting to note that the majority of acute rejection episodes were easily reversed by steroid treatment, thanks, perhaps, to early diagnosis. In addition, for the first time, topical immunosuppressive drugs have been successfully used in transplantation with two aims: firstly, targeting the skin as the most antigenic component of the allograft, and secondly, allowing eventual omission of one or more systemic drugs.

Chronic Rejection

Chronic rejection is the most prevalent cause of graft failure in the first 10 years after transplantation. It is reported in 25 % of patients [14],

occurs months to years following transplantation and is characterized by graft arterial occlusions, which results from the proliferation of smooth muscle cells and production of collagen by fibroblasts. This process results in fibrosis that can cause ischaemia and cell death. These fibrous lesions occur without evidence of an overt cause (such as vascular injury or infection) although it is hypothesised that chronic rejection is really the result of continued, prolonged, multiple acute rejections. Preservation injury may also contribute to its occurrence. The possibility of early diagnosis and all acute rejection episodes in hand transplantation compared with solid organ transplantation could be a great advantage in preventing chronic rejection. To date, no case of chronic rejection has been reported, but the longest follow-up is 7 years for the first American hand transplantation and 6 years for the first bilateral French hand transplantation. Consequently, at present, short follow-up and limited numbers of hand transplantations prevent this possibility.

Conclusions

In conclusion, skin confirmed to be the principal target of rejection episodes, which were frequent although always reversible. Antirejection treatment used in hand transplantation was the same as that employed in solid organ transplantation, except for few rare cases. However, better antirejection strategies must be determined in order to avoid the possibility of developing chronic rejection and complications correlated to antirejection treatment.

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Section 8-c

Monitoring Rejection with a Distant Sentinel Skin Graft

Marco Lanzetta, Luca Rovati

Rationale for Additional Monitoring of the Hand

While an internal organ is hidden but works immediately after the transplantation, a hand is fully visible and takes many months to gradually recover sensation and movement [1, 2]. Assessment of viability of transplanted internal organs is usually done by measuring function, either by biochemical evaluations or routine biopsies [3, 4]. In case of a hand, monitoring is largely based on visual inspection [5–7]. This is a definite advantage compared with internal organs, where an initial rejection may go undetected for some time until the next scheduled biochemical tests or biopsy are carried out. Methods for monitoring rejection in human hand transplantation include visual inspection of skin changes and histological analysis of biopsies. However, repetitive skin biopsies on a relatively small area may pose the problem of leaving multiple visible scars, especially if these procedures must be carried out over a period of many years. Previous experimental studies have shown that skin allografts reject faster and with a greater immune response than composite tissue allografts containing skin [8]. We aimed at using this differential rejection phenomenon to achieve earlier detection of incoming rejection. Our protocol for hand transplantation included extra donor skin transplanted to the hip area of the recipient. This served the purpose of allowing skin biopsies to be taken from a distant area without disturbing the hand. Furthermore, it was our intention to have an additional site for visual monitoring and to evaluate whether both the skin of the transplanted hand and the skin at the hip would simultaneously show signs of rejection or if this event would be somehow different or independent in the two locations [9].

The Distant Sentinel Skin Graft

Upon completion of the hand transplantation procedure, the donor's skin was grafted to the left hip of the recipient. A layer of skin was removed from the patient's hip with an electric blade and replaced with patches of both full-thickness and split-thickness skin graft harvested from the donor's distal forearm. This distant sentinel skin graft (DSSG) measured approximately 12?9 cm in size and was sutured in place with continuous nylon sutures and a tie-over dressing technique (Fig. 1). Patients were given 250 ml of dextran 40 before declamping and 20 ml/h for 7 days. Aspirin 150 mg was administered for 7 days and wide-spectrum antibiotic therapy for 10 days. The induction immunosuppressive protocol consisted of 20 mg of monoclonal antibody anti-CD25 (basiliximab; Simulect) 2 h before the operation, on day 4 and on day 45 postoperatively; FK506 (tacrolimus; Prograf) adjusted to maintain blood concentration between 15 and 20 ng/ml for the first month; mycophenolate mofetil (MMF) (Cell

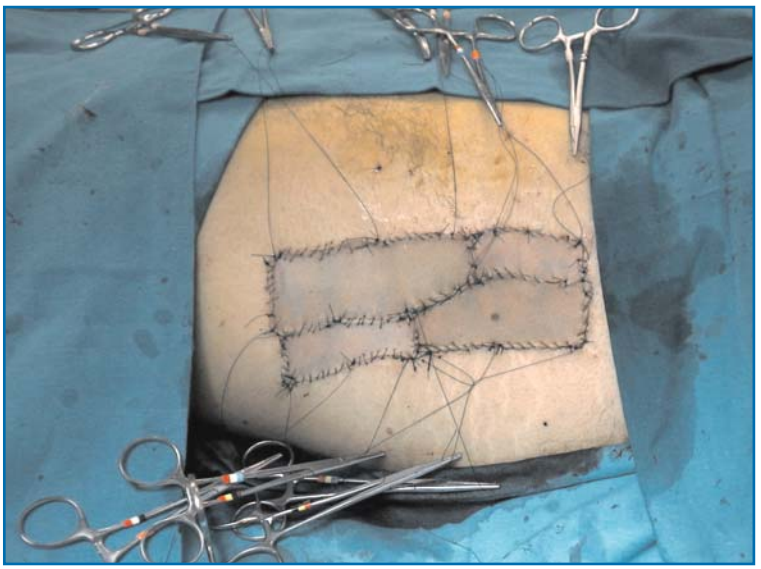

Fig. 1. The "distant sentinel skin graft" (DSSG)

Cept) 2 g/day and steroid (prednisone) 250 mg on day 1 and rapidly tapered to 20 mg/day. Maintenance therapy consisted of FK506 (blood levels between 5 and 10 ng/ml), MMF 1 g/day in 2 cases and 1.5 g/day in the last case and prednisone 10 mg/day.

The transplanted hands, either during hospitalisation or at home, were constantly monitored by means of a newly designed portable monitoring device connected to the Hand Unit via modem. Real-time information through this system included blood pressure, heart beat and PO2 saturation. Monitoring rejection in our patients did not pose particular problems provided the patients were complying with the agreed-upon protocol. Skin changes always correlated well with histological observations of lymphocytic perivascular infiltration. The DSSG used in our patients proved to be of great benefit in announcing an incoming rejection of the hand at least 7 days earlier, which allowed for appropriate treatment to be started immediately [1] (Fig. 2).

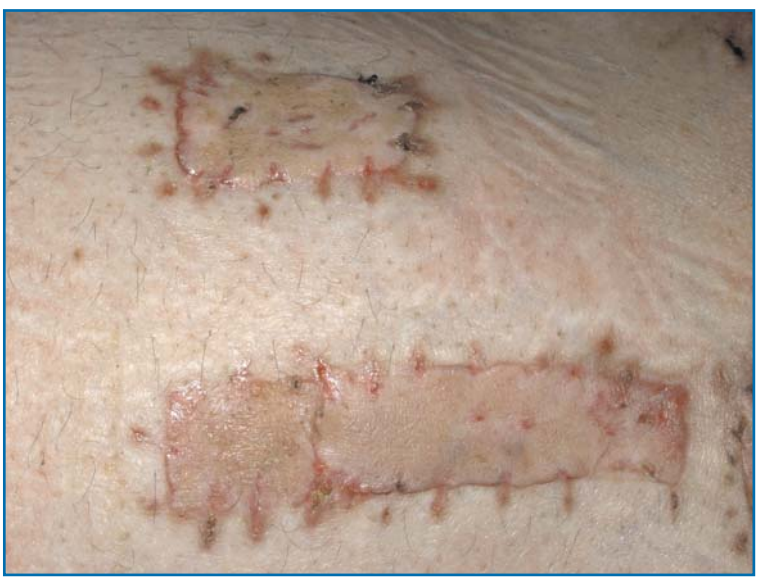

Fig. 2. Redness and oedema of the DSSG showing rejection

The Clinical Experience

Case 1

On day 67, routine skin biopsies obtained from the hand and the DSSG were normal. On day 76 postoperatively, the patient's drug regime was FK506 4 mg/day (with a blood concentration of 10 ng/ml), MMF 2 g/day and prednisone 25 mg. Blood tests were normal [white blood cell count (WBC)=10,200; platelets=179,000] as well as inflammatory indexes (VES=5; C-reactive protein=negative). While the transplanted hand was showing no visible skin alterations, including color changes, the DSSG was showing a definite redness and some mild oedema. Five random punch skin biopsies were taken from both the hand and the DSSG. The skin graft from the hand showed a pattern of normality while the one from the DSSG revealed some perivascular dermal infiltrate of mononuclear cells consistent with rejection. Immunohistochemical analysis confirmed the presence of T cells in the infiltrates (Fig. 3).

Only a week later, on day 83, the skin of the hand started to show a mild erythema in the dorsoradial area. Skin biopsies of the hand were positive for a very mild perivascular infiltrate of mononuclear cells not involving the epidermis. Immunohistochemical analysis confirmed the presence of T cells in the infiltrates. At this stage, blood test showed a moderate increase in the WBC number value (WBC=12,000; neutrophils=87%). These findings were interpreted as consistent with a mild rejection episode and treated according to our protocol. This consisted of administration of steroids 250 mg the same day and 125 mg the following day, and an increase in the dose of FK506 to achieve blood concentration between 13 and 18 ng/ml. In addition, we started topical immunosuppression with tacrolimus and clobetasol. Skin changes resolved within 5 days at both locations. Skin biopsies taken 7 days after the beginning of therapy showed significant improvement in histological patterns. No further instances of rejection have occurred to date.

Case 2

At 60 days postoperatively, the DSSG showed some definite redness and slight swelling while the skin of the hand looked normal. Biopsies were taken at both sites, and histological evaluation confirmed mild rejection of the DSSG with perivascular infiltrate of mononuclear cells while there was no evidence of any rejection at the hand. Based on our experience with the first patient, we decided to start antirejection treatment immediately. This consisted in steroids 250 mg for 2 days and 125 mg the following day, together with an increase in the dose of FK506 to achieve blood concentration between 13 and 18 ng/ml and use of topical cream. Five days, later a new set of biopsies showed that rejection had been completely reversed at the hip while at the hand there was no evidence of infiltrates (Fig. 4).

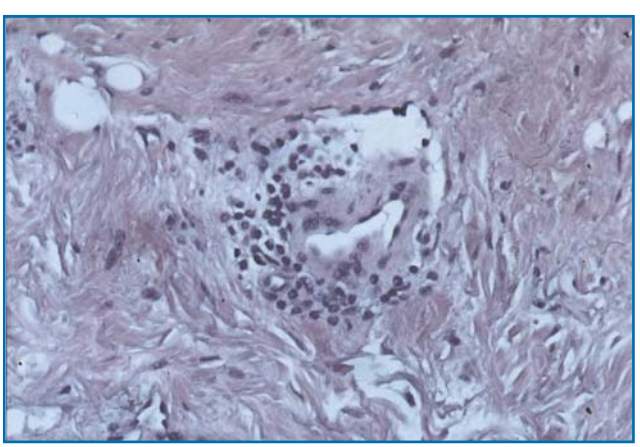

Fig. 3. Histology of the DSSG: perivascular dermal infiltrate of mononuclear cells consistent with rejection

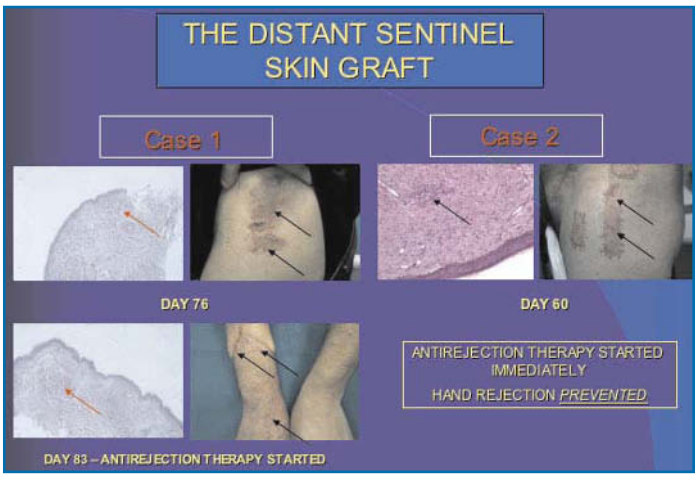

Fig. 4. Examples of correlation between clinical appearance and histological findings of the DSSG in 2 Italian recipients

Case 3

Experience gathered from cases 1 and 2 was used to monitor rejection of the hand in case 3 using the strategy of starting appropriate therapy as soon as there were signs of rejection at the DSSG, thus reducing consistently the appearance of skin changes at the hand level. Using this approach, it was possible to maintain satisfactory control over the hand, and no significant episodes of rejection have been encountered in this patient to date.

A hand transplant consists of multiple tissues with varying degrees of antigenicity, which express different amounts of major histocompatibility complex (MHC) antigens and tissue-specific antigens primarily responsible for elicitation of the host's cellular mediated response (rejection). The skin particularly presents an extreme challenge to the immune system. Because of its complex immunological structure, the skin is the component that develops the most severe rejection. This is due to the abundance of dendritic cells within the epidermis and dermis. These cells are at least 100 times more effective than other major cell subclasses. They have been shown to be the most potent stimulators of the mixed leukocyte reaction, an in vitro analogue of transplant rejection [10]. In the past, because of the perceived need for more selective and effective immunosuppressive drugs, it was even suggested that a possible solution would be to skin graft a transplanted hand with recipient skin [11].

When studying the ability of isolated skin and kidney cells to activate allogeneic lymphocytes in incompatible rat strains, kidney cells displayed a weaker reaction compared with skin epidermal cells and limited localisation of lymphocyte-activating determinants [12]. Because of the different vascular and lymphatic supply among tissue components of the allograft, antigen recognition and targeting by the host immune system also differs among the allograft tissue elements (differential rejection) [13]. In experimental limb transplantation, while the skin component may show histological and clinical signs of severe rejection, the underlying tissues can still present a normal architecture without infiltrates, as they may be differentially protected by the immunosuppressive therapy [14]. At this stage, the vascular anastomoses may also still be completely patent.

Histologically, acute rejection is characterised by the presence of leukocytes dominated by equivalent numbers of macrophages and T cells within the graft. Early acute rejection displays only a few focal regions of perivascular infiltration, which can progress to large, widespread pools of lymphocytes that disrupt the tissue architecture. Early changes in the epidermis include basal cell vacuolation, with infiltrates of keratinocytes and lymphocytes in the dermis. In later and more severe stages of rejection, there is also microscopic evidence of tissue destruction and necrosis of the epidermis, including pyknotic nuclei and cellular debris. The normal architecture of the skin disappears [15–20].

While hand rejection is easily monitored by visual inspection of possible skin alterations and by repetitive skin biopsies, the earlier the onset of rejection is detected, the earlier a change in the immunosuppressive drug scheme can be initiated and thus prevent possible damage to the various tissues of these transplants. Previous attempts to investigate additional methods to monitor early subclinical rejection in experimental limb transplantation models have proved unsatisfactory. Peripheral blood gases, glucose, and lactate were not useful as factors for rejection. Laser Doppler flowmeter values could not predict an initial onset of rejection before clinical signs were evident [21]. In a study done to correlate transcutaneous oxygen and the survival of skin allografts, transcutaneous oxygen levels fluctuated independently of allograft status [22]. Based on these considerations, our rationale for transplanting extra skin distant to the hand has been to provide an additional hidden area for biopsies, and more importantly, to have a cutaneous area that would be likely to present earlier rejection compared with the hand. Previous experience with single and a double hand allografts has shown that a bigger skin area would not need more immunosuppression, confirming previous experimental data [23].

The time discrepancy in rejection between the transplanted hand and the DSSG confirms experimental data, which showed that the skin component of a composite tissue allograft rejects slower than skin only [13]. Antigen competition, induction of enhanced antibodies and activation of suppressor T cells might contribute to the lesser rejection of the composite allograft. A mechanism of "consumption" phenomenon, as the immune system may have to deal with different antigen loads, may be also be taken into consideration [13].

The value of the DSSG has been clinically very important in our experience. Onset of rejection of the DSSG preceded similar clinical and histological signs at the hand by at least 72 h, extending to 7 days in our second case. This early detection allows for appropriate treatment to be started in a subclinical stage when changes are minimal and consequently potential damage is minimised or avoided altogether. When rejection is suspected by onset at the DSSG, prompt treatment will not alter the scheduled rehabilitation programme, which would have to be discontinued at least partially in case of rejection of the hand. A slight adjustment in the therapy is sufficient to reverse skin changes at the DSSG while rejection of the hand will not even start or progress. The DSSG was used for skin biopsies without disturbing the transplanted hand, minimising inevitable aesthetic problems due to multiple scars. No complications were encountered from the use of this technique.

However, as time goes by, the value of the DSSG diminishes, as the skin tends to undergo a sort of creeping substitution, and after a few months, it is not possible to rely on the grafted skin area, as most of the donor's skin has been replaced by the host. Thus, it is realistic to say that the value of the DSSG is limited in time but is of great value in the early stages of hand transplantation when rejection episodes should be avoided if possible.

Conclusion

In conclusion, there is no doubt that monitoring the transplanted hand by macroscopic clinical findings must be coupled with serial histological evaluations to detect rejection. This is a definite advantage compared with internal organs where changes in laboratory tests are the main indicator for the onset of rejection. In these patients, an initial rejection may thus go undetected for some time until the next scheduled biochemical tests are carried out. Based on these preliminary data, we suggest that the use of the DSSG in hand transplantation may be considered as a valuable method for monitoring and early detection of rejection. In addition to this, we hypothesise that such a technique could be applied to solid-organ transplantation where an "external" or "superficial" monitor would be a useful means to be aware of "what is going on inside". We intend to continue to use this technique as more clinical cases are needed before final conclusions can be drawn.

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9. Functional Recovery of Transplanted Hands

Section 9-a

Bone Healing in Hand Transplantation

Markus Gabl, Sigurd Pechlaner, Martin Lutz, Rohit Arora, Michael Blauth, Michael Rieger, Marina Ninkovic, Hildegunde Piza, Stefan Schneeberger, Raimund Margreiter

Introduction

Biology of Bone Healing

Various bone disorders can affect the ability of bone cells to structure organic and inorganic components. Avascularity can cause osteonecrosis, with death of haematopoietic cells, lipocytes and endothelial cells. Repair of osteonecrosis is the time needed for the process to replace necrotic bone. Callous fracture healing is a regenerative process consisting of three stages of inflammation: development of soft callus, of hard callus and remodelling [1, 2]. During inflammation, new blood vessels are induced, enhancing angiogenesis, which can be investigated by Doppler ultrasound. Following inflammation, fibrous and cartilaginous tissue known as soft callus develops, which can be observed by grey-scale ultrasound. In the hard callus stage, cartilaginous tissue converts to woven bone, which will finally be remodelled to lamellar bone.

In primary bone healing under rigid plate fixation creeping substitution can be observed histologically after 4 weeks. Following the Haversian system, osteoclast activities are first necessary to enable cone formations and ingrowth of bridging osteoblasts. This remodelling takes time and weakens the bone for 1–2 years. In the remaining tiny gaps, blood vessels and osteoblasts grow in within the first 2 weeks, forming a lamellar bone that is osteoconductive and bridged at week 4.

Healing of Bone Grafts

Healing of nonvascularised autologous, cancellous and cortical bone shows inflammatory response with vascular ingrowth. With increase of fibrous granulation, in 2 weeks, repair of cancellous grafts differs as osteoblastic new bone is apposed onto necrotic trabeculae, correlating radiographically with an increase in radiodensity. At month 6, this graft is completely repaired, with the necrotic trabeculae resorbed by osteoclasts. The osteoinductive and osteoconductive graft is initially stronger due to apposition of new bone, but strength declines to normal when the necrotic bone is resorbed.

Nonvascularized autologous cortical grafts are incorporated by creeping substitution at a lower rate due to the greater amount of osteonecrosis. In humans, graft healing is prolonged, with loss of 50% graft strength within the first 6 months, maintaining this strength for another 6 months. Radiographically, density is reduced due to bone porosity. Graft strength can be regained up to the second year. In humans, fatigue failures occur between month 6 and 18 [3]. The osteoconductive graft is not completely substituted but remains as a mixture of necrotic donor bone and new host bone. Healing of the osteoconductive graft depends on compression and oxygenation, which can be improved by vascularisation. Such vascularised autologous cortical grafts contain less necrotic bone and show the identical pattern of repair. Strength and stiff-

ness, however, were found to be accelerated, making them superior to nonvascularised grafts [4].

Allogeneic Bone Grafting

In nonvascularised allogeneic cancellous bone, incorporation lasts longer, with increase of vascular response and with the granulation tissue becoming loosely structured. This web is filled with inflammatory cells rather than with fibroblasts and blood vessels. Bone resorption and bone formation are delayed, and the graft may incorporate incompletely.

Nonvascularised allogeneic cortical grafts are osteoconductive and show creeping substitution to be markedly prolonged. Allografts differ from autografts, as vascular penetration and bone formation are slower and resorptive activity is more extensive. Primary lymphocytes dominate, and fibrous tissue encapsulates the graft. The inflammation can either disappear or become chronic. The initially vascular network around the graft becomes occluded, leading to periosteal necrosis and thereby prohibiting appositional bone healing, with more necrotic bone existing than new bone to be formed.

Immunological Response to Allogeneic Structured Bone Grafts

There is much evidence that bone is immunogenetic. The marrow contained in bone, endosteal and periosteal cell-surface antigens as well as bone matrix have been suggested to be responsible for immunogenicity [5]. Cell-mediated immunity is considered to play a minor role in rejection of composite tissue allografts and of bone alone as compared with antibody-mediated response. There is some evidence that cytotoxic antibodies directed against bone allografts do, indeed, appear and may coincide with cellular immunity although they seem to not be directly involved in the rejection process. Bone healing after allotransplantation may proceed normally. Chronic repair is characterised by greater incidence of nonunion or delayed union, peripheral resorption or loss of graft size. In some cases, the graft can be resorbed completely [1].

Vascularized Allogeneic Cortical Grafts Under Immunosuppression

In contrast to avascular allografts, primary vascularisation of limb-tissue allograft is reported to change the pattern of rejection into considerable humeral response early after transplantation [6]. The various components interact with the host immune system in a complex pattern, eliciting less immune response than an individual tissue allograft. Radiographs and histology can be indistinguishable from autograft healing as long as sufficient immunosuppressive drugs are taken. After withdrawal of the immunosuppression, both vascularised and nonvascularised allografts can be rejected quickly [7]. In experimental studies with vascularised bone marrow transplantation, stromal and marrow cells act early after transplantation, circulate to the lymphopoietic system of the recipient and are reported to generate tolerance in long-term survival [8]. Factors affecting chimerism in bone allotransplantation are still unclear. Allogeneic vascularised knee joints have been transplanted under immunosuppression with cyclosporine and azathioprine and corticosteroids with good early results [9].

Biomechanical Properties of Bone Grafts

Incorporation of cancellous bone grafts with new bone formation upon necrotic trabeculae results in early graft strength. In cortical bone grafts, initial graft resorption causes graft porosity with reduced strength, which only slowly improves. It is suggested that human segmental cortical bone grafts loose almost half of their biomechanical strength within the first 6 months and remain weakened for another 6 months. This hypothesis is supported by the high number of graft failures between 6 and 8 months after transplantation. Creeping substitution is significantly prolonged in allografts, with fracture of

large-segment allografts occurring after 26 months [10]. Vascularised bone allografts under immunosuppression show superior biological and biomechanical behaviour with higher rates of bone integration [11, 12].

Case Presentation

In the following, we report on our experience in bone healing of our first patient with double hand transplantation [13].

Patient

The hands of a 47-year-old policeman were traumatised severely by the explosion of a bomb he was trying to deactivate. Both hands had to be amputated at the wrist. Soft tissue coverage of the stumps was poor. Tendons and muscles of both forearms were retracted. Double hand transplantation was performed in March 2000 [14]. For bone reconstruction, a proximally based flap of the interosseous membrane together with the periosteum was created at both recipient forearms proximal to the osteotomy site, which was located at the distal third of the forearm. Donor forearm bones, which had a diameter 2 mm greater than the recipient bones, were stabilized to the recipient bones with compression using 7- and 8-hole low-contact dynamic compression plates and 3.5-mm screws. No additional autologous bone grafts were used. The periosteal flap was positioned to cover the osteotomy sites before vascular reconstruction of the transplanted limb was completed. Forearms were splinted for 4 weeks to protect tendon healing.

Induction therapy with antithymocyte globulin (Fresenius Medical Care, Bad Homburg, Germany) at a dosage of 2.5 mg/kg for 4 days was started during surgery and continued until day 3. Before revascularisation, 500 mg of methylprednisolone was given intravenously. An additional 250 mg of methylprednisolone was given on day 1 and 125 mg on day 2. Steroids were then switched to oral prednisolone and tapered rapidly to 25 mg on day 8. Prednisolone was further reduced to 7.5 mg at 1 year. Tacrolimus (Fujisawa, Munich, Germany) was started at a dose of 0.20 mg/kg body weight in 2 oral doses and then adjusted to maintain levels of 15 ng/ml during the first month after surgery, 12 ng/ml between months 2 and 6 and 10 ng/ml thereafter. In addition, the patient was given 1 g of mycophenolate mofetil (MMF) twice a day (Roche, Basel, Switzerland). Maculopapulous lesions typical of rejection of the skin became apparent on day 55 and were treated successfully with 750 mg and 2 doses of 500 mg methylprednisolone and topical tacrolimus and steroid ointment. After 30 months, when graft function had reached a high level, immunosuppression (IS) was changed according to a previously designed protocol: steroids were withdrawn, and rapamycin started at 2 mg/day, aiming for trough levels of 4–8 g/ml. Simultaneously, tacrolimus was reduced to trough levels of 3–4 g/ml. Over the following 3 months, tacrolimus was slowly tapered and then discontinued, leaving the patient on rapamycin and MMF.

Method

As human bone biopsies are difficult to obtain, bone healing was assessed by ultrasound and radiography. Onset and course of early blood vessel ingrowth, development of soft tissue callus and late ossified callus formation were investigated at the osteotomy sites. Homogeneous union was defined as uniform bone structure on all projections; missing union was defined as radiolucency at the osteotomy site without calcification and was differentiated from a calcified filling called hard callus. Stability of the forearm bones was determined by radiological signs of hardware loosening.

The type of bone healing was classified on radiographs according to Burchardt, as follows: type I, bone healing identical to autografts with remodelling and incorporation of the graft and no fatigue failure; type II, chronic repair with delayed union or nonunion, peripheral resorption with loss of graft size, internal resorption and decrease in mechanical strength; type III, no healing and complete graft resorption [1].

Results

Vascular invasion and early callus formation were visible by colour Doppler ultrasound at week 3. Vessels approached the osteotomy sites from the median side where the periosteal flap was positioned. At week 7, soft tissue callus formation was identified by grey-scale ultrasound. Hard callus of the forearm bone first appeared at month 4. Osseous union was observed between month 7 and month 11. At 1 year, homogeneous osseous union of the 4 forearm bones was terminated. All grafted bones were incorporated fully without any signs of chronic healing. The grafts showed healing Type 1. There were no signs of instability, with no loosening of the hardware devices in either donor or recipient bone.

Fracture Healing

The same patient sustained a distal radius fracture at his left wrist on November 2003 during a motorcycle accident while travelling in South America. He first showed up for X-ray control at our hospital on 2 December 2003. The fracture was treated conservatively by splinting. The radial styloid fracture fragment was dislocated and appeared as a radiolucent fracture line. The metaphyseal area was compressed, the intra-articular fracture pattern showed no significant steps. On 14 January 2004, some radiolucency at the metaphyseal area was visible, with beginning radial appositional bone formation. In March 2004, the metaphyseal compression was hyperdense and showed trabeculae bridging the fracture fragments. At that time, immunosuppression consisted of rapamycin (serum trough levels 4–8 ng/ml) and MMF (2 g). Time course of this metaphyseal, cancellous bone healing was delayed compared with normal fracture healing, which may have been caused by the target of rapamycin (ToR) inhibitor he was taking.

In January 2005, the patient fell while walking on a snow-covered footpath and sustained a fracture of his left radius. The fracture line at the shaft was at the former osteotomy site. The plate showed slight bending without loosening of the screws. The frontal fracture was beneath a spared-off plate hole. There was no fracture sign proximal or distal of the plate. On 15 March 2005, the radiolucent gap was visible in posteroanterior and lateral radiographs. On 6 June 2005, the fracture gap remained radiolucent with signs of calcified filling and a beginning of appositional bone formation. As rapamycin was suspected to slow bone healing, dosage was reduced to achieve trough levels of 2–4 ng/ml and tacrolimus restarted (trough levels 3–5 ng/ml). After the fracture had healed completely, on 9 September 2005, tacrolimus was withdrawn and rapamycin again increased to achieve serum trough levels of 4–8 ng/ml. In addition, splinting and low-pulsed ultrasound was used to improve bone healing. Time course to bone union was delayed and was achieved at month 8.

Discussion

The biological process of bone healing in hand transplantation can adversely be influenced by instability at the site of osteotomy or by impairment of the vascular supply. From experimental studies, the vascular supply of the diaphysis of radius and ulna is known to be primarily supported by the palmar and dorsal interosseous artery. The nutrient foramina are mainly located at the interosseous margin and the palmar aspect of the radius, with the vessels intruding the bone from a periosteal network, which is supplied segmentally [15–17]. Following hand amputation, the level of osteotomy for rigid stabilisation by plates will be proximal to the distal fourth of the length of the forearm bones and so be in a poorly vascularised region. Soft tissue damage due to the initial trauma, and consecutive scarring and fibrosis can additionally impair vascularity and thus influence biological healing capacity.

Apart from these critical biomechanical and biological aspects, the complex process of bone healing in hand transplantation is additionally impaired by immunological reactions and possibly by immunosuppressive medication. Bone healing in hand transplantation is healing of a vascularised, allogeneic cortical graft under immunosuppression. Immunology and the lack

of precise monitoring still leave many aspects of this biological process unknown.

Stability

Bone stability is essential for musculotendinous function and therefore a primary goal in rehabilitation programmes. Due to the immunogenicity, bone strength in hand transplantation remained a challenge in rehabilitation over the years. Close observations are required to calculate the risk of graft failure or fracture. Strategies applied to optimise early stable bone union in an undefined immunological environment are based on the principle of maximal stability at the osteotomy site and the idea to optimise the biological circumstances. Compression stimulates bone healing. Primary bone healing was intended in all patients and attempted to be achieved by different plate systems. No attempts have been reported to achieve union by indirect bone healing using intramedullary stabilisation or external fixation.

The Lyon group [18] used 7-hole plates and 4.5-mm screws for fixation of both forearm bones in their two patients. Although rejection episodes occurred, bone healing was reported to be normal [19]. The forearm bones of the first Louisville patient were stabilised with 3.5-mm metal plates [20]. Two rejection episodes 6 and 20 weeks after surgery did not impair bone healing. In the patient presented in this chapter, 7- and 8-hole plates with 3.5-mm screws were used for bone stabilisation. Unfortunately, no further reports are available as of today about bone healing in the other recipients. All plate systems were stable enough to tolerate the individual rehabilitation protocols. No hardware failure or loosening was reported in the early postoperative period.

To optimise local conditions at the osteotomy site, it was attempted to improve oxygenation at the fracture site or offer osteoinductive and osteoconductive elements. The French group used recipient cancellous bone chips from the iliac crest to support bone union. Radiographs at 3 months confirmed solid callus formation. Nonvascularised autologous cancellous bone grafts under sufficient immunosuppression showed solid healing at the donor–recipient bone junction. To improve bone healing in the early period of repair, a vascularised periosteal flap from the recipient to cover the osteotomy sites was used in the Austrian patient [21]. The fact that the onset of callus formation with first signs of vascular ingrowth occurred where the local periosteal flap was positioned may be evidence that this strategy was helpful.

Time Course of Bone Healing

The time course of bone healing in sufficiently immunosuppressed patients with hand transplantation is delayed compared with nonimmunosuppressed patients but similar to that seen after replantation. Despite their known effect on bone metabolism, glucocorticoids seem not to delay bone healing compared with the time course in replantation. In the Austrian patient the time course of bone healing was not affected by a rejection episode even though the onset of antirejection treatment was delayed a few days because of misinterpretation of skin histology. Also, repeated acute rejections, as reported by other centres, showed no negative impact on bone healing. In our patient, primary bone healing was achieved at month 11. Bone bridging by the use of autologous cancellous grafts can be achieved at month 3, as reported by the French group [18].

Bone Strength

Unfortunately, there is no parameter to estimate bone strength by radiological means. The composition of necrotic and new bone in cancellous bone grafts, as we observed in the distal radius fracture, and the porosity of creeping substitution, as we had to deal with in the radius shaft fracture, remains largely unknown. The use of primary bone grafting for the treatment of forearm fractures is not obligatory. However, in hand transplantation with prolonged intervals of primary bone healing, additional bone grafting can improve bone union and probably bone strength at the osteotomy site.

In our very active patient, we had to deal with the problem of fracture healing under pharmacological immunosuppression. Though fractures in immunosuppressed patients are not rare, fracture healing in transplanted donor hands has not been reported hitherto. Union was delayed after both fractures of the distal radius metaphysis and the cortical radial shaft, respectively. Union of the distal radius metaphysis took 3 months, which is comparable to the bridging of the cancellous bone grafts reported in the French patient. The shaft fracture demonstrates the dilemma of estimating bone strength radiologically even after 5 years. In united bone fragments with a plate in place, a fracture usually occurs at the perimeter of the hardware, as the locus of *minoris resistentiae*. In our patient, these locations seemed to be more stable than the primary osteotomy site, which is still supported by the plate. Thus, active hand recipients should be informed about the impaired stability of the osseous components of their graft.

Immunosuppression and Bone Healing

Immunosuppression was based on tacrolimus in all patients and included steroids and MMF. Tacrolimus is reported to induce alkaline phosphatase, a marker of osteoblast activity, and also to enhance osteoblastic differentiations induced by bone morphogenic protein (BMP)-4 [22]. The proper use of tacrolimus is essential to lower the dose of glucocorticoids, thereby positively influencing bone mass evolution [23].

Early callus formation and early revascularisation imply that immunosuppression had no adverse effect on vascular ingrowth. Not only the initial phase of the bone healing process but also the disappearance of vascular ingrowth and callus maturation to collagen and ossified structures were comparable to bone healing after replantation. Thus, immunosuppressive drugs used so far after hand transplantation seem not to impair cellular biology during maturation of soft tissue callus to chondral and ossified callus. No direct influence on bone healing by the applied immunosuppressive regimes or by early rejection episodes was observed on ultrasound or radiographs. Whether or not rapamycin delays fracture healing remains to be studied.

Conclusion

The primary goal of forearm bone reconstruction in hand transplantation is to achieve and maintain stability enabling early motion rehabilitation. In all reported hand transplants, bone union was achieved by direct bone healing using various plate systems. Biology at the osteotomy site was in some patients supported by primary bone grafting and periosteal flaps, which seemed to be beneficial. The time course of bone union at the osteotomy site was equivalent to replantation and delayed in comparison to normal fracture healing. Immunosuppression was based on tacrolimus in all patients and included steroids and MMF and did not adversely influence bone healing. Time course of bone healing following fracture was delayed compared with normal fracture healing. Bone strength at the osteotomy site seems to remain reduced over 5 years.

Acknowledgement

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Section 9-b

Return of Sensibility and Motor Recovery of Extrinsic and Intrinsic Muscles

Graziella Urso, Luisa Stroppa, Tatiana Barchitta, Paola Cossa

Introduction

Hand transplantation requires immediate and careful rehabilitation. Therapy to reeducate the patient must begin 48 hours after the operation and must be planned with the surgeon, the anaesthetist, the psychologist and the physiotherapists. Initially, it must include monitoring posture in order to prevent oedema and rigidity of the areas near the arm and hand. Recovery of sensibility is introduced later: gliding of extrinsic musculature tendons, recruitment of intrinsic muscles and functional recovery. Integration of the new limb into the body image has a fundamental role in transplantation reeducation, without which there would be no point in the rehabilitation programme and, above all, the final results would be less successful.

Return of Sensibility

The return of sensibility is the most important goal of the rehabilitation programme. To be able to have a hand again, to use it and, through it, feel again, are the goals of this operation. This implies, in itself, "active touching": moving the hand in order to explore and interact with the external world. Motor and sensory reeducation must therefore follow a parallel path. A hand that does not feel is not encouraged to move, and a hand that does not have active movement does not increase the return of sensibility. The more a hand is used, the more it recovers a good level of sensibility. Assessment of sensibility of the transplanted hand starts during the third postoperative month. Reeducation must start when protective sensibility has returned. If the patient has not regained this sensibility, he or she is taught how to observe the protection guidelines mentioned by Brand. Clinical tests to document the gradual return of sensibility are those suggested by B. Rosén, OT. Msc Department of Hand Surgery, Malmö University Hospital (*see* Section 9-i).

Test battery:

- Reinnervation: Semmes-Weinstein monofilaments test (Fig. 1)
- Tactile gnosia: classical static 2-point discrimination (s2PD) test; shape–texture identification (STI) test
- Pain–discomfort: scale for problems linked to hyperesthesia and intolerance to the cold

Each test is carried out every 3 months.

Monofilaments Test: In 1960, Semmes-Weinstein studied a scalable instrument for assessing tactile sensibility. Each monofilament is coded with a colour, and a number that ranges from 1.65 (the thinnest) to 6.65 (the thickest). The dysfunctional area is assessed, and data are registered on a preestablished chart (Fig. 2).

Static 2-Point Discrimination (s2DP) Test: This test measures innervation density of the slow-adaptation receptor-fibre system (Merkel complexes). The Boley gauge or the Dellon Disk-Criminator is used. With eyes closed, the patient must recognise a slight pressure of 1 or 2 points with a distance of 15–6 mm between them.

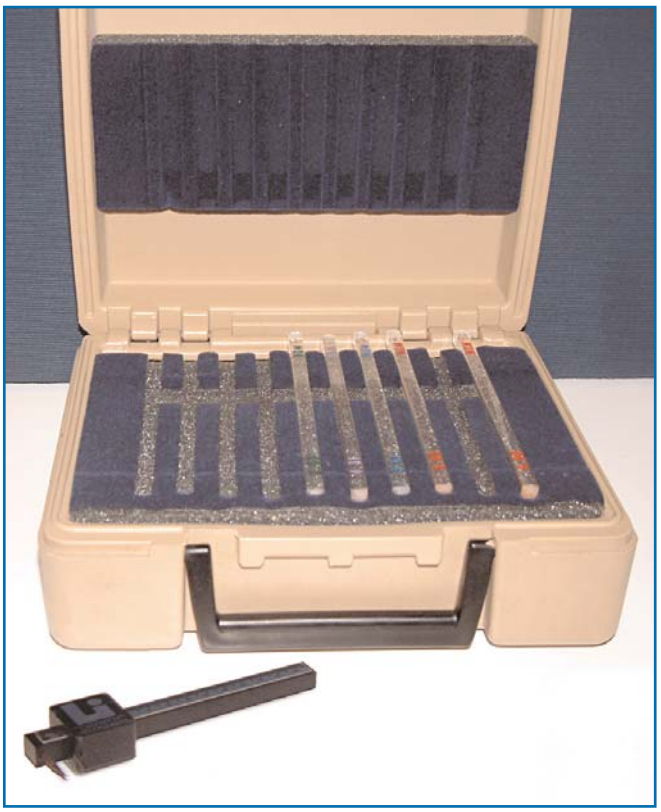
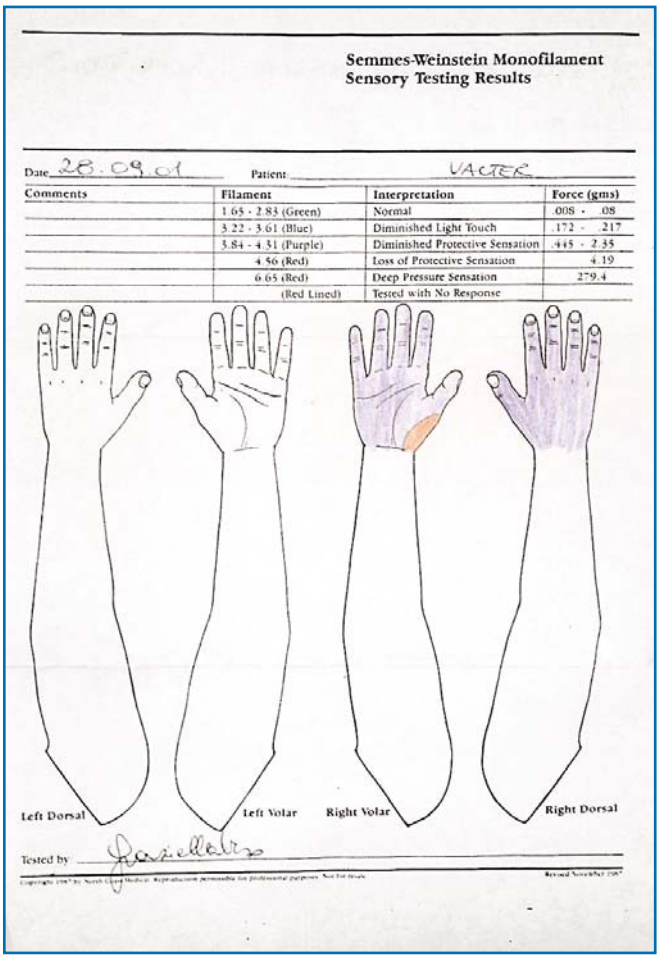


Fig. 1. Semmes-Weinstein monofilaments kit and Boley gauge



Semmes-Weinstein Monofilament
Sensory Testing Results

Date 28.09.01 Patient VACTER

Comments	Filament	Interpretation	Force (gms)
	1.65 - 2.83 (Green)	Normal	.008 - .08
	3.22 - 3.61 (Blue)	Diminished Light Touch	.172 - .217
	3.84 - 4.31 (Purple)	Diminished Protective Sensation	.445 - 2.35
	4.56 (Red)	Loss of Protective Sensation	4.19
	6.65 (Red)	Deep Pressure Sensation	279.4
	(Red Lined)	Tested with No Response	

Left Dorsal Left Volar Right Volar Right Dorsal

Tested by

Fig. 2. Results table

Shape-Texture Identification (STI) Test: Studied by Lundborg and Rosén (*see* Section 9-i), this test consists of identification of shapes and textures without the help of sight. There are 3 degrees of difficulty represented by the measurement of shapes (15, 8, and 5 mm) and distance between one point and another in the textures (15.8 and 4 mm). Identification is carried out with the index finger according to a standardised procedure, and results are recorded with a score of 0 to 6.

Pain-Discomfort: This is a subjective assessment of the patient with respect to hyperesthesia and intolerance to the cold. The patient is asked to describe the pain or discomfort felt with normal touch, when using the hand and when exposed to the cold. The scale includes four answers:

- No pain
- Moderate pain
- Disturbing pain
- Pain that prevents carrying out everyday activities.

Sensibility Reeducation

Treatment must be carried out in ambient conditions that favour concentration. Each sensibility retraining session must last from 5 to 10 min. During the first phase, the goal is the return of dynamic touch perception, and this can only happen if the patient perceives static touch during the Semmes-Weinstein monofilament 4.31 test. Discrimination training also accompanies localisation (Fig. 3). The second phase envisages recovery of tactile gnosia (recognition of objects without the help of sight). Recovery progress is registered periodically through the number of objects identified correctly during an established period of time.

The Tactile Glove

The tactile glove (Lundborg-Rosén) is a glove with small microphones applied on the back of the finger tips. When a surface is touched, the

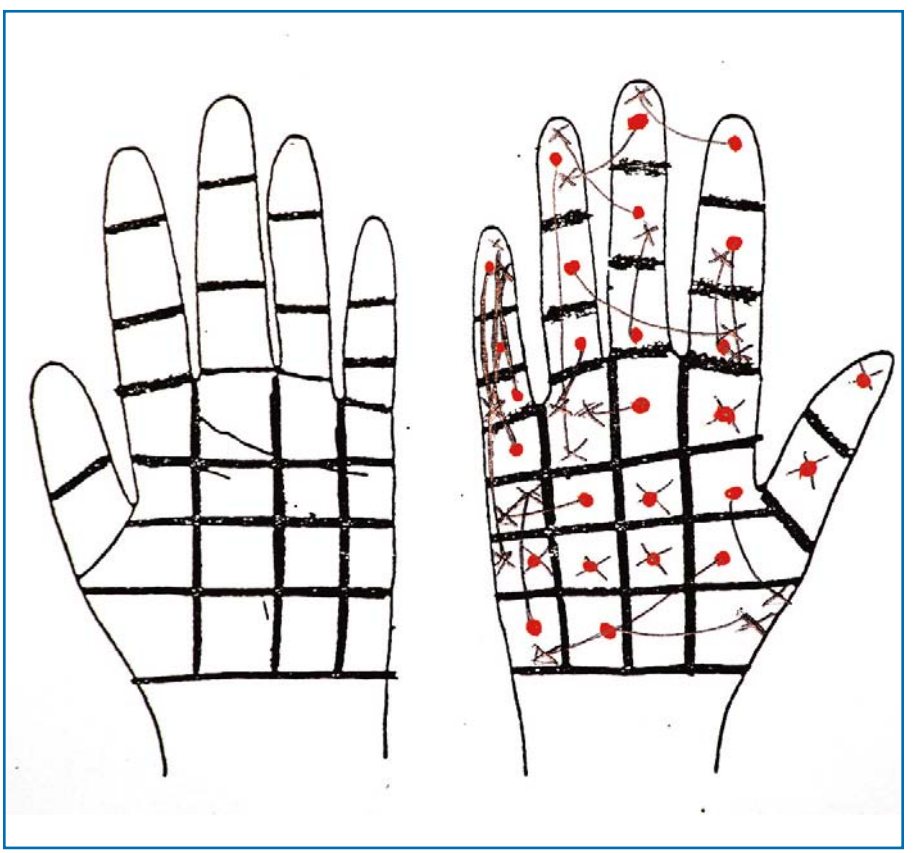


Fig. 3. Localisation assessment table: the red dot corresponds to the point that is being stimulated. The cross corresponds to the point where the patient perceives the stimulation

tactile stimulus generated by friction of the fingertip is transformed into an acoustic stimulus, collected and amplified by the microphone. The various surfaces can be recognised (Fig. 4) by the specific sounds generated, and the individual fingers can be distinguished according to spatial localisation of the afferents, transmitted with different intensities through a small stereo system. This glove is also used during the preoperative phase and is worn on the aesthetic prosthesis. The goal is to establish a flow of specific substitute afferents, starting from the "ghost" hand or the still denerved hand, that can facilitate cortical reintegration of the hand itself and, therefore, sensibility relearning. Exercises with the glove are performed for 5–10 min 3–4 times a day.

Desensitisation

Hypersensibility, frequent above all in the scar areas, can be reduced by planning this programme as soon as possible. The goal is to reaccustom the transplanted hand to contact with the external environment. The exercise consists of rubbing the parts concerned with materials that become gradually more and more unpleasant (1 min of rubbing for each surface starting from the most pleasant to the most unpleasant and vice versa). 4 to 5 surfaces are used, and the exercise is repeated 4 times a day.

Motor Recovery

The approach methodology is the most successful strategy in such a long and complex programme. Rehabilitation starts during the preoperative phase and consists of articular and muscular assessment of the shoulder and elbow; any compensation in the shoulder due, for example, to incorrect usage of the prosthesis is examined, and the strength of flexor and extensor muscles in the forearm are assessed. During the post-

Fig. 4. Sticks with different surfaces for tactile sensibility training

operative phase, each rehabilitation step is discussed and confirmed by the team, especially during the first 8 weeks. Session frequency is: during the first 6 weeks, the patient is treated twice a day 7 days out of 7; from the 2nd to the 8th month, 5 days out of 7; from the 9th month to the 3rd year, the patient is treated and monitored every other day. From the 3rd to the 5th year, treatment is reduced to monthly checkups that may become more or less frequent depending on the progress of recovery and the patient's requirements in relation to returning to work, hobbies and everyday life.

From the 2nd Day to the 3rd Week

Assisted active mobilisation of the shoulder and elbow begins 48 h after the operation. The posture of the entire limb and the transplanted part are checked with sloping wedges with a soft surface. The nursing staff is shown how to check the posture every hour. During the 1st week, a static protection splint of the resting position type is made, which the patient wears 24 h a day (Fig. 5). During this week, treatment of the oedema must be understood as control and prevention. During the 2nd week, passive mobilisation of the wrist and hand begins. The goal is to reinstate the wrist–hand tenodesis effect as soon as possible: extension of the wrist, bending of the fingers and vice versa (Fig. 6). Initially, this will be passive in order to favour tendon gliding, but as soon as possible, it must be carried out in an assisted active way in order to achieve cortical recruitment of this important motor system. A checkup of the skin and scars is introduced in the 3rd week.

From the 3rd to the 6th Week

The splint is modified in order to adapt it to tensions and lengths of extensor and flexor tendons. Formation of scars and adhesions increases, especially in the muscle–tendon joint; blunt dissection

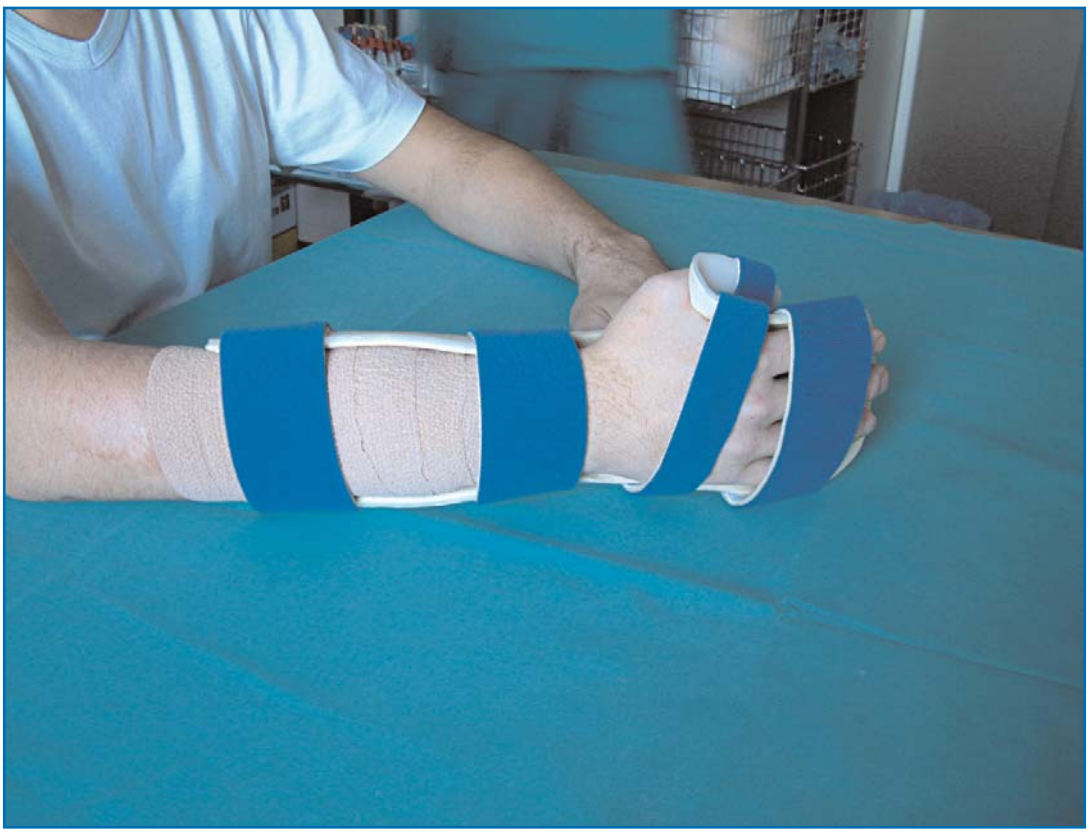


Fig. 5. Resting-position splint made to fit the patient during the first postoperative week

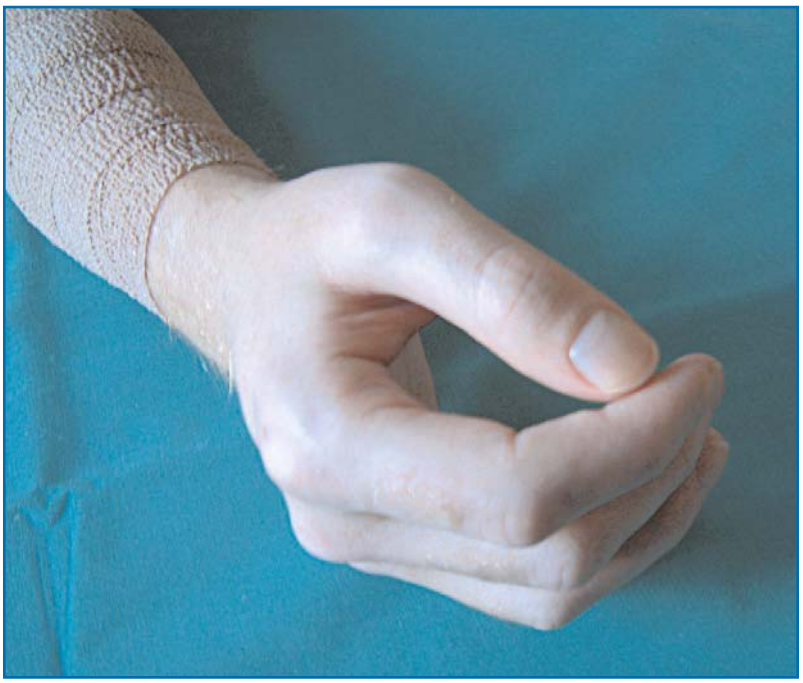


Fig. 6. First tenodesis effect active recovery exercises: the wrist is extended and the fingers bend. Initially, they are performed without gravity with the forearm in lateral decubitus

and remodelling of scars is increased. A medium-compression Coban type bandage is applied from the wrist to the muscle–tendon joint (Fig. 7). During the 4th week, active bending–extension movement of the wrist is stimulated with the fingers in a position that protects the tendon sutures. In week 5, very cautious assisted active work begins in order to extend and bend the long fingers and thumb with the wrist in a position that protects the surgical sutures.

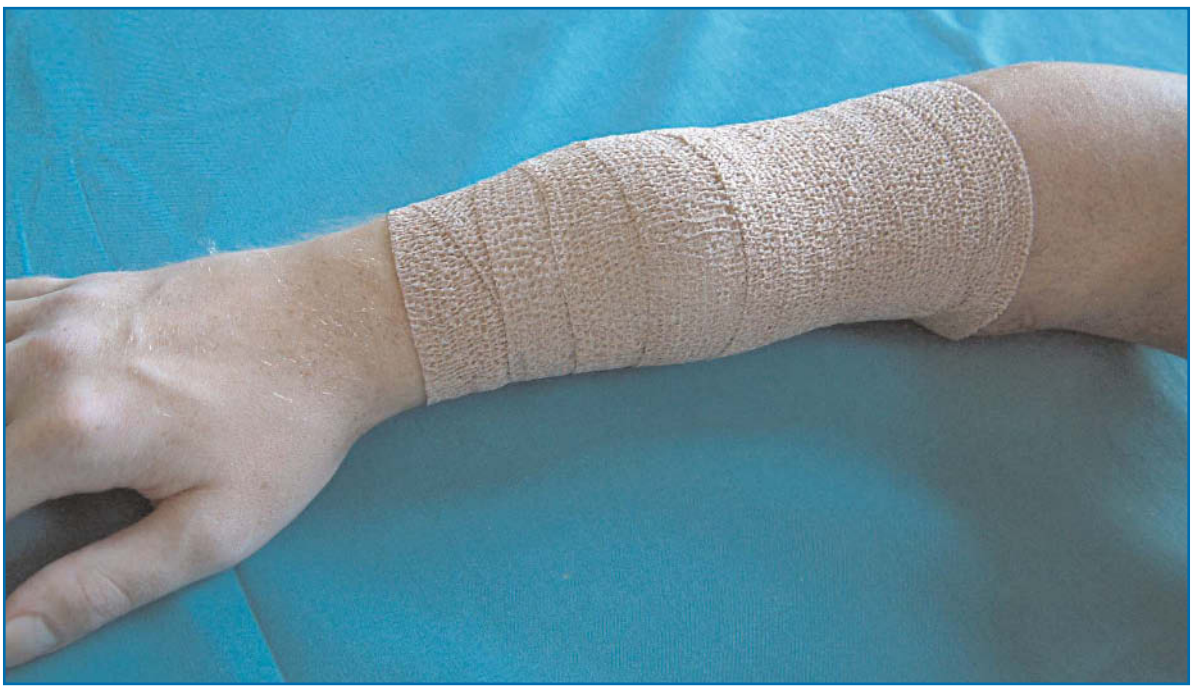


Fig. 7. Coban bandage for remodelling the scar at the muscle–tendon joint

From the 6th to the 8th Week

Active movement of the wrist and fingers improves, and the aim of the session is to improve coordination of wrist and finger movements during light, everyday activities. Active recovered movements must fall within the corporeal image, and the purpose of each movement must be for the patient's own body and the external world. Gripping exercises are studied on the basis of tendon length, and emphasis must be put on measuring gripping strength and on releasing the object (Fig. 8). Use of the resting-position splint continues during the night and at intervals during the day. During this period, a decision is made about whether it is appropriate to introduce a static wrist splint with the fingers free (Fig. 9) to correct axial distortion of the wrist and improve finger function.

Fig. 8. Exercise to grip a medium-size object

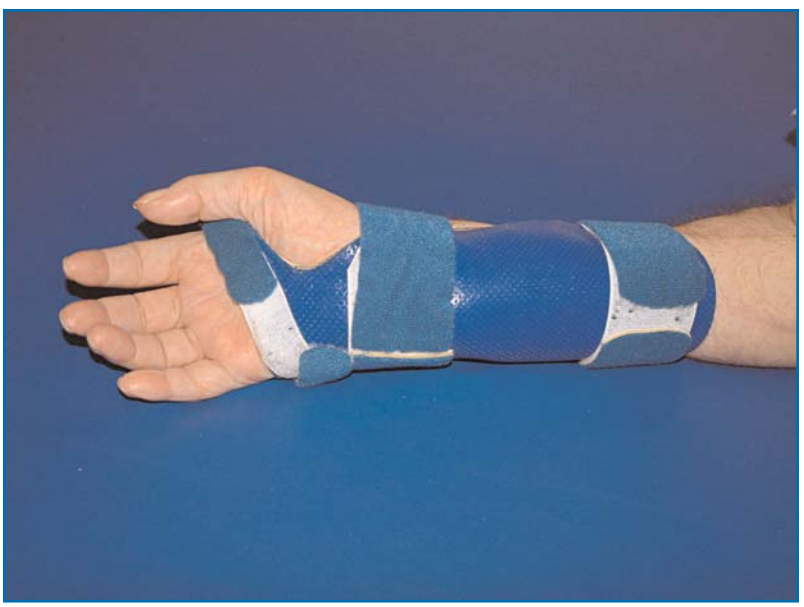


Fig. 9. Static wrist splint to stabilise the radiocarpal joint and improve the bending function of the fingers

From the 8th to the 12th Week

During this period, great attention is paid to increasing tendon gliding, flexor–extensor balance and recovery of gripping strength. During the 8th week, electrotherapy is introduced to strengthen the extrinsic musculature of the forearm.

From the 3rd to the 6th Month

Targets during this period are to introduce the hand into everyday activities and improve wrist stability and function. Treatment also envisages the use of large handles and any writing aids (Fig. 10). Furthermore, functional assessment is carried out as well as assessment of the appropriate use of the aids themselves. Shoulder and elbow compensation studies must also not be neglected (Fig. 11).

From the 6th to the 12th Month

Specific work is carried out on remaining functional deficits, with inclusion of dedicated splints and appropriate exercises. The patient's needs with regard to everyday life and profession are considered, and we proceed with a work-hardening schedule dedicated to the type of life and working activity.

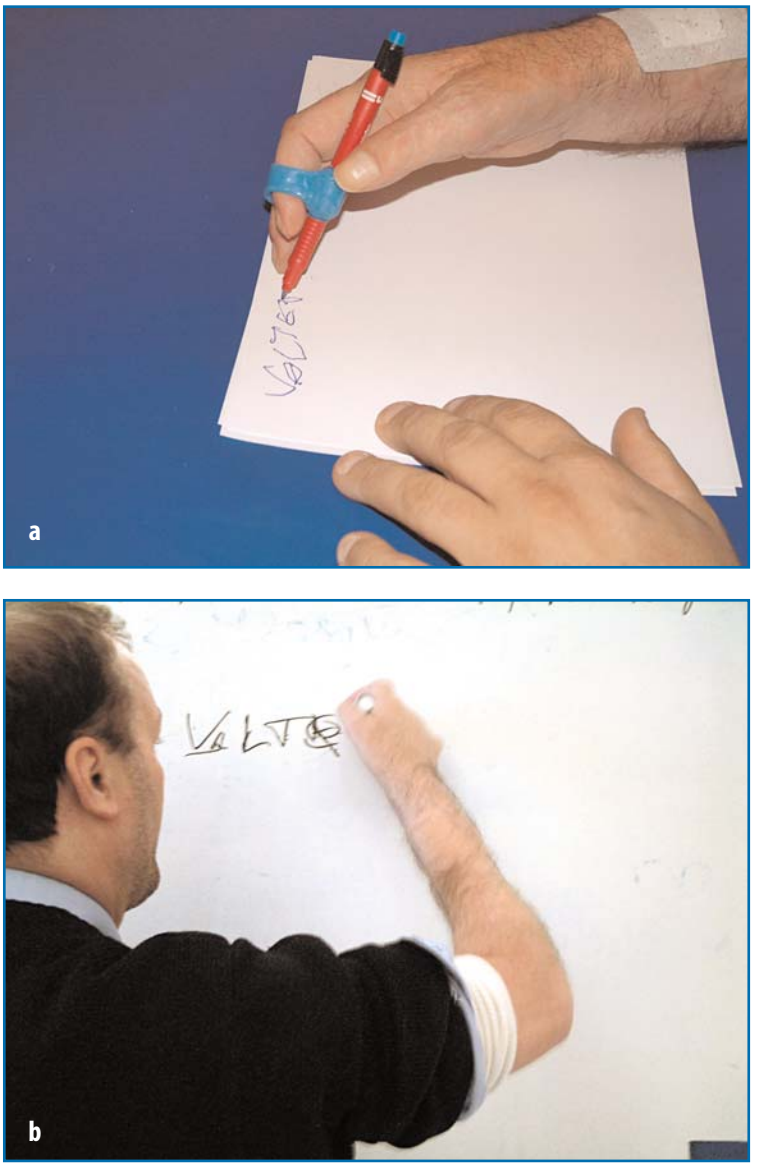


Fig. 10a, b. Writing exercises: first with aid, and then without aid

Fig. 11a-c. Verification of the type of aid suitable for the main everyday activities and study of compensations caused by a wrong choice of aid

From the 12th to the 24th Month

Treatment is reduced to just a few sessions a month, above all to check that results are maintained and to correct wrong postures and overuse of the hand.

From the 24th to the 36th Month

The patient keeps in touch with the physiotherapists to carry out checkups on strength and postural attitudes and to prevent inflammatory syndromes caused by overuse.

Extrinsic and Intrinsic Muscles

Recovery of the extrinsic musculature of the transplanted hand is closely linked to surgery since the choice of flexor and extensor lengths can condition protection times, the type of protection and the result of the grip. Sutured flexors with greater tension have given better results in terms of gripping strength, but it was fundamental to monitor splint position frequently in order to exploit to the fullest the elasticity of tendons and muscular belly. For this reason, from the 12th to the 24th month after the operation, the patient wore a night splint that fosters greater gliding and a gradual recovery of lengths.

When the flexors are sutured with greater length and less tension, it is more difficult to reinstate the complete closing strength of the fist. For this reason, a programme of electrotherapy was used to strengthen the muscles of the flexor tendons with the splint in a position that locks the metacarpal–phalanxes in order to make the surface flexor and deep flexor stronger. During the 6th postoperative month, the transplanted hand begins to involve the palm in medium-sized gripping movements: in actual fact, this happens above all because the wrist has acquired greater coordination in the tenodesis effect and extends when the fingers bend to grip the object. This new gripping image highlights the initial appearance of the intrinsic muscles. From appropriate studies carried out during the 6th postoperative month (*see* Section 9-e), it was shown that 11 months after transplant, the first clear motor unit potential train was detected from the abductor digiti minimi muscle. Twelve months after transplant, abductor pollicis and opponens pollicis muscles showed surface electromyogram (EMG) activity. After 15 months, the first dorsal interosseous muscle showed the first active motor unit. Therefore, by abducting, the 5th finger makes it possible to grip larger and spherical objects, but the appearance of a slight contraction of the opponens pollicis allows considerable increase in recovery from all points of view: cortical, sensitive, motor and relational. In order to stimulate functional activity of the opponens pollicis, a gradual light static splint was made that extends the thumb with medium abduction. Its aim is to replace muscle fibres that are not yet reinnervated, stimulate use of the thumb cortically and shorten the muscle itself, therefore allowing it to contract more easily (Fig. 12). The same principle was applied during the 12th month for the lumbrical muscles, by making an intrinsic plus type splint to help shorten them and improve the hand's gripping strength (Fig. 13). These splints are used during the day, especially during gripping exercises and everyday activities.

The rehabilitation programme for the transplanted hand and the results achieved confirm the importance of working with the patient as soon as possible and tackling various different aspects simultaneously: sensibility, motility, function and reinclusion of the hand in the corporeal image and everyday life. Twelve months after the transplant, patients are able to carry out all the most important everyday activities, drive a car, ride a bike or motorcycle and start to return to their jobs.

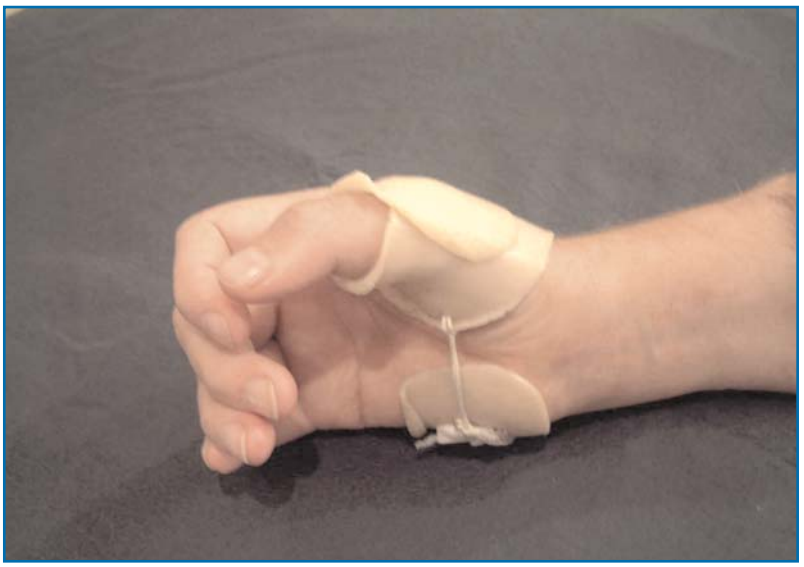


Fig. 12. Splint for thumb abduction

Fig. 13a, b. Intrinsic-plus splint to separate the lumbrical muscles and subsequent splint to stimulate intrinsic muscles to work harder

Section 9-c

From Silent Neuroma to Reactivation of Axonal Growth: How a Peripheral Nerve can Start to Regenerate into a Transplanted Hand?

Lars B. Dahlin, Göran Lundborg

Introduction

Neuronal injury with subsequent axonal outgrowth following transection and repair of a nerve trunk puts great demand on the neuron, thereby representing a situation of enormous complexity [1]. Transection of a peripheral nerve trunk has immediate as well as long-term physiological, biochemical and cellular effects on multiple levels of the nervous system, ranging from targets, such as sensory receptors and muscles, in the periphery and all the way up to somatosensory and motor brain cortex [1–4]. Such a situation constitutes one of the most challenging and difficult reconstructive problems faced by surgeons. Even a simple cut of a digital nerve represents a major problem for the patient. In spite of immediate repair of the injured nerve, there is usually a suboptimal functional recovery in an adult patient, creating extensive costs for society [5]. This is even worse when large nerve trunks or parts of the brachial plexus are injured. From the neurobiological point of view, the problem of peripheral nerve injuries is complex. The inferior outcome is based on multiple factors, such as posttraumatic cellular death, particularly of sensory neurons, long regeneration distances, atrophy of end organs and misdirection of axons at the repair site resulting in functional cortical reorganisational changes [1, 3, 4].

A somewhat different situation is an "old" nerve injury (weeks, months or even years, such as in hand transplantation) where a secondary or late nerve repair of the injury may be done. The proximally situated nerve cell body and its axon have been disconnected from the target organ over an extended time period. The time lapse from injury to repair has a negative influence on nerve cell bodies, such as a continuously decreasing number of surviving sensory neurons [6, 7]. Furthermore, distal nerve segments as well target structures have been denervated during the corresponding period. In the distal nerve segment, there is, in the long term, downregulation of factors in Schwann cells [8, 9], temporal change in macrophages [10], cyclic expression pattern for cytokines [11], invasion of fibroblasts and formation of fibrosis as well as secondary changes in the targets of the neurons (receptors and muscles [1]). The primary question arises whether it is possible to "wake up" a sleeping neuron by resecting the formed neuroma but also whether denervated Schwann cells can support regenerating axons. In experimental studies, various nerve transfer situations have been designed to shed light on these questions and highlight the inherent biological, cellular and molecular reactions in the main actors of the regeneration theatre: neurons, Schwann cells and other non-neuronal cells [12].

An even more unique and complex biological situation for neurons and Schwann cells, in view of recent achievements in transplantation, is reinnervation of a transplanted limb. The recipient of a hand transplant is an amputee, often

with an extremely long time lapse from injury to transplantation (up to 22 years [13]). Neurons in the amputee's arm have been devoid of contact with target organs for an extended time period and have formed a neuroma in the distal part of the amputation stump. During the surgical transplantation process, the neuroma is excised with an additional injury to the neuron, and the nerve segments in the amputation stump of the recipient are connected to the freshly cut distal nerve segments in the transplanted arm with fresh targets. A corresponding situation seldom occurs in routine clinical praxis – the closest analogy would be secondary reconstruction of an old nerve injury where a previously axotomised nerve segment is sutured to a distal, fresh, vascularised nerve graft. However, with limb transplantation, more complex factors further complicate the picture: the distal nerve segment is an allograft, and the regeneration process is influence by various immunosuppressive drugs, commonly the immunophilin ligand FK506 [14–16]. Excision of a neuroma as part of the transplantation process is also a potential threat to neurons. A secondary injury may not only stimulate a regenerative response but may also theoretically arouse pain mechanisms. thereby disturbing the patient. In the present review, we summarise intraneuronal and other alterations in cells induced by a nerve injury, the mechanism involved in nerve regeneration in a "known and foreign" environment and the influence of immunosuppressive drugs with relevance to nerve regeneration in hand transplantation.

Nerve Injury and Regeneration: General Aspects

The biological basis for axonal regeneration after injury and repair and after nerve reconstruction has been extensively delineated in the literature [1, 4, 17]. In the present review, processes relevant for axonal regeneration initiated after a certain time after the injury similar to waking up sleeping neurons in hand transplantation is covered. The outcome of repair and reconstruction is dependent on multiple factors such as age, repair technique, time lapse from injury to repair, type and level of injury and status of the end organs. However, the fundamental requirement for axons' ability to regrow after injury is the intracellular processes that take part in the neuron and the interaction between their supporting cells, i.e. Schwann cells and macrophages in the nerve trunk and satellite cells in the dorsal root ganglion (DRG). These processes are dependent on extensive biochemical events in the neurons occurring from the distal part of the axon up to the nerve cell body. Alterations are triggered by intraneuronal signal steps following nerve transection. The neuron switches from a status of "maintenance" to a status of regeneration, and protein synthesis in the cell body switches from new transmitter-related substances to those required for axonal reconstruction [18–20].

The Initial Signals in Neurons After Nerve Injury

The initial steps after nerve injury are signals initiated by the inhibition of retrograde axonal transport, the premature return of conformationally changed substances and, maybe very importantly, "positive and negative" injury signals incorporated at the tip of the transected axon. The signals elicit a number of steps with the aim of waking up the gene programme, with subsequent production of adequate substances necessary for regeneration. Events from the initial signal to physiological alterations with numerous steps, such as mitogen-activated protein kinase steps (MAPK) (Fig. 1a), leading to a specific physiological outcome are called signal transduction. The research area is presently the target for extensive research [1]. One interesting step is the Jun N-terminal kinase (JNK) pathway (Fig. 1b) in which the transcription factor c-Jun is rapidly activated by MAPK JNK after injury, which is necessary for axonal outgrowth but not for survival in adult sensory neurons [21]. c-Jun forms dimers with other transcription factors, such as activating transcription factor 3 (ATF3), and it is plausible that the expression of this dimerisation partnership could regulate the

physiological effect of c-Jun activation. Furthermore, such dimers may promote survival response in neurons and stress situations by inducing the expression of antiapoptotic proteins such as the heat shock protein 27 (Hsp27) [22].

Components of the JNK signal pathway are axonally transported from the injury site to the cell body of sensory neurons, thereby contributing to the nuclear increased c-Jun activity [23] (Fig. 1c). This is interesting in view of how a neuron becomes aware of an injury inflicted on the axon. One explanation is that a decrease in the return of trophic factors from the target organs could function as a messenger [24]. Another important mechanism by which information could be conveyed to the cell body as a signal of an axonal injury is the return of proteins from the injury site [25, 26]. Importins facilitate retrograde injury signals in an injured axon, thereby forming axoplasmic complexes with nuclear localisation signal (NLS)-bearing proteins, which can be conveyed to the nerve cell body eliciting the whole spectrum of genetic modulation [27]. The JNK signalling components aggregate with the scaffold protein JNK-interacting protein (JIP). Such JIP complex is transported axonally in a retrograde direction, however, by a hitherto unknown mechanism (Fig. 1c) [23]. The functional consequences of such mechanisms, with retrograde axonal transport and subsequent nuclear activation of c-Jun, is induction of, for example, the ATF3 gene, which is induced by binding the c-Jun to the ATF3 promoter [21]. As a response to the c-Jun-ATF3 heterodimerisation, the antiapoptotic factor Hsp27 is induced, thereby acting as a survival factor [28]. By these mechanisms and other central pathways in the MAPK module (Fig. 1b), an extracellular stimulus can induce cell responses leading to survival and regeneration or apoptosis. The induction of JNK, c-Jun and ATF3 is very rapid in neurons (and in Schwann cells), usually within hours after a nerve injury [21]. Interestingly, the above-mentioned transcription factor ATF3 is down-regulated with time (weeks) in motor neurons and Schwann cells. In contrast, sensory neurons still

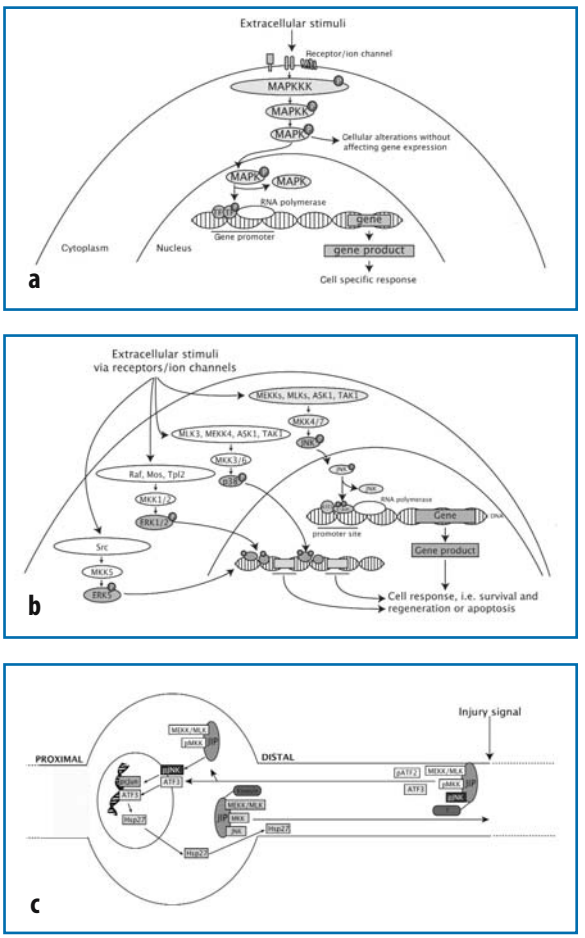


Fig. 1. Simplified and schematic illustration of the mitogen-activated protein kinase (MAPK) signalling pathway (**a**) and details of the four central MAPK pathways [Jun N-terminal kinase (JNK); p38, extracellular signal-regulated kinase (ERK)1/2 and ERK5], which give rise to specific responses in the nucleus (**b**) and an injury-induced signal (**c**). The pathways are initiated by stimulus extracellularly via receptors/ion channels in the cell membrane (**a**). Via three kinases downstream, a final kinase, the MAPK, can induce long-lasting changes by affecting gene transcription through phosphorylation of, for example, transcription factors or short-term alterations by transient phosphorylation of cytoplasmic proteins (**a**). Via the JNK signal pathway, c-Jun and activating transcription factor 3 (ATF3) can be activated, leading to a regenerative response in the neuron (**b**). Endogenous proteins, including JNK and JNK-related signalling proteins, convey information of a distal injury to the neuronal cell body with a retrograde axonal transport of JNK up-stream kinases and the JNK interacting protein (JIP) (**c**). The JNK module may, together with axonal transport of various transcription factors, lead to initiation of the cell body response delineated in (**a**) and (**b**). Such mechanisms are most likely to be initiated in a neuron, maybe even many years after the initial injury, in connection with hand transplantation. Reproduced with kind permission from Charlotta Lindwall, Lund University [22]

show expression of ATF3 in the nuclei up to 6 months after nerve injury [9]. Following activation of the intraneuronal signal steps, there is a profound alteration in gene expression as early as 12 h after an axonal injury in sensory neurons [29, 30]. For review of the detailed mechanism by which genes are initiated [1].

The signal transduction steps are interesting also in view of the fact that they most probably can be reactivated in neurons that have been injured or transected many years ago. By the above-mentioned mechanisms, a sleeping neuron can be woken up. In reconstruction of a previously injured nerve, either in connection with a nerve graft procedure or with hand transplantation, the results will be regeneration of neurons out into a new environment.

Programmed Cell Death After Nerve Injury

Morphologically, the cell body reacts after a nerve injury with unspecific changes, such as change in cell-body volume, displacement of nucleus to the periphery and dispersion of the Nissl substance (chromatolysis) [31, 32]. Cell body reaction varies with age, species, lesion proximity, cell size and nature of the lesion [33]. However, in some cells, the programmed cell-death cascade mechanisms, via activation of caspases, are initiated, and the cell goes into apoptosis, which includes fragmentation of DNA, cell shrinkage, condensation of the chromatin, fragmentation of the nucleus and the cell and formation of apoptotic bodies. A severe problem with the sensory neurons specifically is this neuronal death, a phenomenon that is more pronounced in young individuals (pre- and postnatal) compared with adults [34]. Even after immediate microsurgical repair, as much as 20–50% of neurons in the DRG may die [35–43]. Sensory neuronal cell death is related to mitochondrial activation by their release of preapoptotic molecules that can trigger the cascade of various caspases. Interestingly, N-acetyl-cysteine may substantially decrease the amount of cell death in both motor neurons [44] and sensory neurons [7]. The substance is a glutathione substrate and is neuroprotective, probably by enhancing mitochondrial protection. This indicates that dysregulation of the mitochondria in axotomy-induced neuronal death is important [7]. However, there is a time window in which the treatment has to be initiated (within 24 h; [7]). There is an increasing, continuous, ongoing death of neurons with time, and in rats, up to 29% and 50% of the motor and sensory neurons, respectively, may have died at 16 weeks [45]. The type of injury also influence the amount of neuronal loss since an avulsion of the spinal nerve root may give a 53% death while rhizotomy at the same level only induces a 26% death [44, 46–49]. The extent of posttraumatic cellular death may also be influenced by the timing of the nerve repair. An immediate nerve repair may substantially reduce the loss of sensory neurons compared with when the nerve repair is performed [50], especially if the repair is done early (at 1 week postinjury) compared with delayed nerve repair (8 weeks) [45]. Even if there is continuous neuronal cell death after injury, there is most probably a steady state in the reduction of the number of cell bodies in a chronic axotomy when there is no adaptation of a distal segment to the proximal nerve segment.

Axonal Outgrowth After Injury

After transection and repair of a nerve trunk, significant changes take place in normal morphology and tissue organisation proximally and distally to the lesion. In the proximal segment, axons degenerate for some distance back from the site of injury – a retrograde degeneration – which may extend over one or several internodal segments depending on the state of the affected Schwann cells. After an initial delay, axons in the proximal segment produce a great number of collateral and terminal sprouts, which advance distally. In the growth cones, delicate mechanisms orchestrate the filopodia that palpate the environment on which they grow, with local signal transduction mechanisms active [51–53]. However, misdirection of axonal outgrowth is a central problem in nerve regeneration. At the suture gap, axons arborise, which allows them to

interact simultaneously with several distal pathways. They can often travel laterally across the phase of the distal segment before choosing a pathway [54]. For instance, in a mouse model, the average unbranched axon has access to over 100 distal Schwann cell tubes. This apparent wandering of axons across the repair zone defies surgical control and constitutes the basis for misdirection of axons following repair [54].

From clinical experience and experimental models, it is known that the neurons in the peripheral nervous system can be reactivated, probably even after several years, if a formed neuroma is resected and the proximal nerve segment is attached to a nerve graft or a distal nerve segment [55]. However, the exact time dynamics and limits for such reactivation of motor and sensory neurons are not known. Fu and Gordon studied the effects of prolonged motor neuron axotomy *per se* for functional recovery after delayed nerve repair in mice. The tibial nerve was axotomised up to 12 months before cross-sutured to a fresh distal peroneal nerve. The total number of motor units in the muscle significantly decreased with progression of time lapse after axotomy. When axotomy was prolonged to more than 3 months, the numbers of motor units were only 35% of controls. This model, with a reduced capacity of motor axons to regenerate but not compromising the number of muscle fibres innervated by each axon [55], is reminiscent of the situation in hand transplantation. In contrast to such long-term, prolonged axotomy, the short-term capacity of axons to regrow is improved if a nerve injury to the same nerve trunk occurred days earlier, i.e. the well-known conditioning lesion effect [56, 57]. Conditioning lesions increase the intra-axonal protein synthetics and degradative machinery both in vivo and in vitro [51, 58, 59].

Distal Nerve Segment After Injury

After nerve transection, the distal segment undergoes rapid metabolic and structural changes involving axons, myelin sheaths and Schwann cells as well as endoneurial collagen. A Wallerian degeneration takes place. There is a granular disintegration of axoplasmic microtubules and neurofilaments due to proteolysis [60]. After initial swelling of axons, myelin breaks up into droplets and over a period of time is phagocytised by Schwann cells and macrophages. Inflammatory cells and mediators invade the nerve gap and the distal nerve segment very early [61]. Macrophages are important components in the degeneration as well as the regeneration process. These cells can stimulate Schwann cells and fibroblasts to proliferate and to produce neurotrophic factors [61–63]. Macrophages release a broad spectrum of substances, including interleukin (IL)-1, which triggers an increased nerve growth factor (NGF) transcription and NGF receptor density in Schwann cells [64, 65].

After transection, Schwann cells rapidly undergo mitosis, preceded by similar intracellular signal transduction steps as described above for neurons, forming Schwann cell columns within the basal lamina, called bands of Bügner. The bands of Bügner are important pathways for the regenerating axons, both as guidelines and sources of neurotrophic substances, stimulating axonal growth. Schwann cells also express immediate–early genes and transcription factors, such as c-Jun and ATF3, and up-regulate the synthesis of several types of neurotrophic factors, such as NGF, brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, NT-4/5 and NT-6 [66–69]. Schwann cells also produce insulin-like growth factor (IGF)-1, ciliary neurotrophic factors (CNTF) and glial-derived neurotrophic factor (GDNF) [70–73]. In addition, synthesis of cell adhesion molecules such as neural cell adhesion molecule (N-CAM), N-cadherin, and L1 is increased in the bands of Bügner [74–76]. Laminins are also of pivotal importance for outgrowing axons since they and fibronectin can influence the behaviour of intraneuronal actin via integrins, thereby navigating the filopodia/growth cone of the outgrowing axons.

All these changes that occur in a distal nerve segment with degeneration, proliferation of the Schwann cell, invasion of macrophages and up-regulation of a number of factors including cell adhesion molecules aim at directing the outgrowth of axons. These changes occur, of course,

in the distal nerve segments in the hand that is transplanted to the recipient. In this way, the end of the proximal nerve segment, which is dissected and cut, is attached to the distal nerve segments in the donor hand, thereby leading to a situation in which a neuron with its proximal axon, which had had its initial injury years ago, approaches a fresh distal nerve segment with all its alterations induced by the transplantation. The environment of the distal nerve segment is not optimal until around 3 days after injury at a time when proliferation of Schwann cells and growth factors have reached more optimal levels [77, 78]. These changes can also modify the extracellular environment, e.g. laminins and fibronectins, of the distal nerve segment, thereby attracting the outgrowing axons.

Nerve Regeneration in Hand Transplantation

In hand transplantation, the time lapse from injury to transplantation may vary substantially. Nevertheless, positive results with reinnervation of intrinsic muscles and presence of sensibility of the hand [13, 16, 79], functionally mirroring that of a hand replantation, have been reported in cases even when the time lapse between injury and transplantation has exceeded as much as 20 years. For instance, in a report by Lanzetta et al. [80], a 35-year-old man with an amputation injury of the right dominant hand at the age 13, underwent a hand transplantation in October 2000. Already at 6 months there was some protective sensibility in the median innervated area of the hand, and functional magnetic resonance imaging (fMRI) at 3 and 9 months showed activations of the contralateral primary motor and sensory cortex with active hand movements. Obviously, regeneration into the transplanted hand had occurred, and a functional connection between hand and brain cortex had been established, which is in accordance with the experience from other reports of hand transplantation. How can the "silent" nerve system in the recipient's amputation stump be reactivated after 20 years, and how can regeneration be initiated again?

Reinitiation of Injury Signals in Neurons in Hand Transplantation

An amputation injury implies a chronic axotomy with no possibilities for reconnection with peripheral target organs. However, in such cases, a neuroma is formed, i.e. a structure constituted by a conglomerate of axons, Schwann cells and fibroblasts and initially also macrophages [81]. Schwann cells are known to have the capacity for synthesis of a large number of neurotrophic factors. It is therefore plausible that the cells in the neuroma, after the initial reduction in number of surviving neurons, are able to substitute for absent target organs and that they, e.g. via retrograde transport mechanisms, can support survival of remaining nerve cell bodies, as mentioned above. However, can the regeneration process be reawakened and initiated from the amputation stump? This requires a conversion from maintenance to regeneration and a switch in protein synthesis towards substances required for axonal reconstruction, as after all other nerve injuries delineated above. Phenotypic changes in neurons are preceded by the expression of immediate early genes and transcription factors, such as c-Jun and ATF3 [21, 82, 83]. These factors, which are involved in the cascade of events leading to regeneration, are reinitiated by a new nerve injury, such as resection of the neuroma. The factors are probably induced via the injury-provoked activation of the various signal transduction mechanisms involved in communication between the newly cut nerve and the nerve cell body mediated via the retrograde axonal transport. Signals to the neuron with the message of a new injury can be produced locally at the site of the lesion ("injury factors") (Fig. 1c). Leukaemia inhibitory factors (LIF) have been suggested as an "injury factor", which are released by nonneuronal cells and transported retrogradely to the nerve cell body to initiate regeneration and modulate neuropeptide expression [84–86]. Even if a long time has passed between initial injury (amputation leading to above-mentioned signal transduction mechanisms) and resection of the later-formed neuroma in conjunction with hand transplantation, signal transduction mechanisms [1] are

most probably reinitiated. This makes the neuron readjust to an additional injury, leading to axonal outgrowth.

Nerve Regeneration and Immunosuppression

From the technical point of view, hand transplantation is a relatively favourable nerve repair situation in which the surgery can be planned in detail as an elective procedure. Tissues can be prepared under ideal conditions with appropriate length to avoid tension at the site of nerve repair. In contrast to conventional nerve repair or reconstruction, the newly formed sprouts from axon tips created by resection of the neuroma of the proximal nerve segment (chronically axotomised neurons) encounter a different milieu with Schwann cells that are foreign. Due to degeneration in the distal nerve segment in the transplanted hand, resident (foreign) macrophages as well as invading (from the host) macrophages are also present in the distal nerve segment. Numerous immunocompetent cells are invading the transplanted hand and thereby rejection may occur unless immunosuppressive drug therapy is used. For many years there has been great interest in systemic immunosuppressive agents and concepts, such as FK506, sirolimus and the conventionally used cyclosporine in nerve allograft transplantation [87–90], with all its different involved mechanisms in transplantation [91–94]. These agents have also potential side-effects but seem to be a minor problem in the hitherto reported hand transplantations. During the last few years, blockade of costimulatory molecules, alone [87] or in different combinations, has gained interest [93], with a potential use in tissue transplantation such as nerve allografts.

Nerve Regeneration and FK506

Use of the immunophilin ligand FK506 raises some questions since the effect may vary with the situation and experimental model [95–98]. Different opinions have been expressed regarding at what rate the nerve regeneration process occurs in patients receiving a hand transplant with concomitant treatment with FK506 [14, 97, 99, 100]. FK506 is a potent immunosuppressant used in organ transplantation. The effect is mediated via binding of the FK506 binding proteins-12 (FKBP-12), resulting in formation of a complex inhibiting T-cell proliferation through inhibition of calcium and calmodulin-dependent protein phosphatase, calcineurin [101–103]. Thereby, dephosphorylation of the nuclear factor of activated T cells (NFAT) required for nuclear translocation and activation of IL-2 gene transcription is prevented, with a subsequent reduction of T-cell proliferation and immunosuppression [87, 104]. However, the immunosuppressive and nerve regenerative effects may be exerted via two different binding proteins: FKBP-12 and FKBP-52. A “positive” effect on nerve regeneration has been attributed to the effect of FK506 on FKBP-52 [105]. In in vitro systems, using freshly explanted DRGs, a new immunophilin ligand that interacts with both FKBP-12 and FKBP-52 (JNJ-460) induces neurite outgrowth, probably by affecting the neuron-glial signalling, interpreted as that the new FK506 derivative can alter Schwann cell gene expression [106]. In a simple experimental nerve-crush model where axonal outgrowth is investigated by different techniques, FK506 and FKBP ligands improve nerve regeneration [98, 104, 107–112]. However, the nerve-crush model is not relevant in a clinical situation. In autologous nerve grafts, improved Schwann cell proliferation and a higher axonal sprouting has initially been found during treatment with FK506, but such an effect subsides over time [113, 114]. In contrast, no effect on the rate of regeneration, tested with neurofilament staining 4–8 days after a nerve-graft procedure, has recently been reported [98]. In a different model, where rats were treated with FK506 and a chronically injured proximal nerve (2 months) was sutured to a freshly cut distal nerve, the higher number of regenerated motor neurons were noted, together with an increased number of myelinated axons in the distal nerve stump after 3 weeks during treatment with FK506 [104]. In contrast, FK506 did not improve a reduced capacity of

Schwann cells to support axonal regeneration after chronic denervation. Data also indicate that a "neuroregenerative" effect of FK506 is markedly diminished if repair and initiation of FK506 therapy is delayed by 7 days [115].

Regeneration in Nerve Allografts and FK506

FK506 has also been used in connection with nerve reconstruction using allografts, and even xenografts, showing a "positive" long-term effect on nerve regeneration [87, 116–120]. An interesting aspect of using FK506 is the risk for infectious difficulties related to FK506 therapy, which has been highlighted recently in experimental studies [121, 122]. Weight loss and poor feeding was noticed in FK506-treated animals. The question of continuous versus discontinuous treatment schedules with FK506 is another important issue [123].

Taken together, a large number of data indicate that FK506 has a regeneration promoting effect after a nerve crush, but studies on nerve regeneration after nerve repair and nerve reconstruction with nerve grafts have shown conflicting results. However, the regeneration in nerve allografts, i.e. as in conjunction with a composite hand transplantation, is positively influenced by FK506 by inhibiting rejection [90, 119, 120, 124]. A combination of FK506 and blockade of costimulatory molecules (or such ligands alone) may be an exciting concept in the future for tissue transplantation [93] and in nerve allografting [88, 125], but potential side-effects are a threat. Furthermore, in contrast to hand transplantation, nerve reconstruction with nerve allografts may require only a temporary period of immunosuppression if the graft is populated with nonneuronal cells (e.g. Schwann cells) from the host [87].

Conclusion

Hand transplantation represents a unique and very special situation with respect to reinnervation of the transplanted hand: nerve structures in the recipient's arm have been subjected to long-term axotomies corresponding to the time lapse from injury to transplantation while the "distal" nerve segment in the transplanted arm is freshly cut. "Chronically" axotomised neurons have to reinnervate the freshly cut nerve segment containing foreign Schwann cells that undergo the same type of changes as in transection and nerve repair with signal transduction steps and proliferation although modified by immunocompetent cells. Resection of the neuroma most likely initiates similar signal transduction mechanisms in neurons as after the primary amputation injury, inducing nerve regeneration. From clinical experience, it is known that regeneration in such situations in a transplanted hand takes place, and that connection is re-established between the end organs of the transplanted hand and the cortex of the brain. Immunosuppressive drugs, especially FK506, may have an influence on the reinnervation process. In the future, drugs that act by costimulation blockade may be used in tissue and nerve transplantation either alone or in conjunction with traditional immunosuppressants.

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Section 9-d

Modified Visual Feedback in Rehabilitation

Hélène Parmentier

Introduction

Functional recovery is the principal goal for hand grafted patients and it seems conditioned by rehabilitation program. Although the majority of these programs are based on the rehabilitative procedures used in replantation [1], new appropriate techniques have been studied and performed for our hand grafted patients. In our experience, passive rehabilitation started early in the postoperative period and lasted three weeks. From four to six weeks, place and hold exercises [2] and active controlled motility exercises were performed respecting tenodesis effect [3] and minimizing tension in repaired tendons. Then, active rehabilitation started allowing the patient to perform daily activities. Hand grafted patients experienced traumatic bilateral amputation, limb phantom sensation, myoelectric prostheses with modification of muscular behavior and, finally, hand transplantation [4, 5].

Hand amputation and allograft may lead a modification in motor and sensitive cortical representation modifying neural, muscular and sensory end-organ components with consequent alteration of sensory inputs to brain [6].

Almost immediately after a limb loss, 90–95% of the patients experienced a vivid phantom and this incidence increases after traumatic loss. The duration is variable, ranging for few days or weeks to decades. Patients report that phantom occupies a "habitual posture" or that spontaneous changes occur following voluntary or involuntary movements of the stump [7]. Phantom may disappear or become shorter reaching progressively the extremity of the stump. The explication of this phenomenon is not clear and it has been suggested that at its basis might be the lack of proprioceptive and visual feedback of the missing arm [8]. It is also interesting to note that several patients are able to evocate and to modify its extension. Our hand grafted patients presented non-painful phantom limbs before transplantation and it is very difficult to know when it disappeared, indeed they reported immediately after transplantation pain of grafted limbs, which are not yet innervated. We can suppose that following transplantation emotional stress and vision of not yet sensible limbs might activate phantom phenomenon. However, at present we do not know if hand allograft replantation several years after traumatic accident, lead to activate or not vivid impression of phantom limbs.

Functional restoration is conditioned by nerve regeneration, furthermore sensory motor rehabilitation, proprioceptive stimulations, imagined motion activations and repeated exercises may improve brain inputs and outputs. Stimulations from rehabilitative intensive upper limb exercises or from natural healing process and environmental confrontation have shown a possible reversibility of modified and acquired cortical patterns of movements by cerebral plasticity [9].

In a normal person all the commands are controlled by proprioceptive and visual feedback from the arm while in amputated patients there is not this verification and the brain is confront-

ed with a flood of conflicting signals, which contribute to phantom limb. We usually interact with our environment and the visual perception of human movements is an important critical cognitive ability [10]. For all these reasons, an active observation of movements made by a substitute of the injured limb should reduce phantom limb sensation and pain. Therefore Sirigu and Giraux demonstrated that in deafferentated patients a visuomotor training ameliorates phantom limb sensation restoring a coherent body image [11]. They exposed the patients to virtual limb movements to induce plastic changes in the cortical representation of the impaired limb. In the same laboratory (Institut des Sciences Cognitives, Lyon) we used the same visuomotor technique to induce motor rehabilitation in a bilateral hand grafted patient.

Visuomotor training protocol

The patient was a 22 year-old-man who underwent bilateral hand transplantation in April 2003. Before traumatic amputation his right hand was dominant. Amputation was at wrist level on the left side and at forearm level on the right side. For this reason better functional prognosis was expected for the left side.

Fourteen months after surgery he performed experimental illusory movement training once a week for eight weeks. The training did not interfere in scheduled rehabilitative program of physiotherapy, and the patient received all information about the protocol.

In a quiet room the patient sat in front of a table and placed the grafted hands below a 45°-oriented mirror. A video monitor placed above the mirror projected the image of hand movements onto the mirror. Sequences of 20 s of hand movements were prerecorded and projected while the patient was asked to reproduce the same movements watching hand movements in the mirror. This system gave patient the illusion that his grafted hands were performing the movements, which he was watching in the mirror. In the experimental protocol, 3 movements (on the right side screwing and unscrewing, grasping an elastic shape with the thumb, and flexing metacarpophalangeal joints of the long fingers with extended interphalangeal joints; on the left side, fingers-to-thumb opposition, displacing the index toward radial and ulnar side and flexing metacarpophalangeal joints of the long fingers with extended interphalangeal joints) were repeated 5 times for each sequence. All sequences were introduced 3 times according to a randomized order for each session.

The effects of visuomotor training were assessed by several tests.

On the right side active range of motion of metacarpophalangeal joints increased over an average of 10°, and motor recovery gained M2 to M4 concerning abductor pollicis brevis, flexor pollicis brevis and opponens muscles while total opposition test improved from 2 to 3.

On the left side active range of motion of metacarpophalangeal joints improved, total opposition test improved from 2 to 3 and the degree of muscle activity of lumbricals modified from M3 to M4.

Weber and Dellon two-point discrimination tests showed a significant improvement except for moving-two-point-discrimination test on the left side. Semmes-Weinstein monofilament test did not modify. The hand proprioceptive representation test on Wynn Parry chart showed a better ability to perceive localized stimulations (Figs. 1, 2). Perdue test showed that the patient gained a better dexterity, particularly the two-hand-coordination test scoring from 0 to 5 within a 30-s timeframe.

Conclusions

Illusory movement training obtained by modified visual feedback allowed a better sensitive and motion recovery of the bilateral hand grafted patient at 14 months after transplantation. The major improvement was a better opposition on both sides, which allowed an effective pinch grip. Active flexion of the metacarpophalangeal joints also improved. Real benefits on dexterity and motor control of executing tasks were shown although stereotyped hand movements

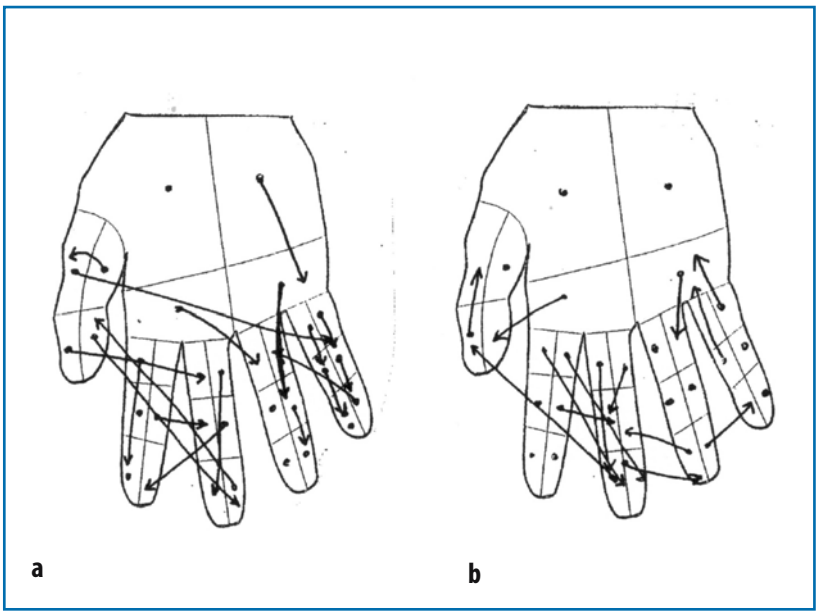

Fig 1a, b. Mapping of the localization (right side). Before (**a)** and after (**b**) the training

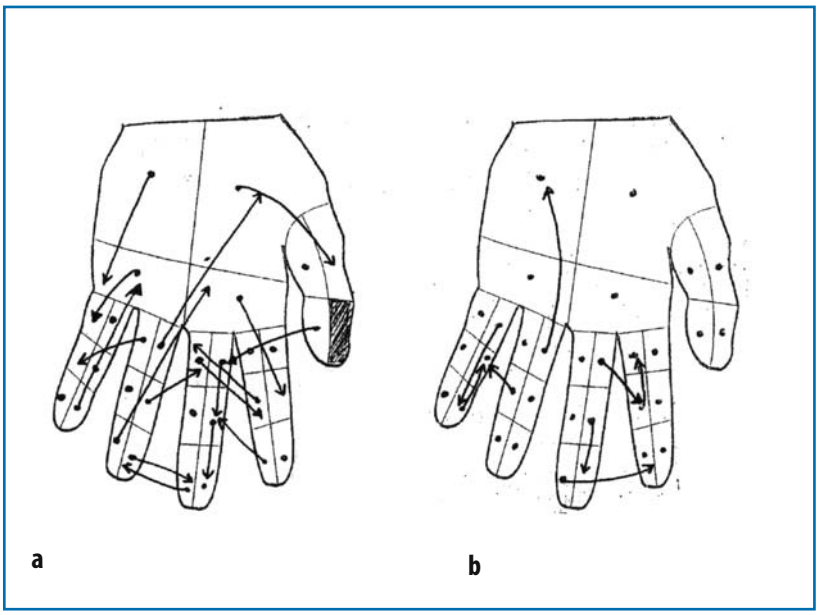

Fig 2a, b. Mapping of the localization (left side). Before (**a)** and after (**b**) the training

and fibrosis of soft tissues limited this amelioration. It is interesting to note that muscles power did not improve. Indeed, visual feedback was modified to give corrected inputs to central nervous system, in order to facilitate sensory motor integration. The training did not facilitate muscular power but it provided a better use of anterior acquired function and dexterity. It is also interesting that there was an improvement of sensitivity recovery, which may depend on a better cortical upper limb representation.

Imaging studies have shown that visual capacity permits anticipation [12] and this training might facilitate motor activity: giving selective movements to memory, it contributes to economize gesture and optimizes experience.

These results showed that the visuomotor training program, providing the motor system with a model of limb movements, introduces novel correlations between inflow and outflow signals and represents a new and efficient tool of rehabilitation for hand grafted patients.

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Section 9-e

Analysis of Motor Unit Reinnervation in Muscles of the Transplanted Hand

Marco Pozzo, Dario Farina

Introduction

Sensory and motor recovery in hand-transplanted patients is conditioned by nerve regeneration [1]. Whereas functional recovery can be evaluated clinically [2], there is a need for tools allowing direct assessment of muscle control in transplanted muscles at the level of their smallest functional units, the motor units (MUs). These should permit, for example, the ability to determine when MUs are innervated and whether their control strategies and physiological properties are similar to those observed in normally innervated muscles. These issues can be assessed by techniques that allow extraction of the electrical activity from single MUs, such as intramuscular electromyography (EMG). However, noninvasive methods detecting EMG signals on the skin (surface EMG) should be preferred in hand-transplanted subjects to minimise possible damage to the allograft.

Recently, advanced EMG techniques (multichannel surface EMG [3]) have been advantageously applied to assess the reinnervation process in intrinsic muscles of the transplanted hand at the finest level of single MU activities. Such methodology allows the detection of early signs of reinnervation in intrinsic muscles of the transplanted hand when only few MUs are reinnervated and the exerted force is too weak to be perceived. In addition, after reinnervation, it allows the investigation of MU physiological and control properties and their functional recovery.

This chapter reviews the most recent findings in the application of multichannel surface EMG in the field of hand transplantation. Due to the innovative nature of this methodology, most of the findings illustrated in this chapter will refer to the postoperative follow-up of one patient [3–5]. The technique has also been applied to a second recipient, of whom preliminary results will be shown in this chapter.

The Multichannel Surface EMG Technique

Muscle fibres are activated by the central nervous system through electric signals transmitted by the motoneurons. A motoneuron innervates a group of muscle fibres (in a range from few tens to several hundreds) that constitute an MU, the smallest functional unit of the muscle, which is controlled independently. MU activation by the central nervous system can be assessed by the detection of electrical signals (MU action potentials) generated before their contraction [6]. Surface EMG signals reflect the electrical activity of the active MUs in a muscle. When an electrical signal reaches the neuromuscular junction through the axon branches, two action potentials are generated at the end-plate region (innervation zone) and travel by active propagation towards the tendon endings at a speed (termed conduction velocity) related to MU membrane and contractile properties [7] and eventually

fade at each tendon. Intracellular action potentials generated in the muscle fibres are the sources of the surface EMG signal detected over the skin.

Classic techniques for the detection of surface EMG signals consist of pairs of electrodes spaced at 20–30 mm and aligned with muscle-fibre orientation. A signal, which is the difference of the electric potentials detected by the two electrodes, is recorded [single differential (SD) or bipolar recording). The surface EMG technique is particularly attractive in the conditions of slow reinnervation processes since in these cases, only a few MUs are active. Despite the lower spatial resolution of the recording with respect to the intramuscular technique, it is possible to separate the interference EMG signal into its constituent action potentials generated by the active MUs.

Surface recording has many advantages over intramuscular detection, avoiding risks of infections and discomfort issues (which are of particular importance in this specific application), despite the fact that it can provide only global indications on muscle activity. More advanced methods for surface EMG signal recording have been proposed [8] with the aim of investigating single MU anatomical, action potential propagation and control properties. These methods make use of linear electrode arrays, i.e. a number of equally spaces electrodes placed parallel to fibre orientation, in which each consecutive electrode pair originates an SD EMG signal (Fig. 1c). Detection of such multichannel EMG signals allows identification of the MU innervation zone location, tendon placement, fibre length, conduction velocity and, in some conditions, discharge patterns [8–11]. Figure 1 shows examples of surface EMG signals recorded by a linear electrode array (16 dot-shaped electrodes, 2.5-mm interelectrode distance), from the abductor digiti minimi muscle of a healthy male subject during a linearly increasing force contraction from 0% to 100% of the maximal voluntary contraction (MVC). During the ramp contraction, the EMG signal amplitude increases (Fig. 1a) as a consequence of MU recruitment and the increase of MU discharge rate [i.e. mean number of MU action potentials generated per second and measured in pulses per second (pps)]. A large number of MU action potentials are present when the force level increases.

In a (re)innervated hand muscle, the location of the innervation zone of active MUs can be assessed by visual analysis of the surface EMG signals and corresponds to the point of inversion of propagation of the MU action potentials [9], as shown in the example from a healthy subject (Fig. 1d) When EMG activity in the allograft is evident, single MU action potentials can be extracted from the signal by means of dedicated signal processing algorithms, which classify MU action potentials based on their shape as belonging to different MUs. It is then possible to identify with precision when a new MU is reinnervated and to analyse the membrane and control properties of each MU individually.

The instantaneous discharge rate of each MU can be calculated as the inverse of the time interval between consecutive discharges. This parameter gives indication on the capability of the recipient to modulate the motor control of the reinnervated MUs in specific tests. MU conduction velocity can be estimated from the highest available number of propagating signals with methods described in the literature [12]. Its value can give an insight into membrane and physiological properties (such as fatigability) of the innervated MUs.

Procedures for Follow-Up Assessment of Reinnervation

Assessment of early signs of reinnervation in the transplanted hand is performed by periodical EMG recording sessions, starting a few months postoperatively, in which evidence of electrical activity from intrinsic muscles is evaluated. The first case analysed with this methodology was a 35-year-old male recipient who had lost his right dominant hand at the age of t13. Recordings of EMG activity started 7 months postoperatively, followed by a second evaluation at 11 months and then monthly thereafter, until reaching 10 sessions. An additional session was then performed 4 months after the 10th session. The sec-

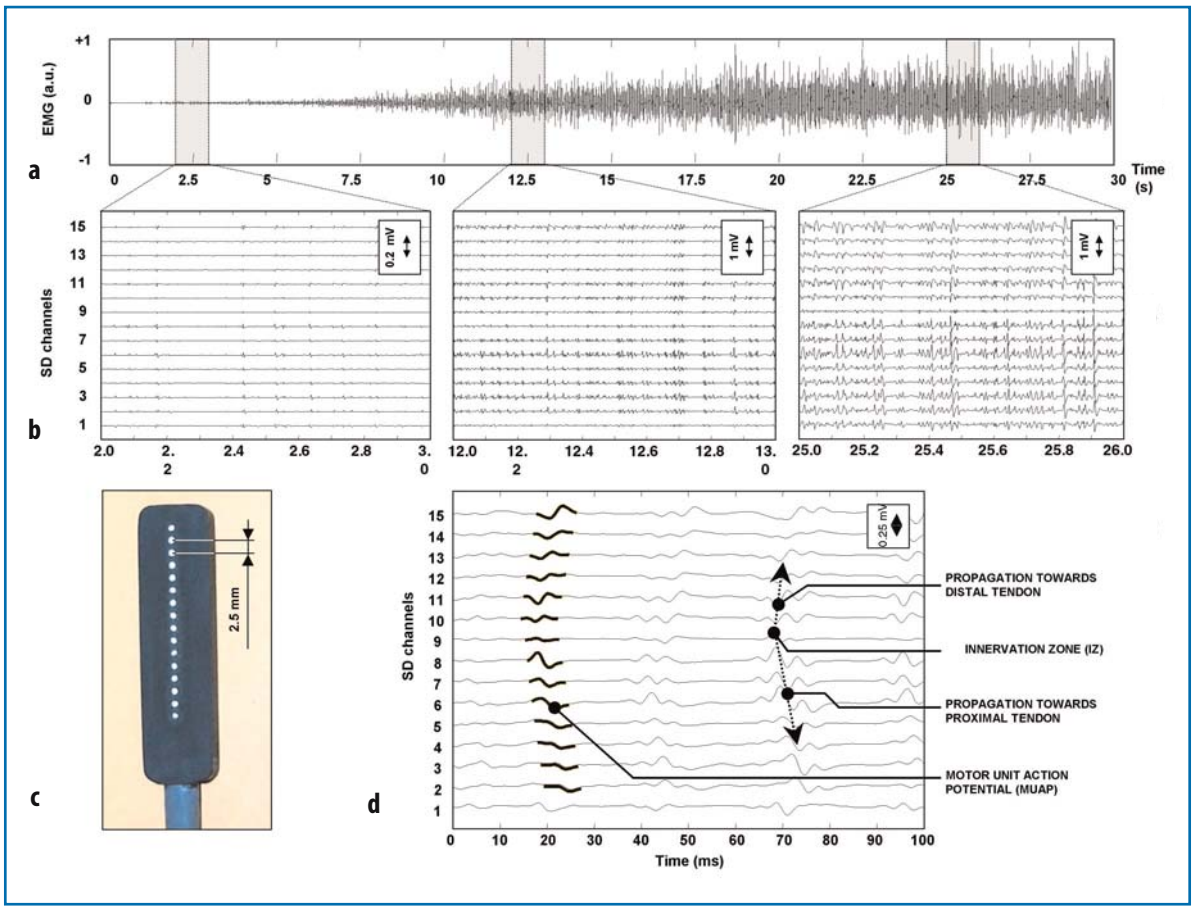

Fig. 1a-d. Multichannel surface electromyography (EMG) signals acquired from the abductor digiti minimi muscle of a healthy subject during a 30-s increasing force ramp contraction from 0% to 100% of the maximal voluntary contraction (MVC). A 16-channel, 2.5-mm interelectrode distance array with silver dot electrodes was used to acquire EMG signals. **a** Time course of one EMG channel, showing an evident increase in the global amplitude. **b** One-second epochs of EMG signals extracted from the recording at the beginning, middle and end of the contraction. Note the increase in the firing rate of active motor units (MU) and the progressive recruitment of larger MUs as the force demand increases. **c** The electrode array used to acquire multichannel surface EMG signals. The silver dot electrodes are equally spaced by an interelectrode distance of 2.5 mm. During EMG acquisitions, the array is positioned parallel to the muscle fibre direction and held in place by applying a gentle pressure on the skin. **d** Sample portion of multichannel surface EMG signals acquired from the abductor digiti minimi muscle from a healthy subject (16 channels, 2.5-mm interelectrode distance array, 10% MVC) showing its features. Each MU, when active, produces a train of MU action potentials traveling from the innervation zone (*IZ*) towards the tendons, originating typical V-shaped patterns. The channel where sign reversal is observed corresponds to the location of the innervation zone. The time delay of potentials travelling under consecutive electrodes is related to the MU conduction velocity, the normal value of which is approximately in the range 3–5 m/s

ond recipient was a 32-year-old male who lost his right dominant hand 7 years earlier. In this case, EMG recording sessions started at month 3 postoperatively, and 3 additional sessions were performed at months 6, 7 and 13. Muscles that can be investigated with this method are the abductor digiti minimi, abductor pollicis brevis, opponens pollicis, first dorsal interosseous and first lumbricalis. Indeed, these are sufficient to provide an overview of the reinnervation status in a transplanted hand.

For the EMG assessment, the skin overlying the muscle to be investigated is slightly abraded with abrasive paste to improve the quality of the skin–electrode contact. The electrode array is held in place by an operator who explains to the subject the specific movement to perform to activate the muscle and provides an appropriate counterresistance. In case of presence of EMG signals, the final location of the array is determined by visual inspection of the signals detected while the subject is performing short test con-

tractions. The best electrode location is defined as that corresponding to the propagation of the MU action potentials along the array with minimal shape changes. In case of absence of EMG activity, the array is placed along the muscle fibre direction, as estimated by muscle palpation.

Surface EMG signals are amplified by a multichannel surface EMG displayed in real-time on a monitor and stored on a computer for further processing and analysis [3]. For each muscle, the subject is asked to perform a 60-s contraction at maximal level and is verbally encouraged to increase the force level. In case no EMG activity is observed, the muscle is considered not innervated, and no other measures on the muscle are performed in the same experimental session. In case clear MU action potentials are identified, the subject is asked to perform three additional contractions, increasing linearly the muscle activity from zero to the maximum (subjective regulation of force). When a single MU action potential train is identified, the subject is also provided with visual feedback that displays the MU instantaneous discharge frequency on a visual analogue scale. Such feedback allows the subject to linearly increase in discharge rate from a minimum to the maximum. This ramp contraction serves to test the MU control strategies in a simple force-production task.

Electrophysiological Evidence of Motor Unit Reinnervation

In the first recipient, the first clear MU potential train appeared from the abductor digiti minimi muscle (Fig. 2a) 11 months after the allograft procedure. Analysis of EMG signals allowed determination of the point in which the axon connected to the muscle fibres (Fig. 2f). Observed discharge rates were within physiological values (with a minimum of 8–10 pps and a maximum of 35–40 pps) [13, 14], except for occasional multiple discharges very close to each other (reaching instantaneous firing rates up to 100 pps). These discharges resembled the double discharges observed both in healthy [15] and pathological subjects [16], but in the investigated subject, more than two discharges often appeared very close to each other. The estimated conduction velocity was within physiological values, in the range 3–4.5 m/s, and it depended on the discharge rate, as shown below. After 13 months, a second MU appeared during maximal contractions of the abductor digiti minimi muscle. Surface potentials of this unit presented significantly smaller amplitudes than those of the first observed MU, indicating either a deeper or a smaller MU. After 12 months from transplant, abductor and opponens pollicis muscles began to show single MU surface EMG activity (Fig. 2b, c). A clear MU action potential train was observed in the opponens pollicis muscle while, at the time in which reinnervation was first observed, at least 3 MUs were detected from the abductor pollicis muscle. Also in these muscles, instantaneous discharge rates were within physiological values. After 15 months, the first dorsal interosseous muscle showed the first active MU (Fig. 2d), made manifest by a train of action potentials. Activity from the first lumbricalis was first detected 24 months postoperatively although the amplitude of the MU action potential train was lower than in the other muscles. For the abductor digiti minimi, abductor pollicis, and opponens pollicis muscles, from the EMG recordings it was possible to clearly identify the MU innervation zones, which could be marked over the skin (Fig. 2f).

In the second recipient, the smaller number of evaluation sessions did not allow determination of the reinnervation sequence with the same precision. However, in this case, the reinnervation process was faster, with the first clear MU action potentials detected on the abductor digiti minimi in the session at month 7 postoperatively. By the fourth measurement session (month 13 postoperatively), the opponent and abductor pollicis and first lumbricalis also showed MU action potential trains. In the case of the opponent pollicis, at least two MUs could be identified while no activity was observed in the first dorsal interosseous in any of the sessions. In all reinnervated muscles, it was possible to observe signal propagation (Fig. 3).

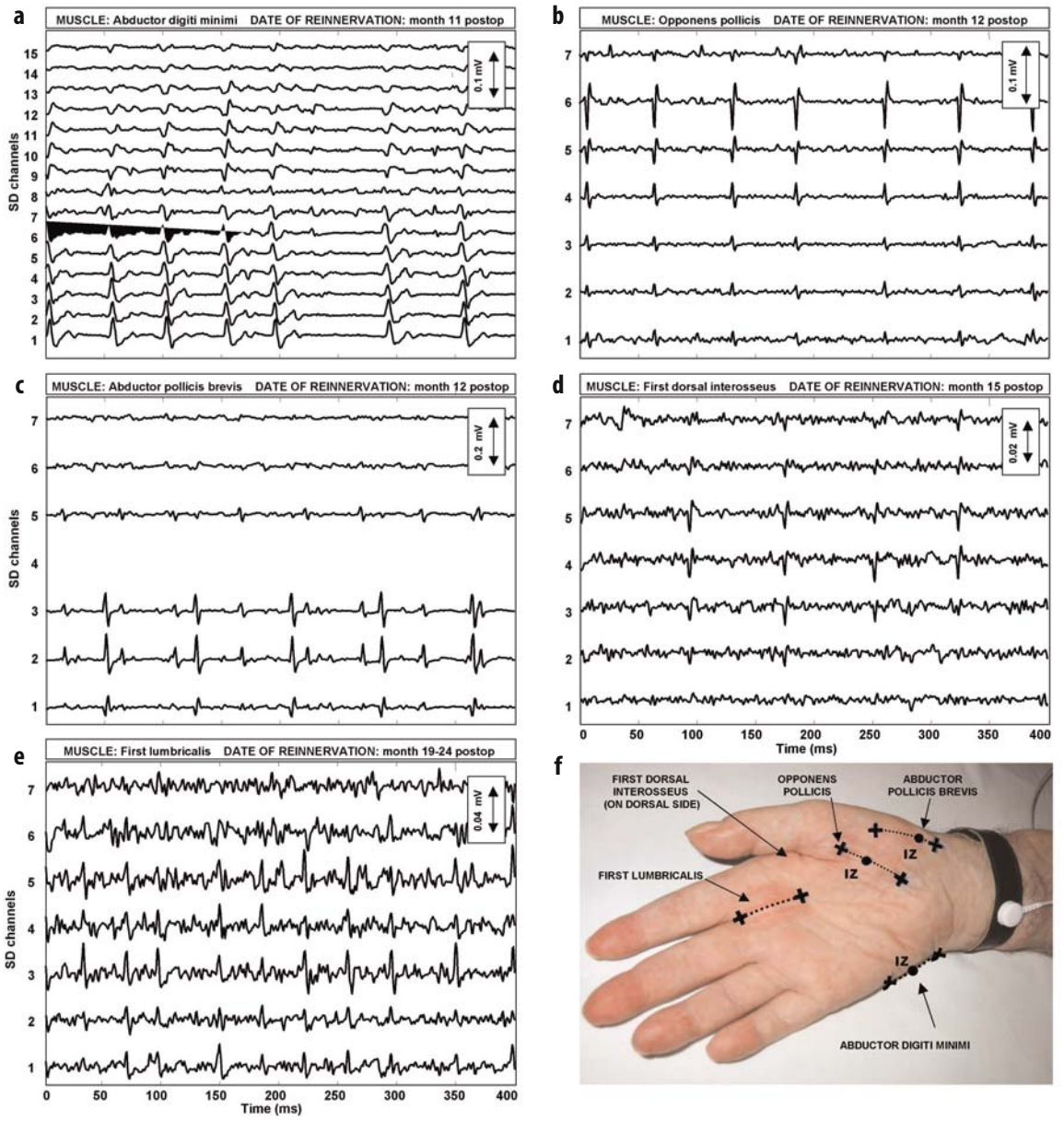

Fig. 2a-f. Multichannel surface electromyography (EMG) signals acquired from intrinsic muscles of the transplanted hand of first recipient during attempted voluntary contractions against the resistance of the operator. For each muscle, the date when voluntary EMG activity was observed for the first time is indicated. Only the channels with high enough signal quality were plotted in each case. A 16-channel, 2.5-mm interelectrode distance array with silver dot electrodes (as shown in Fig. 1c) was used to record EMG signals. Note the different amplitude scale for each graph. The investigated muscles were: (**a**) abductor digiti minimi, (**b**) abductor pollicis brevis, (**c**) opponens pollicis, (**d**) first dorsal interosseous, (**e**) first lumbricalis. **f** Position of the array for the investigated muscles (except for the first dorsal interosseous). For each muscle, the two *crosses* (+) represent the location of electrodes 1 and 16 of the array, and the *dashed line* (- - -) indicates array direction. For the muscles in which signal quality and number of propagating channels was high enough, the estimated position of the innervation zone (● *IZ*) is also marked. From [3], used with permission

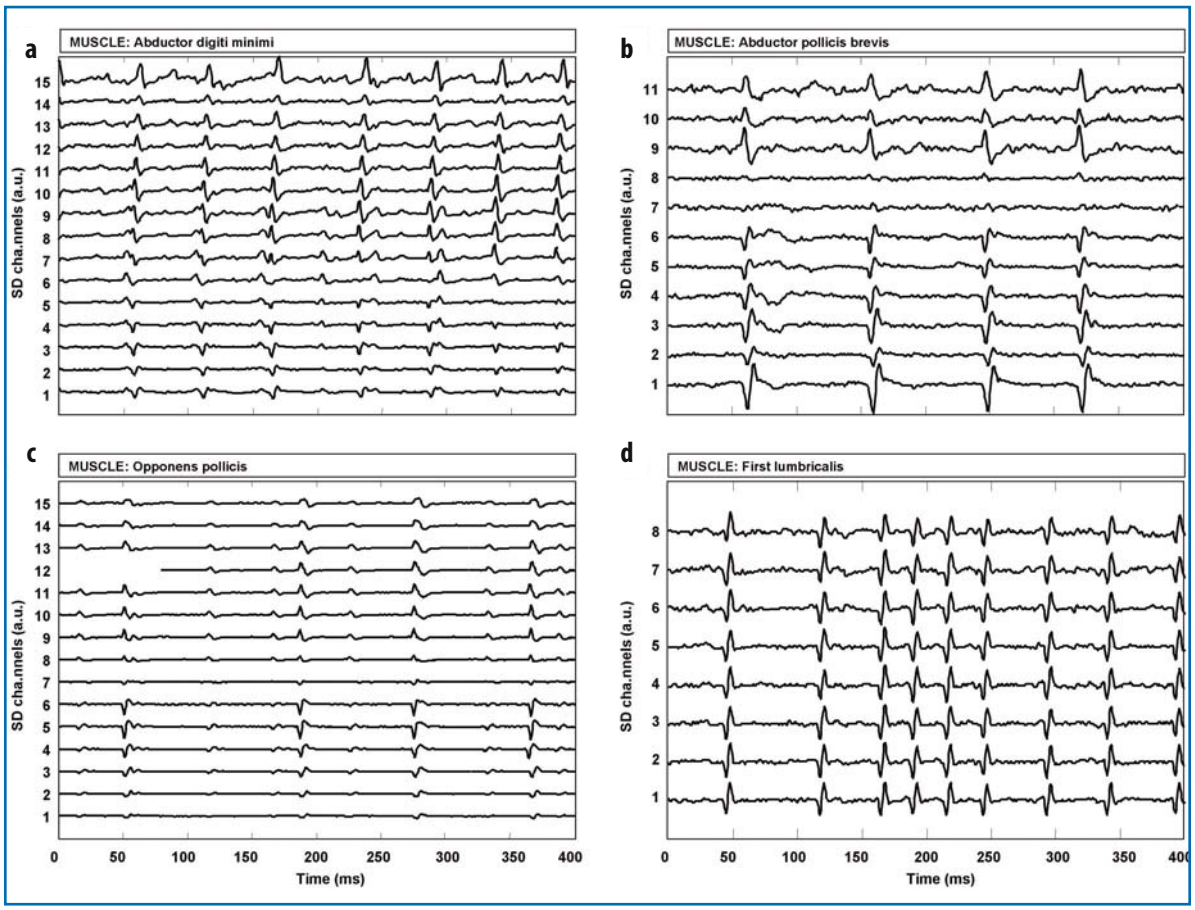

Fig. 3a-d. Multichannel surface electromyography (EMG) signals acquired from intrinsic muscles of the transplanted hand of second recipient during attempted voluntary contractions against the resistance of the operator. Plots refer to EMG recordings obtained at month 13 postoperative from: (**a**) abductor digiti minimi, (**b**) abductor pollicis brevis, (**c**) opponens pollicis, (**d**) first lumbricalis. No activity was detected on the first dorsal interosseous. A 16-channel, 2.5-mm interelectrode distance array with silver dot electrodes (as shown in Fig. 1c) was used to detect EMG signals. Signals are depicted in arbitrary units (AU), with different vertical scales for each muscle for best visualization. Only channels with good signal quality and clear propagation are shown

Figure 4 shows a 1-s segment of surface EMG signals detected from the abductor digiti minimi of the first recipient during a 60-s maximal voluntary contraction. Fluctuation of discharge rate is evident, as is the occasional presence of multiple discharges at high instantaneous rate. In all the 60-s contractions sustained at the maximal level, the mean discharge rate decreased on average, probably reflecting central phenomena of fatigue, despite the verbal encouragement given to the subject to keep it at the initial level. Figure 5 shows a ramp contraction of the abductor digiti minimi performed by the first subject with the feedback on discharge rate. The subject was able to approximately increase the frequency of activation of the MU linearly in time from about 10 up to approximately 40 pps. The occasional high discharge frequency values can be observed from the plot of the instantaneous discharge rate. Interestingly, conduction velocity shows high correlation with instantaneous discharge rate, as it was also observed in normal subjects [17], indicating that membrane properties depend on the time elapsed from the previous discharge. Figure 4e shows the action potentials classified as belonging to the MU under study. The subject was able to perform this simple ramp motor control task (constituted by the linear increase of single MU discharge rate) since the beginning of the reinnervation and with all muscles from which it was possible to extract single MU activities. The minimum discharge rate that could be

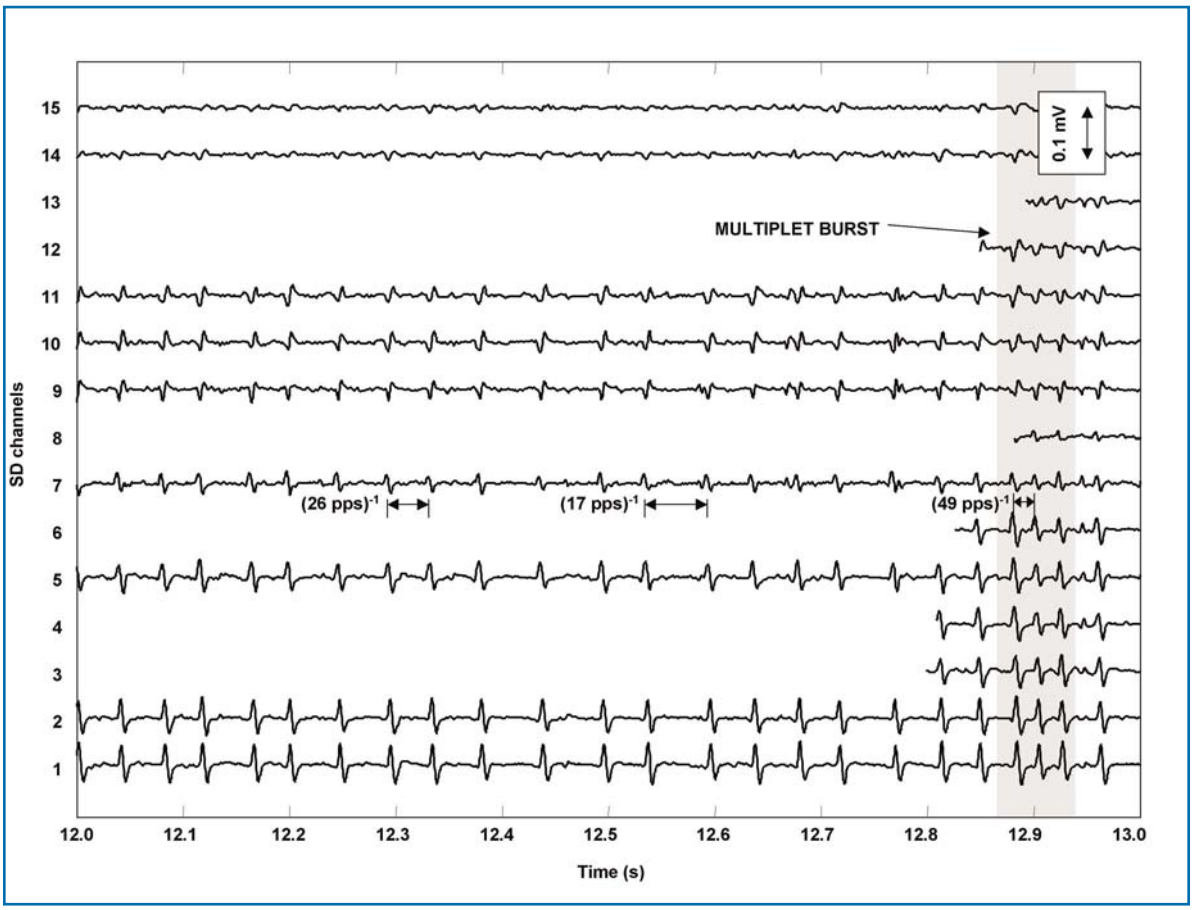

Fig. 4. Multichannel surface electromyography (EMG) signals acquired from the abductor digiti minimi muscle of the transplanted hand of first recipient during attempted maximum voluntary contraction (MVC). The subject was asked to exert the maximum possible force against the resistance of the operator and keep it for 60 s; the subject was verbally encouraged during the contraction, but no feedback was given to him. A 16channel, 2.5-mm interelectrode distance array with silver dot electrodes (as shown in Fig. 1c) was used to acquire EMG signals. One epoch of EMG signals, one second long, at the beginning of the contraction (12–13 s) is shown. Despite the fact that exerted force was almost not perceivable by the operator, the effort of performing a maximal contraction reflects in the high firing rate of the only detected motor unit. Occasional bursts of multiplets (shaded area) with high firing rate (approaching in this case 50 pps) can be observed

sustained constantly was approximately 8–10 pps in all conditions, and the maximum firing rate, sustained for at least 2 s, was never higher than 40 pps, which is similar to those observed in normal subjects. Similar phenomena were observed in the second recipient.

Physiological and Clinical Implications

Analysis of MU properties opens a window on the understanding of central control strategies and peripheral status of the neuromuscular system. Using the technique described in this chapter, the activation of single MUs from intrinsic hand muscles can be followed after the transplant operation. The electrical activity of such muscles shows that small forces perceived by the therapist in the transplanted hand are not only due to synergic efforts performed by extrinsic muscles.

Anatomical information about the muscle can be obtained by localisation of innervation zones of the detected MUs. In addition, physiological information can result from the analysis of both the discharge pattern and conduction velocity of single MUs. Results from the first two recipients analysed showed that the discharge

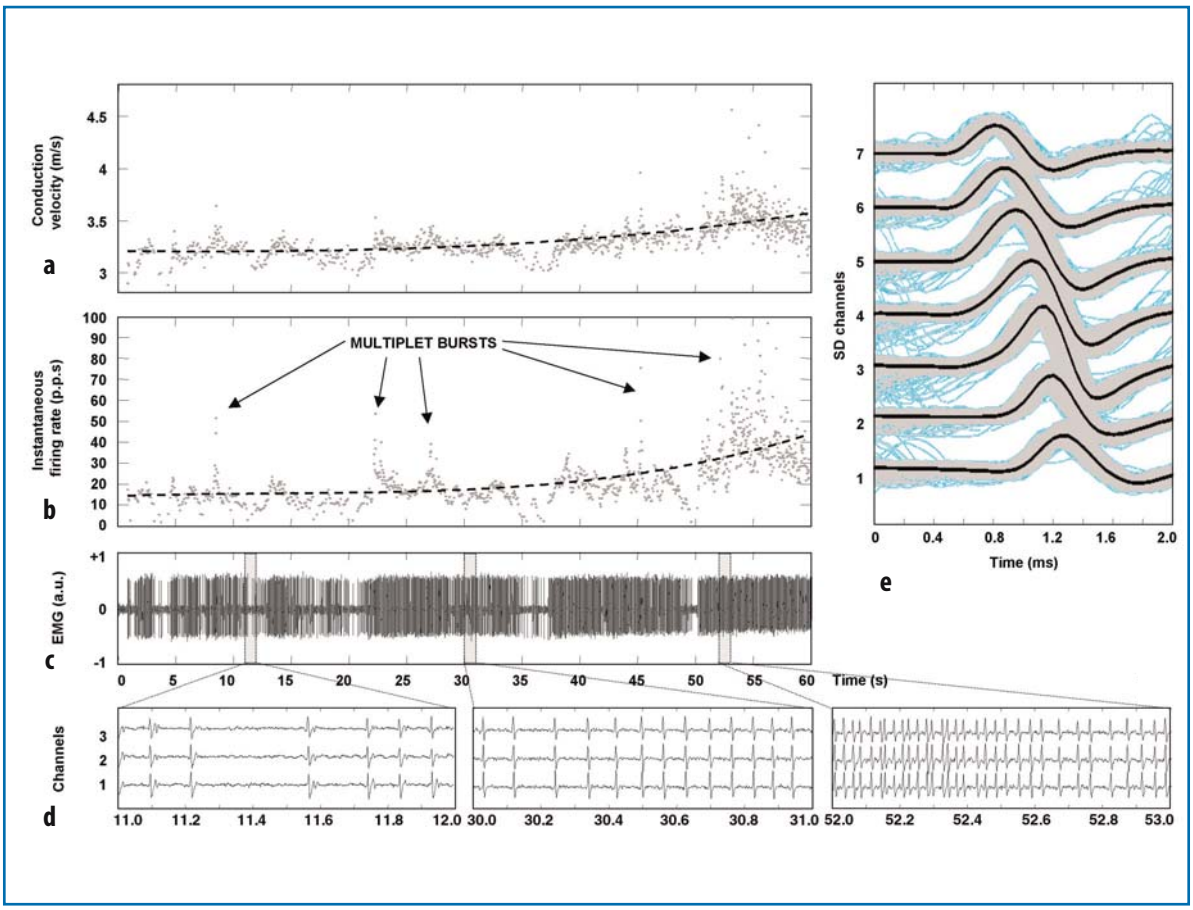

Fig. 5a-e. Single motor unit (MU) parameters of surface electromyography (EMG) signals acquired from the abductor digiti minimi muscle of the transplanted hand of the first recipient during a 60-s voluntary ramp contraction. The subject was given real-time feedback of the instantaneous firing rate of its active MU and was instructed to follow a target, varying at small steps from the minimum to the maximum firing rate that he could exert. **a** Time course of conduction velocity (CV) of the active MU (●) and its interpolating curve (- - -). Note the high and instantaneous correlation between the MU conduction velocity and firing rate (**b**). **b** Time course of the instantaneous firing rate of the active MU (●) and its interpolating curve (- - -). Note that despite the fluctuations the subject was able to increase the MU firing rate as requested. **c** Time course of one EMG channel. Note the constant amplitude with respect to Fig. 1a due to the only active MU contributing to the signal. **d** Epochs of EMG signals (three channels shown), 1 s long, extracted from the signal at the beginning (11.0–12.0 s), middle (30.0–31.0 s) and end (52.0–53.0 s) of the ramp contraction. Note the increase of the firing rate. **e** All the MU action potentials extracted from the signal (*dark grey lines*). All propagating channels used to compute conduction velocity are shown; the average MU action potential is shown superimposed (*black lines*). Note the similarity of all MU action potentials with their average, which confirms that they all belong to the same MU. The jitter in the shape is due to fluctuations of the CV, which are evident in **a**, as described in the text

rates achieved by the patients were within the range of physiological values (8–40 pps). Stable discharge rates were never below 8 pps, which is a finding common to a number of muscles in normal conditions [14]. Occasionally, high instantaneous discharge rates (up to 100 pps) were recorded (Fig. 4). They corresponded to discharges very close to each other, which could resemble "doublets" [15] identified in normal subjects but that in this case involved usually more than two discharges ("multiplets"). Multiple discharges may reflect an attempt of the central nervous system to exert an increasing force when few MUs are available. In addition, the subjects were able, with limitations but with increased skill over the sessions, to voluntarly control the innervated MUs by increasing their discharge rate when requested. For the muscles in which conduction velocity could be estimated, its values were within normal physiological ranges and correlated to MU discharge rate, as it has been observed in normal subjects.

In conclusion, advanced noninvasive EMG techniques can monitor the reinnervation of single MUs in transplanted hands. The location in the muscle in which the neuromuscular junctions are restored can be detected, and the membrane and control properties of the innervated MUs can be investigated and compared with those of normal subjects. Selective assessment of intrinsic muscles in the transplanted hand is thus feasible even at the lowest functional level, the MU. This assessment provides important information from clinical and basic physiological perspectives and discloses new research areas in limb transplants and motor control studies.

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Section 9-f

Role of the Sympathetic Nervous System on Arterial Distensibility

Cristina Giannattasio

Introduction

The sympathetic nervous system has a powerful excitatory influence on the cardiovascular system. Central or reflex activation of cardiac sympathetic nerves increases heart rate, shortens transmission of the electrical impulse from the atria to the ventricles and increases ventricular contractility, thereby improving cardiac function and helping cardiac output to be maintained or increased when this is necessary to provide adequate perfusion to organs and tissues. Central or reflex activation of peripheral sympathetic nerves cause vasoconstriction that can selectively or diffusely increase peripheral vascular resistance, maintaining or increasing blood pressure values to allow organs to be adequately perfused in a variety of behavioural circumstances in which perfusion needs substantial variations [1].

Another important possible effect of the sympathetic nervous system, i.e. modulation of arterial distensibility, has for many years not been addressed because of difficulty posed by properly studying the alterations of the diameter of large elastic arteries in response to changes in intravascular and/or extravascular pressure. This limitation is important because arterial distensibility is a vascular function of great clinical relevance. First, arterial distensibility absorbs the energy associated with the systolic ejection of blood from the heart and gives it back during diastole, thereby maintaining diastolic blood pressure values and ensuring constancy (rather than intermittency) of tissue blood flow and perfusion throughout the cardiac cycle [2–10]. Second, arterial distensibility buffers the increase in systolic blood pressure that would otherwise occur during systole, thereby limiting cardiac afterload and reducing the traumatic (and atherogenic) effect a sudden and marked blood pressure increase would have on the vessel wall. Third, arterial distensibility allows stretch receptors located in some arterial sites (aortic arch and carotid arteries) to be stimulated in response to even small changes in vessel diameter, with a result in powerful inhibition of sympathetic and excitation of vagal cardiac drive. This prevents excessive blood pressure increases and protects against disease and death in several clinical conditions [11–13].

This chapter reviews the evidence that sympathetic activity exerts a major modulatory effect on large-artery distensibility. Reference is made to animals but particularly to human data.

Phasic Influences of Sympathetic Nerve Activity on Arterial Distensibility

Several studies have shown that an acute increase in sympathetic nerve activity is accompanied by a reduction of arterial distensibility. We have seen, for example, that infusion of

phenylephrine in a brachial artery is associated with immediate reduction of radial artery distensibility as assessed by beat-to-beat changes in vessel diameter (echo-tracking device) in association with an increase in the nearby finger blood pressure [14]. Boutouyrie et al. [15] have described by a similar technique a marked reduction of radial artery distensibility in response to cold pressure test, i.e. a manoeuvre known to increase blood pressure and heart rate because of a diffuse central and reflex increase in sympathetic drive [15, 16]. We have finally seen that a reduction in radial but also carotid artery distensibility occurs during cigarette smoking (Fig. 1), i.e. a behaviour that is accompanied by a marked increase in blood pressure and heart rate because of the peripheral (and possibly central) sympathostimulating effects of nicotine and other smoking products [17], as documented by its abolition following blockade of alfa- and beta-adrenergic receptors [18–26]. Although a reduction of arterial distensibility may originate nonspecifically from an increase in blood pressure (due to the stretching of inextensible collagen within the arterial wall), there is little doubt that sympathetic stimulation is capable of increasing arterial stiffness. The effect is a clearcut one in midsize muscular type arteries, such as the radial ones, but large elastic arteries (and thus possibly the whole arterial tree) seems to be also involved (Fig. 1).

Tonic Sympathetic Influences on Arterial Distensibility

A question of primary importance is whether arterial distensibility is reduced only when sympathetic activity is increased or the existing sympathetic drive exerts a continuous stiffening effect on large artery vessels, its influence being thus not just episodic but tonic. We have addressed this question in several studies in ani-

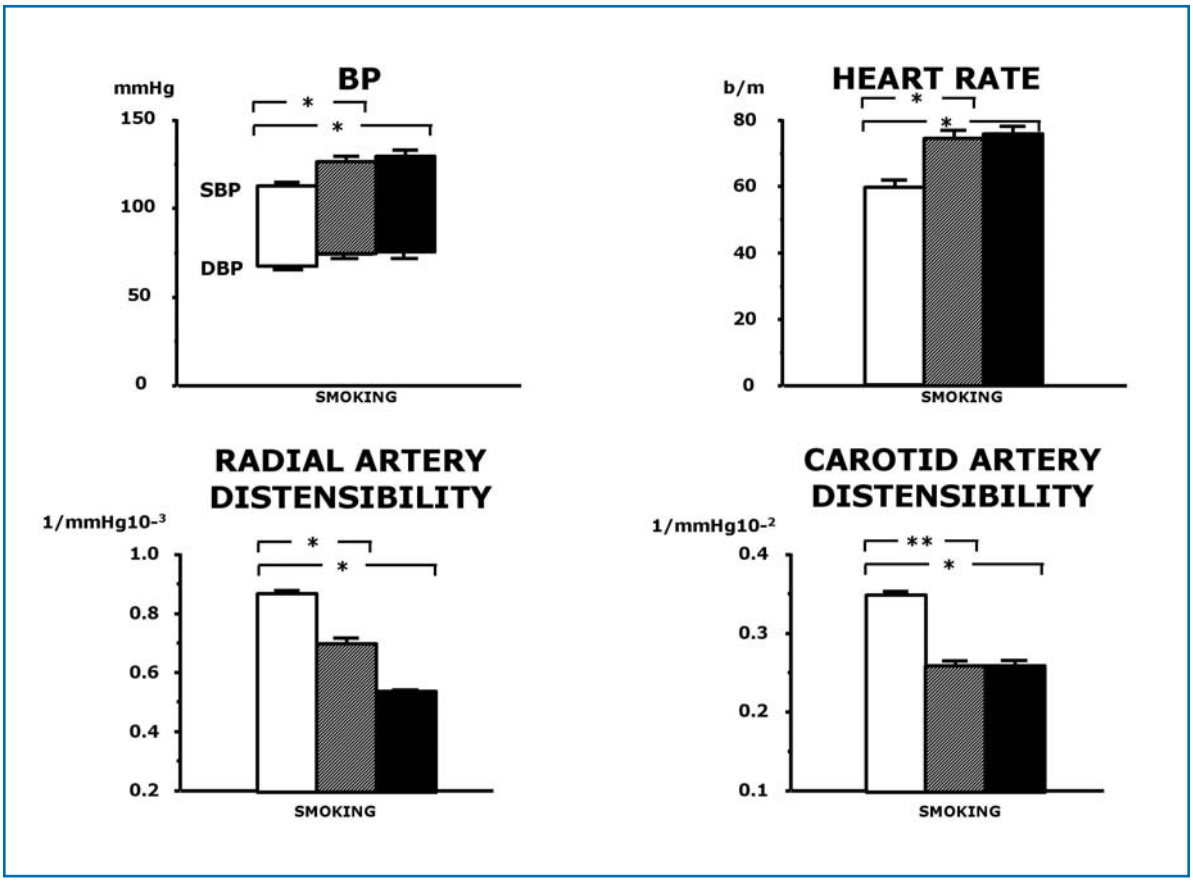

Fig. 1. Arterial distensibility before, during and after cigarette smoking. Modified from [12], used with permission

mals and humans. In one human study, radial artery distensibility was assessed (echo-tracking device) before and after ipsilateral anaesthesia of the brachial plexus in healthy patients prepared for surgical correction of Dupuytren's disease. As shown in Figure 2, the anaesthesia did not cause any substantial changes in blood pressure and heart rate, but it was accompanied in all 8 subjects studied by an increase in arterial distensibility throughout the diastosystolic pressure range [27].

Arterial distensibility was measured also in the femoral artery by the relationship between echo-determined diastosystolic changes in vessel diameter versus pulse pressure values in the brachial artery. Measurements were made before and after ipsilateral subarachnoid anaesthesia in healthy subjects undergoing arthroscopic removal of a meniscal lesion. As shown in Figure 2, subarachnoid anaesthesia was followed by a significant increase in ipsilateral femoral artery distensibility, again with little changes in blood pressure and heart rate, as well as with non-significant changes in blood flow in the contralateral vessel.

Similar findings were obtained in 5 patients in whom femoral artery distensibility was assessed before and 1 month after surgical ablation of the ipsilateral lumbar sympathetic chain due to peripheral vascular disease (Fig. 2). Thus, removal of sympathetic activity is accompanied by an increase in arterial distensibility, which means that the ongoing sympathetic activity exerts a restraining influence on this arterial function [27]. This is the case in midsize or relatively large arteries with muscular structure, such as radial and femoral ones. It is similarly the case in healthy subjects and patients with altered vessel anatomy, such as those with peripheral artery disease [28–32].

The above studies do not answer the question whether sympathetic activity modulates arterial distensibility in large vessels with a prevalent elastic complication, However, carotid artery distensibility as measured by diastosystolic changes in diameter in response to blood pressure changes measured intra-arterially from the contralateral vessel was found to be increased in rats sympathectomised by 6-hydroxidopamine compared with intact animals (Fig. 3) [33, 34]. It is thus likely that a stiffening effect is exerted by the sympathetic nervous system on all arteries, with a resulting tonic restraining influence on overall arterial distensibility.

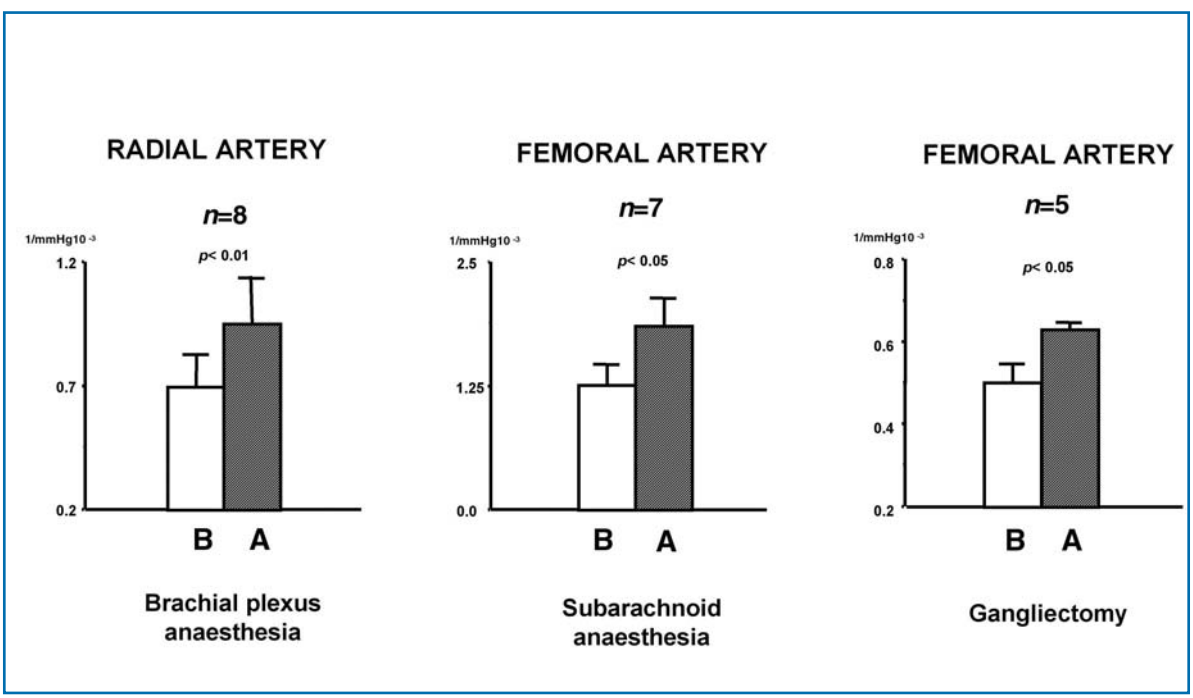

Fig. 2. Arterial distensibility before and after removal of sympathetic drive in humans. Open hystograms refer to baseline. Modified from [7], used with permission

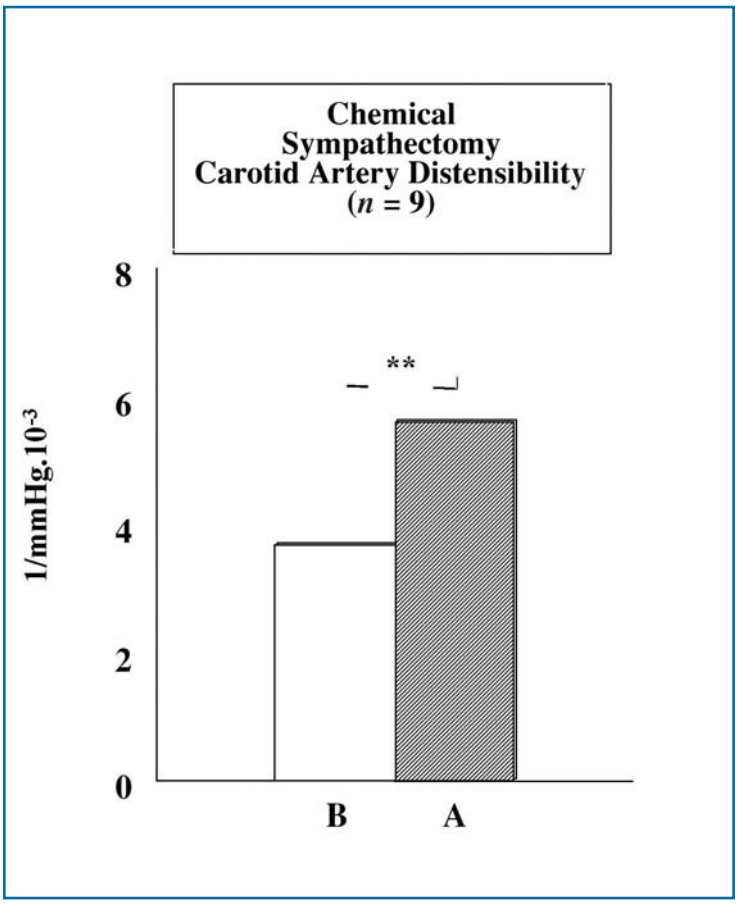

Fig. 3. Arterial distensibility before and after removal of sympathetic drive in the rat. Modified from [33], used with permission

Influence of Sympathetic Activity on Arterial Distensibility in Diseases

The above data imply that in diseases characterised by an increased sympathetic activity, arterial distensibility is reduced. This has indeed been obtained in studies on hypertension, renal insufficiency and heart failure, i.e. conditions characterised by sympathetic activation [35–37]. We have observed that in patients with heart failure, distensibility of the radial artery, the carotid artery and the abdominal aorta is reduced (Fig. 4) [35]. Furthermore, we have seen that there is in this condition a relationship between reduction of arterial distensibility and magnitude of sympathetic activation as measured directly by microneurography. Finally, we have observed that therapeutic interventions that indirectly or directly reduce sympathetic activity, such as those based on ACE-inhibitors or angiotensin II antagonists, are accompanied by an improvement of arterial distensibility [38]. Thus, whenever sympathetic activation is part of cardiovascular disease, arterial stiffening has to be expected.

Arterial Stiffening and Sympathetic Influences: Possible Mechanisms

An increase in sympathetic activity may reduce arterial distensibility through a variety of mechanisms. First, as mentioned above, when the increase is accompanied by an increase in blood pressure, distensibility may be reduced because the resulting increase in vessel diameter stretches the least distensible component of the vessel wall (e.g. collagen), making the relationship an inverse one within a blood pressure range from diastole to systole [2–10]. Second, distensibility can be reduced because of a sympathetic-dependent increase in heart rate, given that this

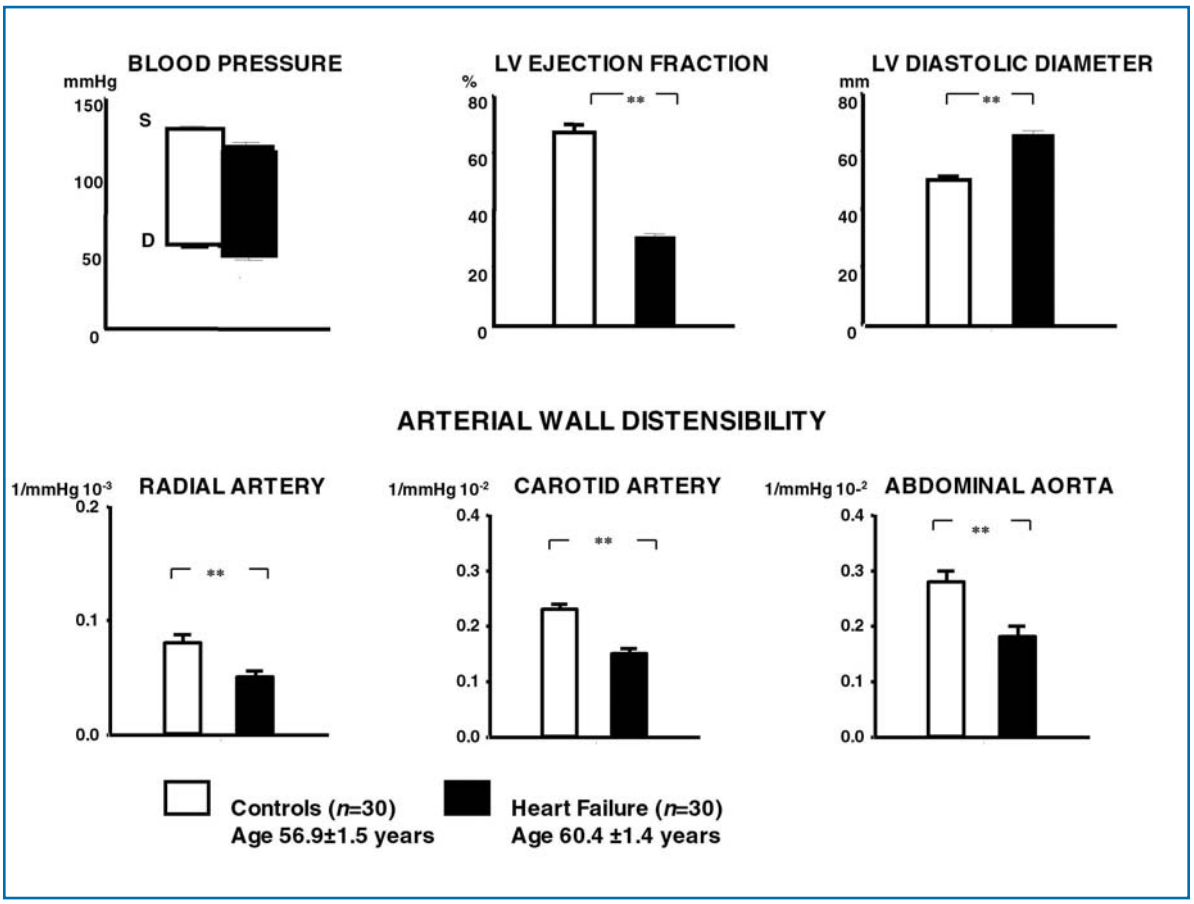

Fig. 4. Arterial distensibility in heart failure

increase is associated with a stiffening of mid-size and large elastic arteries both in animals and humans [33–37, 39–45] (Fig. 5). Third, because sympathetic influences reduce arterial distensibility in absence of any blood pressure and heart rate change (*see following page*), other mechanisms must be involved. We can speculate that the well-known trophic effect of sympathetic influences on arterial wall tissue modifies the arterial wall structure in a way that favours its less extensible components and increases its thickness, as shown in animal studies in which chronic denervation of a carotid artery was accompanied by reduction of its thickness compared with the value of the contralateral intact vessel [46, 47]. We can further speculate, however, that given that sympathetic drive can also actively modify arterial distensibility, additional mechanisms are importantly involved. These mechanisms may consist of contraction of vascular smooth muscle because the elastic modulus of contracted muscle tissue is greater than that of the relaxed one [48–53]. A contracted vascular smooth muscle may also have greater viscous properties, i.e. it may more prominently oppose resistance to vessel distension in relation to tissue. The potential importance of this factor is exemplified by the marked reduction of carotid and femoral artery distensibility that occurs in rats when heart rate is increased, even after sympathectomy [33, 34].

Evidence of Sympathetic Functional Recovery in Allotransplantation of the Hand in Humans

Allotransplantation of the hand is another model for the study of sympathetic modulation of arterial distensibility because the surgical removal of sympathetic innervation at the level of the radial artery is associated with reinnervation approximately 3 months later [39, 40]. We

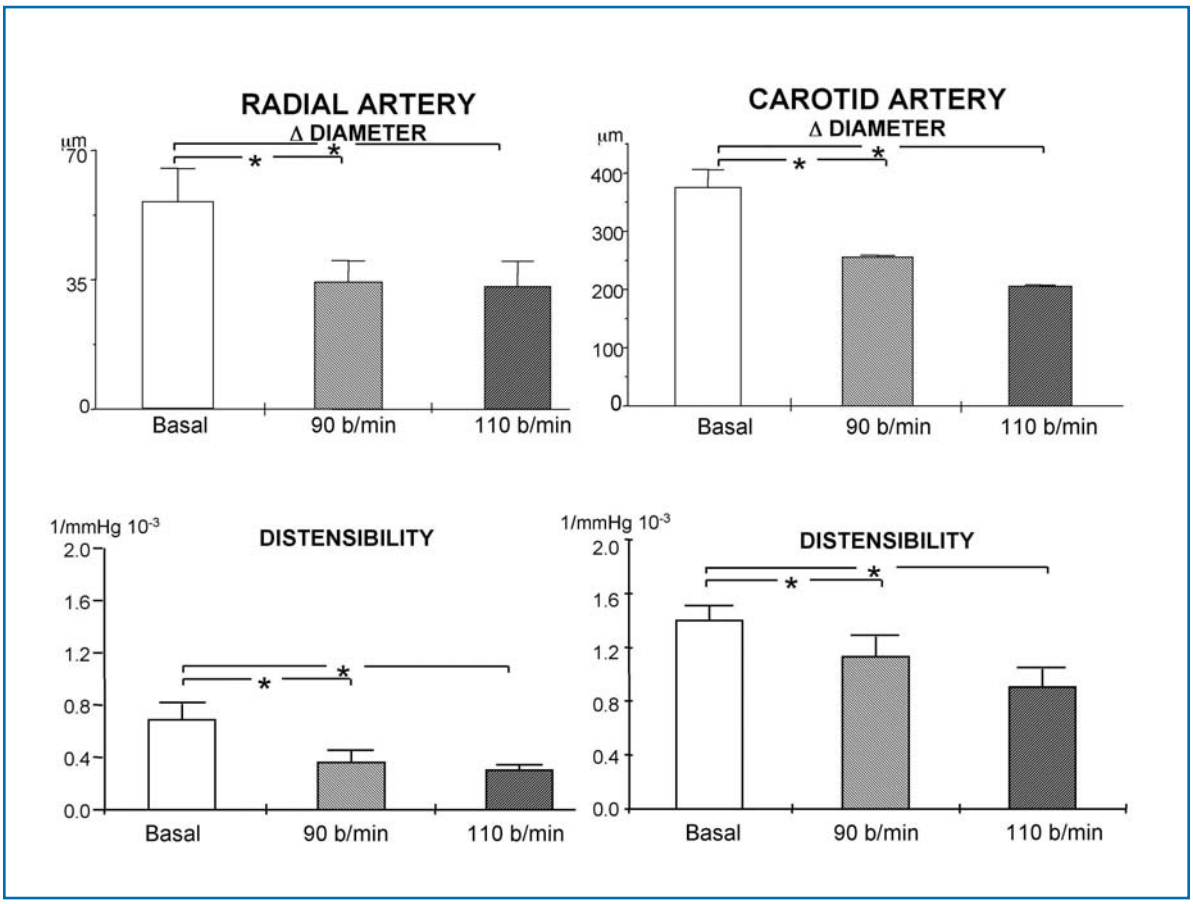

Fig. 5. Arterial distensibility before and during pacing in humans

had the chance to study two patients who underwent allotransplantation of the right hand, which had been lost in a previous car accident. Both patients underwent a routine pretransplantation investigation and morphological and functional testing of the forearm stumps to ensure immunological and mechanical donor-recipient compatibility. They were put on treatment with monoclonal antibodies anti-CD25, FK506, mycophenolate mofetil (MMF) and prednisone immediately after surgery to prevent graft rejection. Maintenance therapy was unchanged throughout the study duration. The posttreatment programme of rehabilitation consisted of physiotherapy, electrostimulation and occupational therapy. Pain, touch and T° sensations as well as ability to perform active movements were examined weekly by conventional clinical tests. Sweat function was examined by application to the skin of laboratory blotting papers on a weekly basis. Radial artery distensibility was measured 40 days after the surgical procedure and then every 4 weeks for the following 6 months. Measurements were made in the wrist 4 cm below the suture as well as at the same wrist level in the contralateral vessel.

Radial artery distensibility was measured by a B-M mode echo-tracking device based on Doppler shift (Wall Track System, PIE Medical, Maastricht, The Netherlands) and on a transducer operating at a frequency of 7.5 MHz [54, 55]. The transducer was mounted on a stereotaxic arm oriented perpendicularly to the longitudinal axis of the vessel under B-mode guidance. After switching to A mode, the backscattered echoes from the anterior and posterior radial artery walls were visualised on a screen, and the corresponding radiofrequency signal was tracked by electronic tracers to allow the digitalised signal of the internal diameter variations to be derived at 50 Hz. The spatial resolution was 300 mm [54, 55]. Blood pressure was measured from the brachial artery at the same time as ultrasound evaluation via a semiautomatic

device (Dinamap 1846 SX/SXP, Critikon, Chatenay Malabry, France), and radial artery distensibility was derived according to the following formula:

$$\text{Dist} = (2\,\Delta D/Dd)/\,\Delta P$$

where Dist is distensibility, Dd the diastolic diameter of the vessel, ΔD the systodiastolic diameter change and ΔP the corresponding pulse pressure. Radial artery measurements were made by a single operator with an intraobserver variability of 4%. Heart rate was measured by the palpatory method (30 s) after each blood pressure measurement.

As shown in Figure 6, in the two patients in whom the right hand was allotransplanted, 1 month after surgery, the radial artery below the transplantation suture showed a distensibility that was much higher than that of the contralateral control artery. Thereafter, however, the distensibility value showed a progressive reduction, with return to the contralateral value 2 months later. This was the time at which the ability to perform active movements, to detect touch, temperature and pressor stimuli as well as to sweat also reappeared, strongly suggesting that somatic and autonomic reinnervation had been reestablished [39]. Thus, transplantation is characterised by a temporary marked reduction in the ability of the arterial wall to resist the distension caused by intravascular pressure as a result of a marked increase in arterial distensibility. Based on previous animal and human data on the stiffening influence of ongoing sympathetic activity on large and medium arteries [3, 4], this is likely to be due to loss of sympathetic innervation, with sympathetic reinnervation of the vessel wall being conversely responsible for return of arterial distensibility to normal.

One may wonder whether the increase in radial artery distensibility that accompanies transplantation has favourable or unfavourable clinical implications. An increase in vessel distensibility is regarded as clinically beneficial because it results in a reduction in pulse pressure (an independent cardiovascular risk factor [56]) and endothelial trauma, with reduced atherogenesis [57]. It is not inconceivable, however, that when the increase in distensibility is as abrupt and large as the one seen in the radial artery after transplantation, the resulting marked increase in systodiastolic vessel excursion may lead to mechanical damage of the vessel.

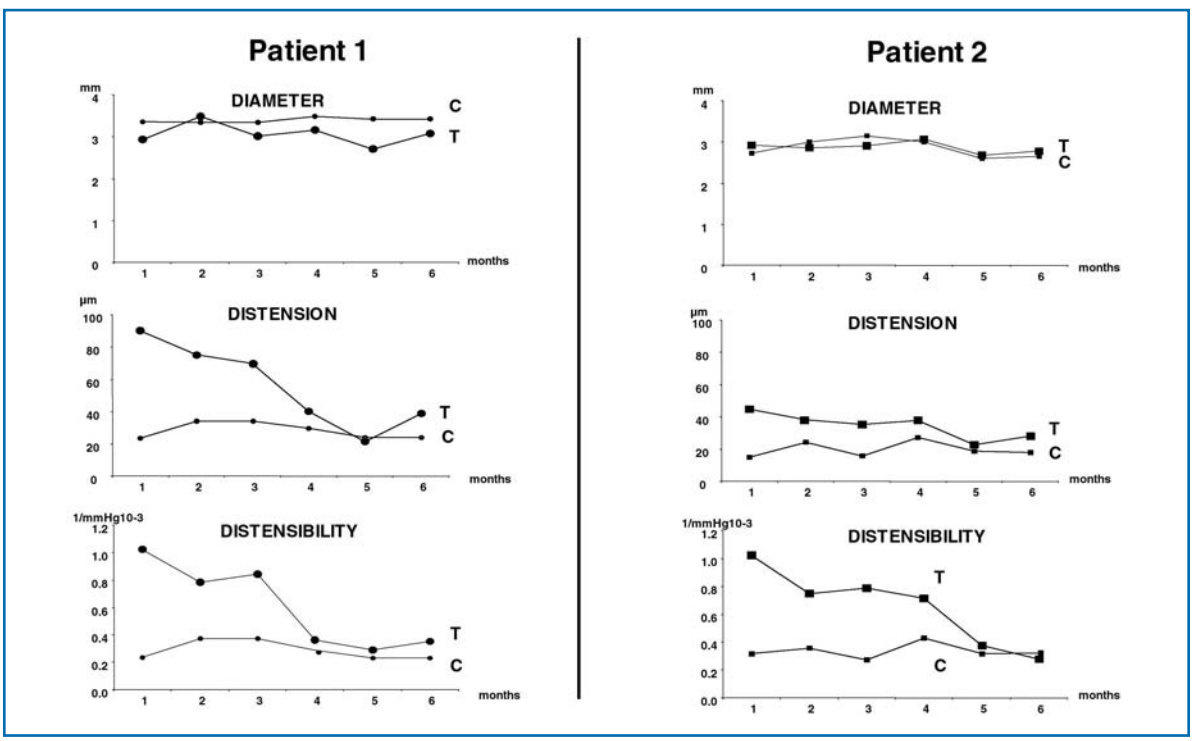

Fig. 6. Arterial distensibility in humans before and after hand transplantation: From [39], used with permission

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Section 9-g

An Instrumental Kit for a Comprehensive Assessment of Functional Recovery

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Introduction

Impairment of, or damage to, the upper limbs, as well as central or peripheral neuropathies, may limit hand function. This creates severe disabilities since it reduces the capacity to manipulate objects and work tools and interface with the environment, which are fundamental motor tasks in daily life. "Hand assessment" is a term commonly used to describe evaluation of hand and wrist function. It involves analysis of kinetic and kinematic properties while investigating fine-motor abilities, such as hand-wrist posture, finger posture, pinch and various grasp tasks (firm, different prehensile grasp of objects daily life, work tools grasp, etc.), as well as hand endurance and how these abilities affect hand function. Hand assessment is important in different fields of application, from clinics, occupational therapy and rehabilitation to biomechanics research.

Many methods have been proposed to assess hand force in daily and work tasks and ergonomics [1–3]. Direct measurement of the force exerted during tasks simulating common physical work or daily activities (e.g. pushing, pinching, grasping) was generally carried out by instrumenting usual tools with force transducers. In association with these devices, tests have been proposed based on tracking specific force targets to assess the perception of effort [1]. Other works investigated specific hand motor tasks, such as precision grasping (between thumb and index) and whole-hand grasping (involving all five fingers) to evaluate muscle or tendon damage and investigate finger coordination and motor-control strategies. Grasp or grip (the terms are interchangeable) has been widely studied both in natural and artificial motor performances (by the latter, we mean electrically driven, paralysed hands). In whole-hand grasping tasks simulating daily activities, force is measured by means of cylinders instrumented with strain gauges [4] or by means of a vertical support with force sensorised contact pads for each of the five finger [5, 6]. Specific grasp types, such as lateral hand grasp and precision grip, were also assessed: the lateral grasp was assessed via instrumented objects constructed to resemble everyday items (e.g. a fork, a glass, a pen) [7] and the precision grip *via* a compressible cylinder held between thumb and index [8].

Most studies dealing with pressing motor tasks focused on the action of the four long fingers (index, middle, ring, little) without involvement of the thumb, and several devices were purposely designed to measure forces. As an example, some Authors produced a set of steel frames instrumented with piezoelectric sensors [9, 10] in order to measure the force expressed by single fingers of one or both hands.

Commercially available devices to measure hand force are mainly intended for rehabilitation follow-up. In general, these devices measure only maximal force delivered during predefined tasks, mostly based on grasping or pinch. The

hand dynamometer for grip force is a consolidated clinical instrument. The first one introduced in the market was the Jamar hand dynamometer – registered trademark of Sammons Preston – presented in 1954 by Bechtol [11]. It measures isometric grip force exerted on an adjustable handle placed in one of the five preestablished positions from 3.5 to 8.6 cm in half-inch increments (range, 0–900 *n*, accuracy 5% or less [12]). Other similar commercial handgrip dynamometers are those by ESA (range, 40–1,000 *n*, accuracy 0.75% and Biometrics Ltd. (Gwent, UK) (range, 0–900 *n*, accuracy 2% f.s.). To measure pinch strength, there are several pinch gauges on the market: the Jamar pinch gauge (range 0–200 *n*, accuracy 1% f.s.) that measures pinch force under different types of prehension and pinch dynamometers by ESA (range, 0–270 *n*, accuracy 0.75% f.s.) and Biometrics Ltd (range, 0–220 *n*, accuracy 2% f.s.). Pressing and grasping are also investigated by means of matrices of force sensors, either piezoresistive (Tekscan, UK) or piezocapacitive (Novel Gmbh, Germany). Measurement accuracy and precision depend on sensor characteristics in terms of dimension, linearity and hysteresis and on their relative distance that determines spatial resolution.

Hand kinematics assessment usually entails evaluation of range of motion (ROM), traditionally by means of manual goniometry. This method is often affected by considerable inter- and intraoperator variability [13] and is very time consuming. In addition, the simultaneous measurement of all finger joints is not possible, nor is overall hand evaluation during a dynamic task.

To overcome these limitations, other techniques, such as optoelectronic techniques and sensorised gloves, have been developed. The optoelectronic technique is based on surface markers and is mostly used to study biomechanical properties of the hand [14–18] through identification of kinematic models and the study of movement coordination among finger joints. The gloves are equipped with angular position sensors that allow for continuous monitoring of finger joints. Since validity and reliability of these measurements are acceptable [19, 20], sensorised gloves may be a useful tool for assessing the degree of impairment and functional ability of the hand. Most of these gloves have been developed for virtual reality applications. When used in rehabilitation, they may also enable remote monitoring and periodic reassessment [21–23].

Unfortunately, measurement instruments capable of fully monitoring hand and finger strength and posture during motor tasks are laboratory prototypes and not commercially available. For instance, an instrumental system for comprehensive hand assessment was designed and constructed within the research project Hand Assessment and Treatment System (HATS) under the Telematics for the Integration of Disabled and Elderly (TIDE) programme of the European Commission [24, 25]. The HATS system consists of a portable set of five electronic instruments for measurement of movement ranges, oedema and grip strength. Grip-strength measurement is achieved by a grip gauge conceptually similar to other commercial Jamar-like instruments. This instrumental system, however, does not enable kinetic or kinematic analysis of individual fingers.

Authors committed to assessment of function recovery of the transplanted hand within a research project approved by the Italian Ministry of Health considered the above-reported deficiencies of commercially available measurement devices and decided to set up an instrument kit consisting of a clinical tool set and associated tests for a wide-ranging hand–finger kinematic and kinetic assessment. The clinical tool set integrates different devices designed and constructed ad hoc and commercially available. These devices are:

1. A commercial sensorised glove for finger-joint kinematics
2. A set of measuring devices designed and constructed at the Authors' laboratory, with tests designed to measure and monitor the force each finger separately exerts under isometric conditions
3. A commercial Jamar-like instrument and a commercial pinch meter.

The choice of tools was based mainly on measurement accuracy requirements, their complementarity to pursue the completeness of the

assessment and the simplicity of preparing the subject. Relevant associated tests were identified and developed. A set of typical results from the assessment of the transplanted hand are hereby reported to show their validity in evaluation of the recovery process and to underline the desired complementarity of the clinical tools and associated tests.

The Instrument Kit

The instrument kit features three clinical tools and associated tests for assessing finger kinematics and kinetics and full hand kinetics.

Hand-Finger Kinematic Assessment

The Tool

The tool for hand-finger kinematic assessment is the Humanglove by HumanWare Srl (Pisa, Italy). PC-based, this tool consists of an instrumented glove (Fig. 1), a software package for transducer calibration, a software package for data analysis and display with virtual reality rendering. The core of the device is the glove instrumented with 20 Hall-effect angular sensors. Four sensors are positioned on each long finger for measuring flexion–extension angles (Fig. 2) of the three interphalangeal joints and the abduction–adduction angle of the proximal joint. The remaining four sensors are arranged for measuring flexion-extension angles of the two thumb joints and the first metacarpal joint while performing thumb opposition and abduction-adduction movements. An electronic circuitry drives the sensors. Their output is sampled at 16 samples/s, 12-bit A/D converted, and fed to a PC via a standard serial port. The calibration software package contemplates automatic and manual calibration. Manual calibration requires fixed angles to be imposed on all joints and was preferred for its higher accuracy. We designed and constructed an ad hoc object to impose 0° and +45° angles. Data analysis and display software is based on open GL applications that enable data recording during the tests and three-dimensional (3D) virtual reality display (Fig. 3). This software also yields several exhaustive graphic visualisations of the angular excursion for each interphalangeal joint as a function of time and relevant maximal and mean values of angular

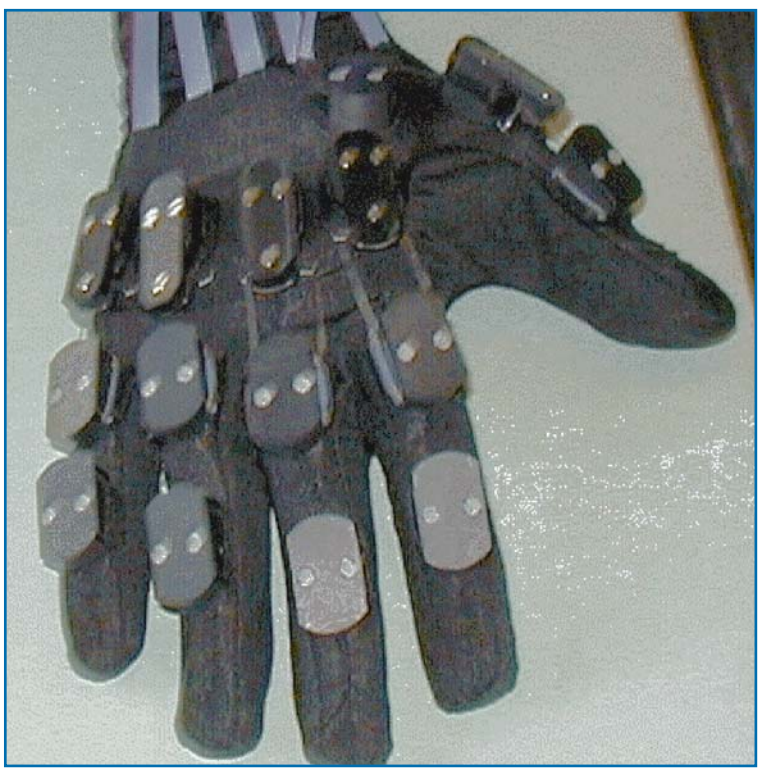

Fig. 1. Instrumented glove

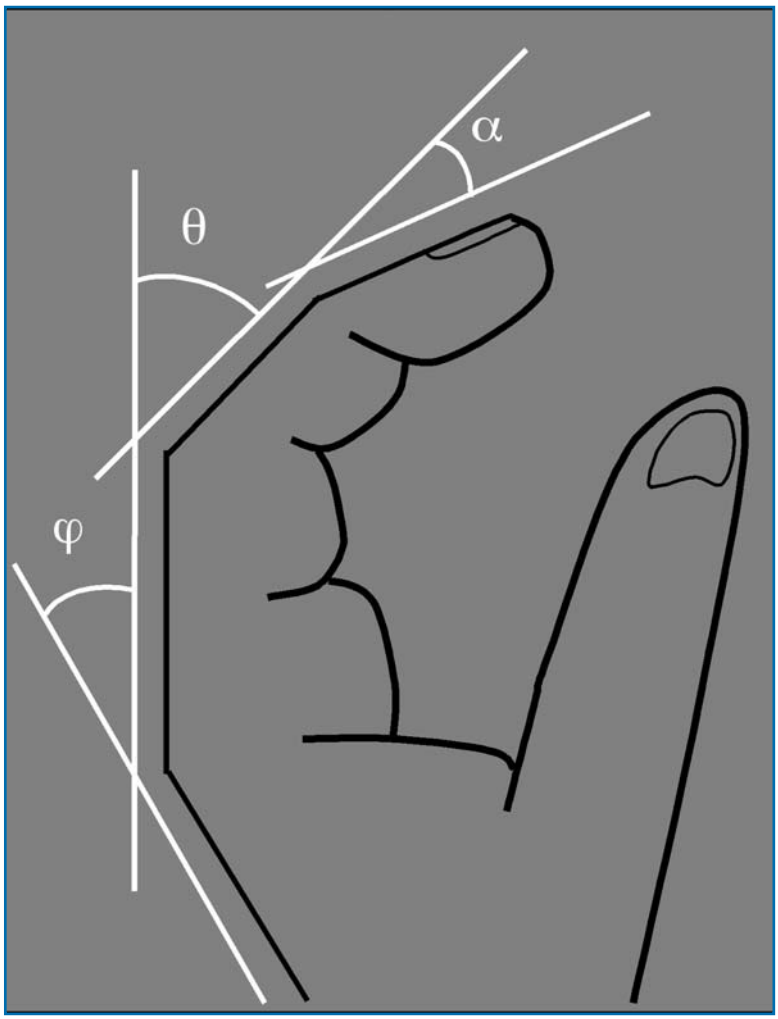

Fig. 2. Interphalange joint flexion–extension angles: α, distal; θ, middle; φ, proximal

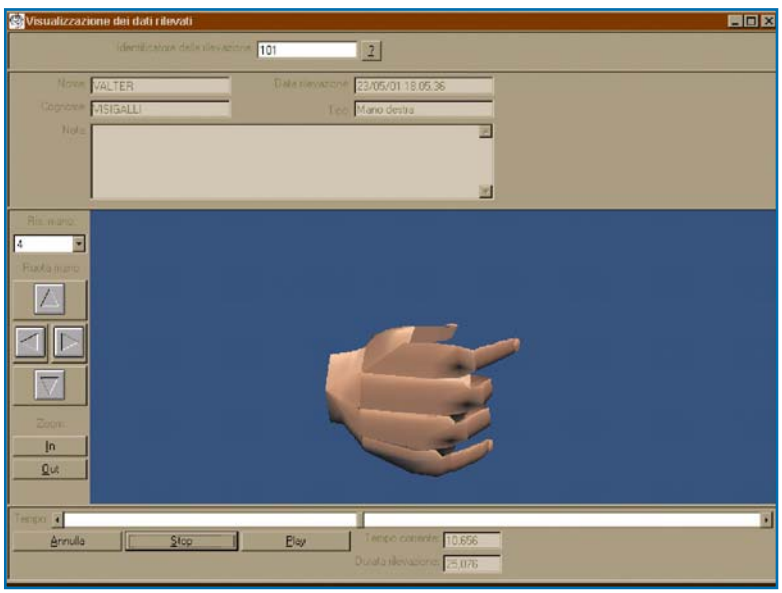

Fig. 3. Three-dimensional virtual reality display

velocity and acceleration. The recorded data can be also exported in ASCII files.

Associated Tests

Figure 4 shows a transplanted patient during a session for hand-finger kinematic measurement. Five principal associated tests were designed to assess flexion-extension angular range of each finger joint during the performance of everyday movements:

1. Cycles of active flexion and extension of all fingers but the thumb (30 s)
2. Cycles of active flexion and extension of the thumb (30 s)
3. Cycles of active flexion and extension of all fingers (30 s)
4. Cycles of closing and opening the hand as performing a pinch (30 s)
5. Picking up a ball.

The last test assesses the evolution of the picking-up strategy during the rehabilitation process. Ball size was standardised to fit hand size by setting its diameter at about 60% of the mean length of the four long fingers. The virtual glove added the necessary quantitative measurement to the pure subjective medical evaluation when examining recovered hand dexterity.

Hand–Finger Kinetics Assessment

The Tools

Two devices for the analysis of finger kinetics have been designed and constructed at the Authors' laboratory. One is an instrumented keyboard (IK), the other is a mouse-like tool (MLT). Figure 5a shows the IK. It features 5 instrumented aluminium keys that transduce the forces each fingertip exerts during pressing tasks while the palmar aspect of the hand is in contact with the keyboard support. Key positions can be adjusted to fit a wide range of hand sizes – from a 6-year-old child to an adult man. The key is an aluminium cantilever beam (63.5-mm long, 13x13-mm pressing key area) (Fig. 5b). It is equipped with two extensometric half bridges (EA-13-062TT-350, Measurements Group, Inc., Raleigh, NC, USA) close to the fixed end (Fig. 5c). Signals from the extensometers are electronically conditioned. A low-pass Butterworth second-order filter is used, with a cutoff frequency of 20 Hz. Data are acquired with a 12-bit A/D converter (DAQ-PAD 6020E by National Instruments) at 100 samples/s and fed to a PC for analysis and presentation. The MLT (Fig. 6) embodies a single key, equal to those of the IK,

Fig. 4. Transplanted patient during a measurement session with the instrumented glove

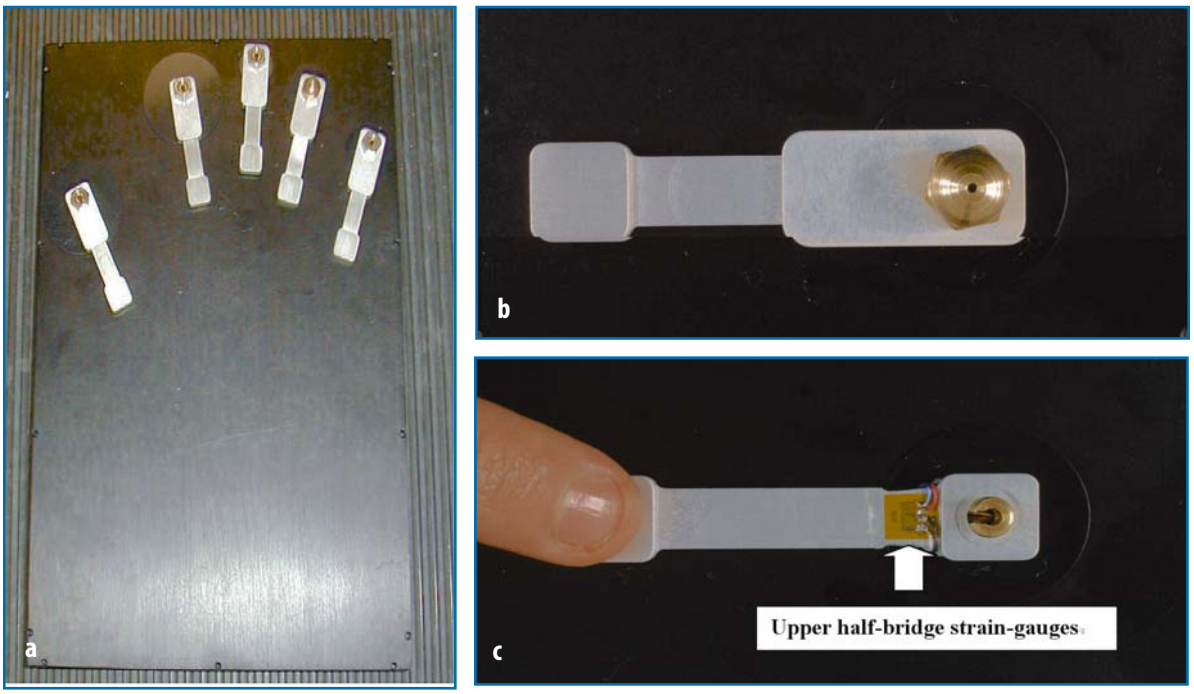

Fig. 5a-c. a Instrumented keyboard. **b** Aluminium key is a cantilever beam. **c** Positioning of the half-bridge strain gauges

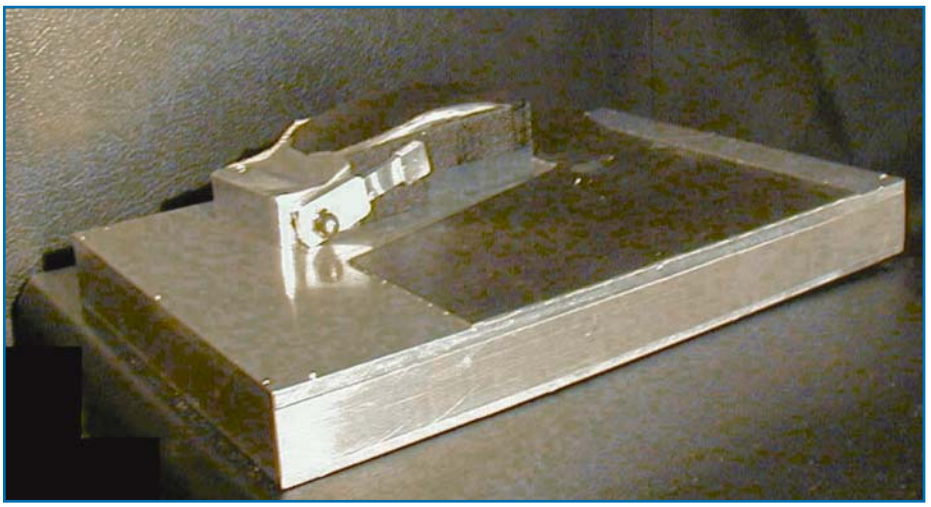

Fig. 6. Mouse-like tool

placed on the internal vertical facet of a mouse-like support. It measures the force expressed by the thumb when isometrically adducting or, alternatively, by the little finger when isometrically abducting with the hand fixed beside the MLT. The transducing conditioning is identical to that of the instrumented keyboard. During measurement, two straps keep the wrist and forearm in a fixed position.

Associated Tests

Figure 7 shows a transplanted patient during a clinical session of hand–finger kinetic assessment using the IK. Dedicated software (developed using the Matlab programming environment) manages the tests proposed to the subjects and provides for data acquisition and processing. Two test sets were delivered:

1. Maximal voluntary contractions (MVCs): The

Fig. 7. A hand transplanted patient during kinetics assessment. Experimental setup and hand positioning on the instrumented keyboard

subject performs a sequence of isometric MVCs lasting 10 s, with a 60-s interval in between: (a) one finger on the IK, (b) all fingers of one hand simultaneously on the IK, (c) both hands simultaneously on the IK, (d) one or both thumbs simultaneously on the MLT(s), and (e) one or both little fingers simultaneously on the MLT(s).

2. Long-lasting submaximal contractions with biofeedback: The test lasts 20 s, with a 120-s interval between trials. Visual feedback is used here: a real-time display of the total force of all fingers of a hand while trying to follow a sigmoidal force profile, the maximum value of which is set at 60% of the MVC (Fig. 8). The subject is asked to follow the sigmoidal force profile during unilateral and bilateral tasks with all five fingers on the IK or with the thumb on the MLT.

Kinetic Assessment of the Hand as a Whole

The Tools

Two commercial devices by Biometrics are used for kinetic assessment of the hand as a whole.

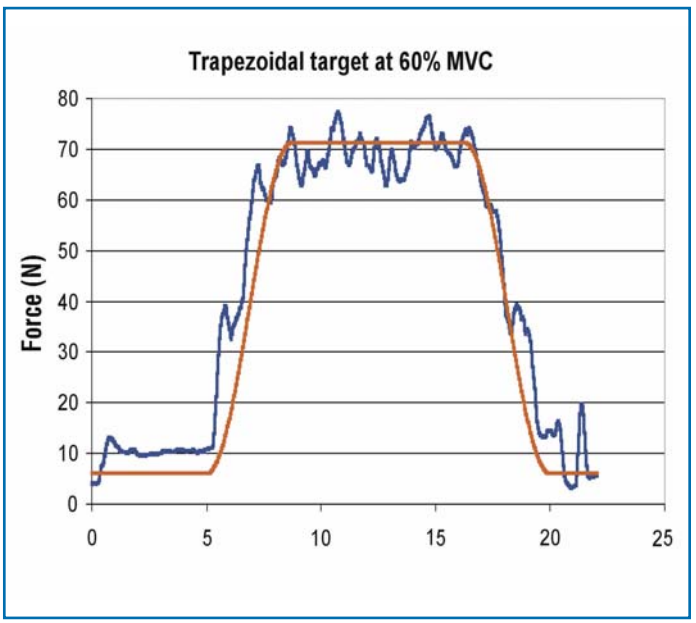

Fig. 8. Example of a test with visual biofeedback. Sigmoidal target (*red line*) and all-finger contribution (*blue line*)

One is a standard hand-held dynamometer with 5 positions for measurement of grip strength. The other is a pinch meter.

Associated Tests

Tests with the hand-held dynamometer consist of three trials conducted in each of the five positions, alternating the hands. Tests with the pinch meter consist of three "jaw" trials and three tip-to-tip trials for each thumb-to-finger opposition for both hands.

Results

Calibration Results and Instrumentation Accuracy

The IK and MLT were calibrated and characterised by means of a precision dynamometer (DPU 50K, Imada, Japan), 0.1-N resolution, 500-N range. The nonlinearity of the sensor key was found to be better than 0.4% f.s.; accuracy was ± 1.3% f.s. Hysteresis (<0.2% f.s.) was computed on the basis of loading–unloading slow cycles. Drift was eliminated by means of zeroing algorithms; each channel was independent of the others, and no cross-talk was detected. The claimed features for the Biometrics pinch meter and hand-grip dynamometer were confirmed during bench tests conducted by means of the same dynamometer in the force range of interest. HumanWare claims the following characteristics for the glove: 0.2° sensor resolution for the whole measurement range, 1° accuracy and 1% f.s linearity. These features were confirmed by imposing different known angles.

A Few Relevant Measurement Results

Finger Kinematic Evaluation

Figure 9 refers to the maximal angular ranges of middle- and ring-finger interphalangeal joints as assessed in three measurement sessions. A significant recovery was documented.

Postprocessing of data investigated temporal involvement for each finger's interphalangeal joint during the task. Figure 10 shows temporal involvement of each joint for the middle and ring fingers in task 3, which involves all five fingers.

Progression of rehabilitation is clearly documented. Middle joints do not flex in the first two sessions. Of interest seems to be the progression in maximal angular velocity and acceleration expressed by finger joints during the same task 3, not reported here.

With the glove, we also investigated the hand's strategies while picking up an object. The sum $\varphi+\theta+\alpha$ of the interphalangeal joint of flexion-extension was chosen as investigation parameter. For each finger, we investigated the angular contribution of each joint with reference to the sum of the ROM of the three joints. Figure 11 shows the evolution of the angular contribu-

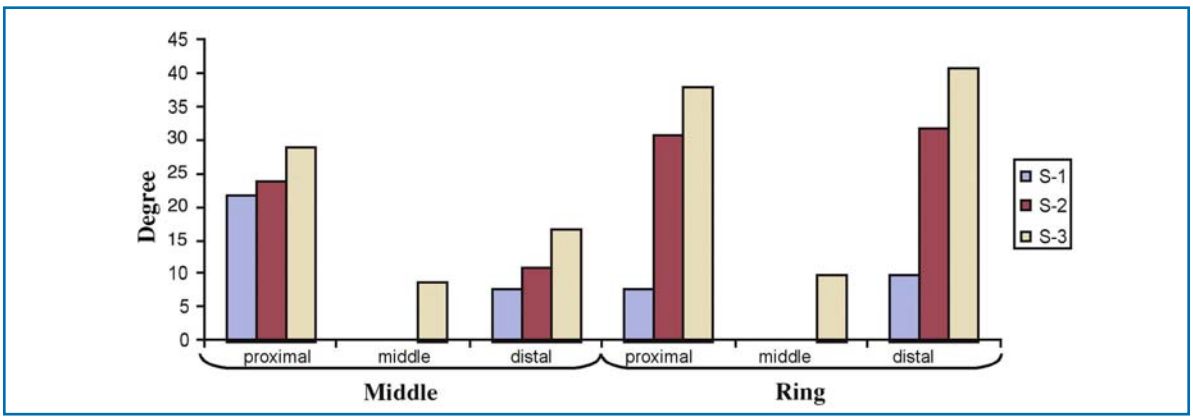

Fig. 9. Maximal angular flexion–extension of middle- and ring-finger interphalangeal joints of the transplanted hand in three sessions. Time interval between sessions was approximately 3 months

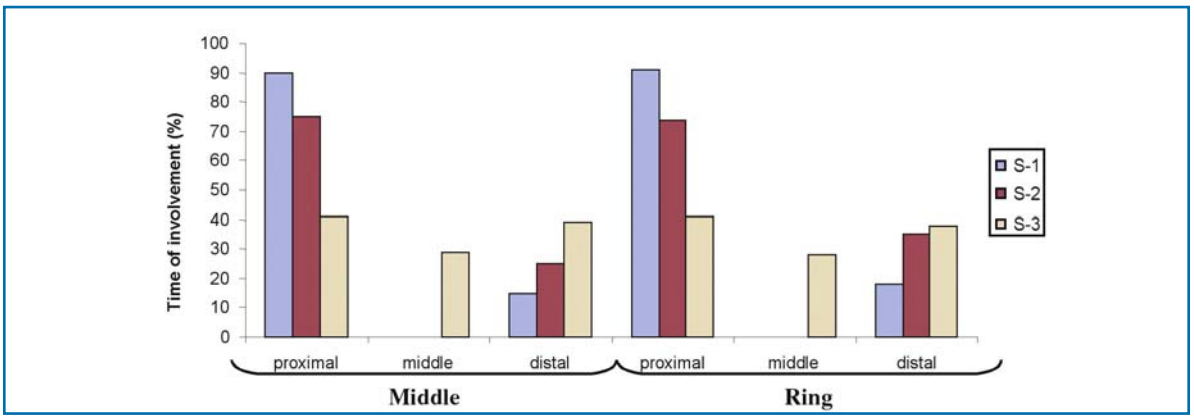

Fig. 10. Temporal involvement of all interphalangeal joints of the ring and middle fingers as a percentage of the flexion-extension cycle duration. One trial of task 3 in three sessions

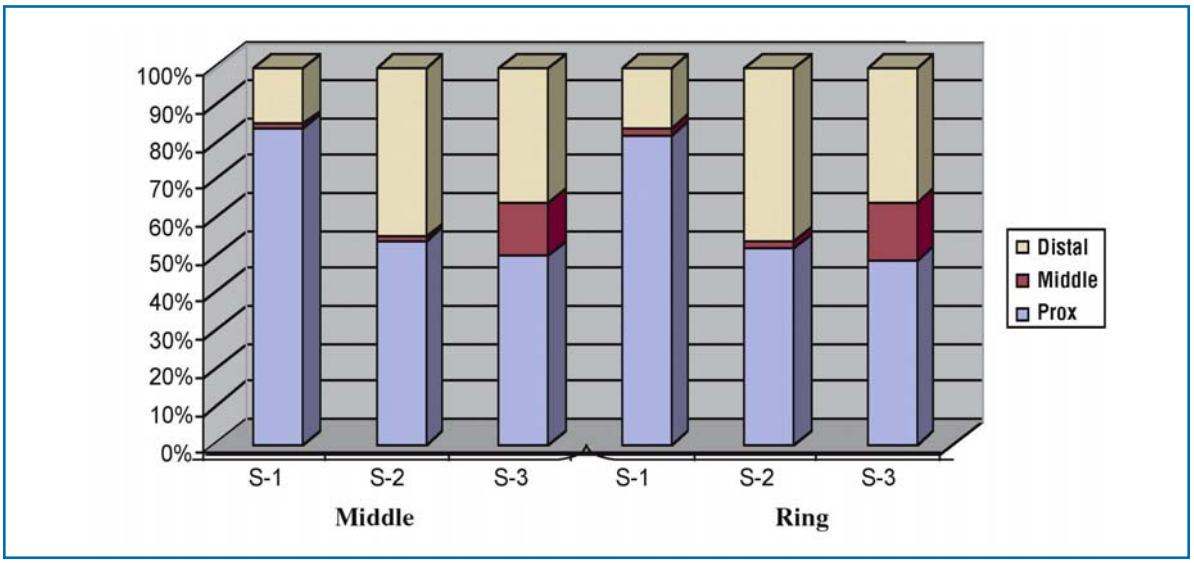

Fig. 11. Picking up a ball. Range of motion (ROM) of each joint of the ring and middle fingers along the three sessions, expressed as percentage of the sum of their ROM

tion for the ring and middle fingers as recorded along the three measurement sessions. For instance, if we focus on the intermediate joints and cross-reference results from this task with the previously described ones, we can trace recovery of their functionality – from a low to a significant angular and temporal contribution in all tasks.

Finger Kinetics Evaluation

For the sake of brevity, we hereby report only some preliminary measurements, which show the full complementarity of the instrument kit. For the maximal voluntary contraction trial, we report IK measurements on the middle and ring fingers in three sessions. Recovery of finger function is shown (Fig. 12) during a task where the hand works flat on the IK.

Other dynamic tasks by which recovery of middle- and ring-finger function is highlighted are those conducted with the hand-held dynamometer that contemporarily involves all fingers. While with the IK we dynamically investigate how the fingers work when flat, this task

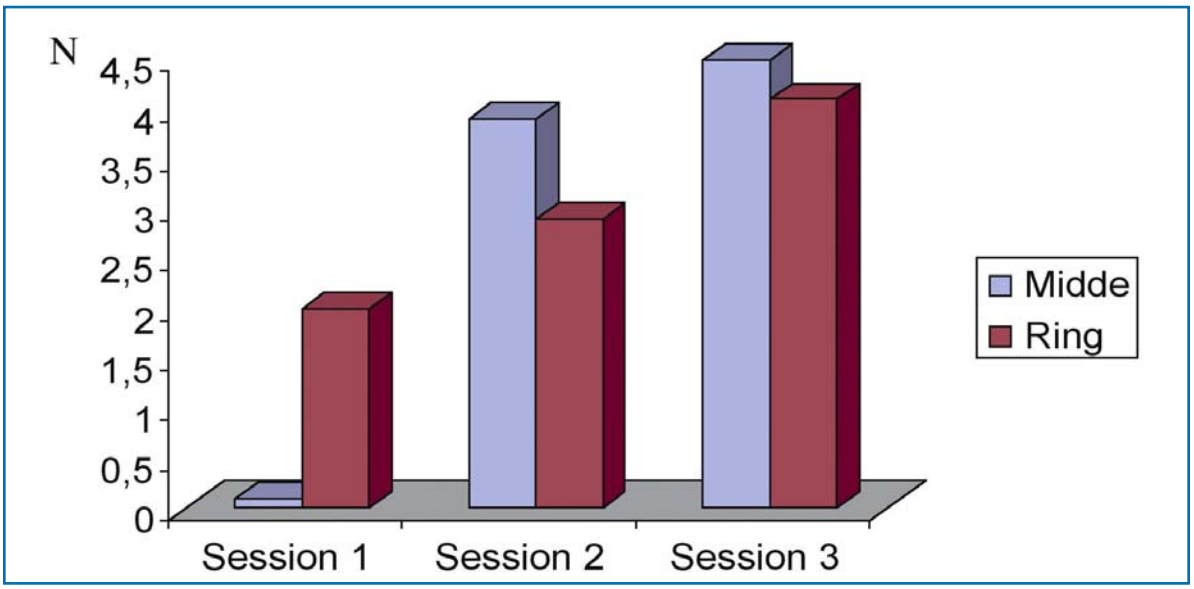

Fig. 12. Middle- and ring-finger force during maximal voluntary isometric contractions on the instrumented keyboard in the three sessions

allows us to investigate how these fingers work jointly during grasping, a task where both the middle and ring fingers play an important role. Thanks to recovery of function of the middle joints of the middle and ring fingers (as documented in the previously described kinematic trials), in the third session, the patient was able to perform this specific task, which requires significant finger ROM unattainable in the early rehabilitation phase. Figure 13 shows forces for the five dynamometer positions. Even in position 1, the most critical in terms of ROM requirement, the hand exerted significant forces.

With the test set based on visual biofeedback, we investigated other functions directly correlated with the recovery strategies, in particular, single-finger force modulation in order to maintain the total force value required by the task. For example, Figure 14 shows the capacity of five fingers to exchange involvement, depending on the level of fatigue, during tracking of the 60% MVC force task in the third measurement session.

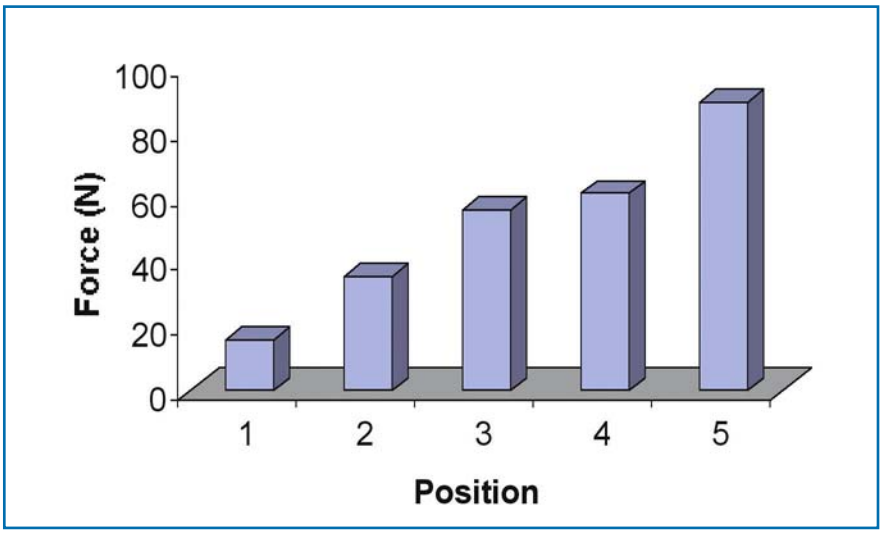

Fig. 13. Hand forces exerted in the five dynamometer positions

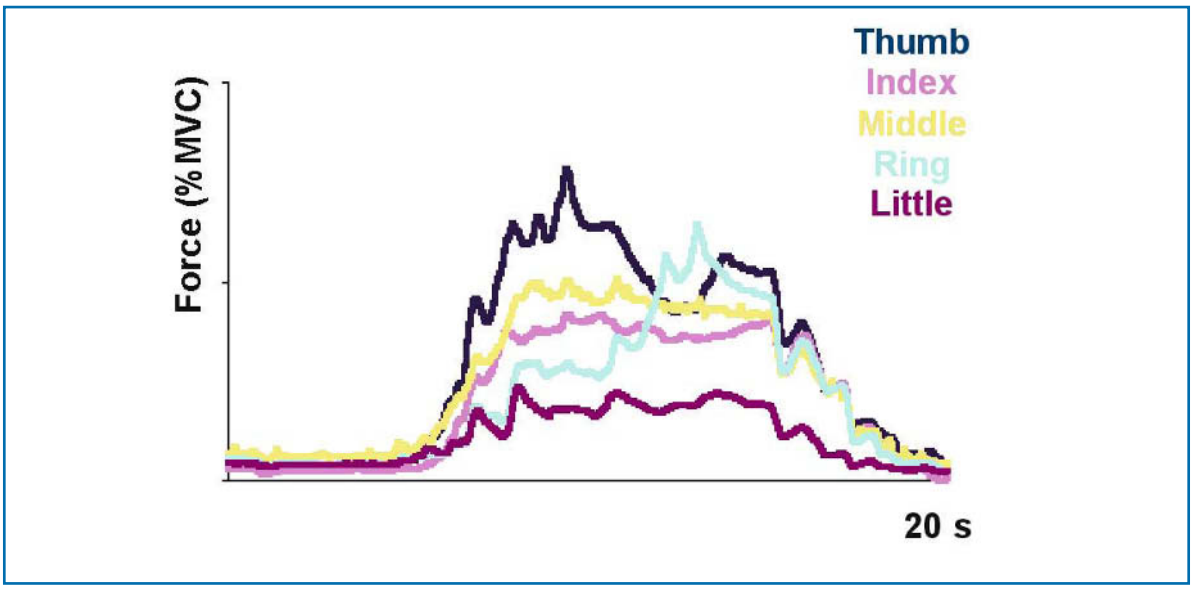

Fig. 14. Each finger contribution during the force profile tracking test. As force exerted by the thumb and middle fingers decreases, force exerted by the ring finger increases

Discussion and Conclusion

Summary

In order to investigate recovery of hand function after transplantation, a quantitative assessment should be performed. We designed a clinical tool set with associated tests for a wide-ranging quantitative assessment of hand functional recovery. Both kinematics and kinetics quantities are considered. For assessment of finger forces, a special set of instruments was designed and constructed to measure the force the fingers can exert under isometric conditions. One measures the force each finger exerts, with joints at 0° angular position, against an IK; the other measures the force the thumb exerts while grasping a mouse-like support. The dynamic tests associated with the keyboards are driven by dedicated software that manages test execution, data analysis and presentation. A commercial device kit by Biometrics is also used to assess hand function as a whole, such as in grip and pinch.

For the kinematics assessment, a commercial device (Humanglove by HumanWare) is used. The associated tests were designed to investigate functional recovery in terms of joint ROM, timing, angular velocity, acceleration and dexterity in the execution of tests reproducing real-life demands, such as displacement of objects of different shapes and dimensions. A virtual reality software package is used for data analysis, presentation and recording. In particular, 3D virtual reality recording enables construction of a database with minimal memory requirements, which is a valuable tool to help the physician interpret both kinematic and kinetic data. The methodology proved to be efficacious and the measurements accurate and suitable for the purpose.

It is known that even simple, everyday life hand motor tasks, such as pressing or grasping, entail complex muscle activation. Motor strategies, both during maximal and biofeedback-driven contractions, show the implication of complex finger coordination, especially the thumb in its critical role of opposing the long fingers. Kinematics analysis is necessary for a more complete and objective assessment of finger functionality.

From a general point of view, results enhance complementarity of information that the clinical tool set may furnish. For instance, it highlights the importance of the thumb, less investigated in literature if compared with the other fingers. As for kinematics, if we focus, for example, on the simple task 3 (open/close all fingers), the virtual

glove and associated tests show not only angular ranges (as does goniometry) but also how an interphalangeal joint works in terms of timing of activation, velocity, acceleration and finger phalange phases [21, 23] (not shown here). Furthermore, useful information comes from the quantitative data furnished by the glove during ball pick up; this information is correlated to overall ability, implying recovery of suitable brain internal neural model.

On the other hand, kinetic assessment not only furnishes quantitative information regarding hand forces when considering the hand as a whole (as many commercial devices do), but also gives the contribution of individual fingers, the thumb included, which is seldom addressed in the literature. The introduction of biofeedback tasks allows investigation the "force modulation" ability of each finger when tracking different force profiles.

Future Perspectives

The proposed methodology described here was developed to follow the recovery of the transplanted hand, but can be applied to other medical investigations into hand functionality; for instance, recovery from an injury or rehabilitation from crucial occupational diseases or from nervous-system pathologies such as stroke, multiple sclerosis and traumatic brain injuries. In this respect, special care was dedicated to software portability: it can be installed on a PC under different operating systems, e.g. Windows ME, 2K, WX.

Acknowledgments

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Section 9-h

Human Brain Plasticity after Bilateral Hand Allograft

Antoine Aballéa, Pascal Giraux, Marc Schieber, Jean-Michel Dubernard, Angela Sirigu

Introduction

Neuropsychological studies suggest that somatic perception and awareness of bodily movement emerge from the activation of multiple, coordinated, dynamic representations of the body at different levels of the central nervous system, commonly called the "body schema". Among the regions involved in this process, the primary sensory and motor cortices contain the most detailed maps in the cerebral cortex.

This body representation in the primary sensory (S1) and motor (M1) cortex were classically conceived as "somatotopic". The concept of the "homunculus" (literally, "little man"), as described by Penfield and Rasmussen [1] refers to anatomically and functionally independent representations of body segments within the central sulcus. However, recent electrophysiological data in monkeys and functional neuroimaging results in humans converge to a more dynamic model of body representations in M1 and S1. Though the somatotopic organization of M1 clearly includes separate representations of the face, arm, and leg within each of these major representations, studies have shown considerable overlap and intermingling of representations of smaller body parts. In monkeys, a given neuron may be active during movements of distinct digits, and the cortical territories containing neurons active during movements of different digits show considerable overlap [2]. Functional magnetic resonance imaging (fMRI) studies in normal subjects confirm the existence of spatial overlap in the motor cortex for movements that involve adjacent corporal segments, such as fingers, wrist, and elbow [3, 4]. Thus, although large body segments such as head, limb and trunk occupy distinct territories, there seems to be a mosaic-like representation of muscular groups [5]. In contrast to the classical somatotopically organised model, these data suggest that movement execution depends on a distributed network in the sensorimotor cortex, constituting an efficient way of coding multisegment motor synergies [6]. These representational maps could undergo considerable plastic reorganisation in response to behavioural use, for instance, or amputation of the peripheral sensory and motor apparatus.

Reorganization of the sensorimotor cortex has previously been shown in animals and in humans after peripheral injuries, such as deafferentation, peripheral lesions, or amputation [7–12]. In the case of amputation, the absence of a limb may lead to strong phantom sensations accompanied in most cases by pain. The phenomenon of phantom limb, defined as the persistence of sensorimotor perceptions associated with the missing body part, has been interpreted as reflecting reorganisation in the sensorimotor cortex [13–16]. In parallel, an extension of the primary sensory representation of the face [17], elbow [18] and trunk [19] towards the hand has been found in S1. Some studies have shown that representation of unaffected muscles expands such that representation of the stump invades

portions of M1 previously dedicated to the amputated segment [20–24]. Thus, after amputation, the cortical territory that has been deprived of its afferent sensory input, like its motor effectors, reorganises to represent remaining nearby body parts [25–27].

The human brain is able to reorganise and adapt to all new situations. But little is known about the reversibility of such plastic reorganisation months or years after amputation when neuronal degeneration and regrowth of peripheral axons to innervate aberrant targets have all had time to occur [28–32]. Thus, human subjects whose amputated body parts are replaced by transplantation have provided a new and unique opportunity to study that reversibility of neural plasticity after such long-term changes.

Previous studies in unilateral transplant recipients indicated that reinnervations often remain incomplete, even after many years [33]. From animal studies, it has been established that regrowth of peripheral sensory nerves following a peripheral nerve cut is a very gradual and often quite imprecise process [34, 35]. Human subjects whose amputated body parts are replaced by transplantation provide a unique opportunity to examine the reversal of long-standing, amputation-induced reorganisation in the motor cortex. In a recent study [36], we investigated directly the nature and time course of cortical rearrangement of body motor representation produced by hand allograft. We tested patient CD, who received in January 2000 a bilateral hand transplant in Lyon, France [37]. We performed six identical fMRI examinations, the first 6 months before the graft and then postoperatively 2, 4, 6, 12 and 18 months afterwards. The task required the subject to perform flexion/extension of the last four digits of the left or right hand and flexion/extension of the left or right elbow. Before surgery, we monitored flexion and extension of the missing fingers by palpating the corresponding extrinsic muscle contractions at the forearm level. In the presurgery exam, movements of both right and left hand activated the most lateral part of the hand area in M1. This activated region is close to the face representation. Six months after the graft, the hand representation expanded medially and reoccupied the normal hand region (Fig. 1).

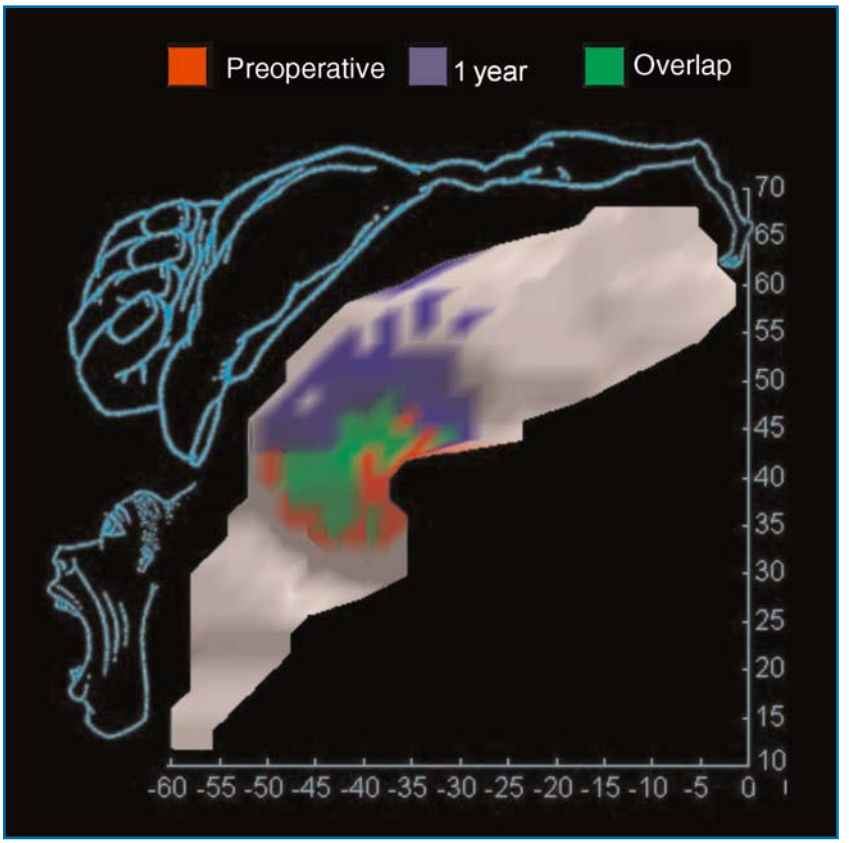

Fig. 1. Activation maps in the primary motor cortex (M1) obtained for right-hand movement condition. The surface of both the right and the left central sulcus was manually extracted from the subject using high-resolution T1-weighted magnetic resonance imaging (MRI). Boundaries of M1 areas were defined within a space of 6 mm in front of the central sulcus. Activated voxels within this defined space were considered as M1 activations and subsequently projected onto the three-dimensional surface on the nearest point. The schematic location of the hand area on Penfield's motor homunculus matches the "hand knob" region, as described previously, whereas the other body parts were scaled proportionally to the length of the precentral sulcus

Direct statistical comparison between the first (preoperative) and 6-month examination indicated that lateral M1 sites that were active for hand movements prior to the graft were less active following the graft and that a medial site that was not active before became active after [36]. Interestingly, this medial M1 site corresponds to the anatomical "hand knob" within the central sulcus, which marks the functional sensorimotor hand representation in normal subjects performing a similar task [38]. Analysis of centre of gravity (COG) coordinates for hand activations showed a spatial displacement between the pre- and postoperative phases. Before graft, COGs were close to the face area but shifted towards the classical hand area after the graft. More important, hand movement COGs recorded at 18 months postsurgery were similar to those recorded 6 months earlier. This demonstrated that spatial displacement of hand activations was not accomplished randomly. Thus, it seems that once hand neurons have recognised their target (i.e. the hand area), between major representations (i.e., face and hand) decrease. This cortical stability is probably achieved thanks to major inputs (sensory but also visual) and outputs (potential movements) necessary to reactivate the hand representation (Fig. 2).

Elbow movements produced a pattern of motor activations that evolved over time in parallel with hand motor representation. Before surgery, movements of either elbow triggered extensive activation in a contralateral central region of M1, corresponding to the normal location of hand motor representation. Left elbow movements, in addition, activated a more medial area. At 6 months postsurgery, elbow activations had migrated towards an area situated in the upper part of the limb representation and classically defined as the arm region [1]. Statistical comparison between the first (presurgery) and 6-month exam demonstrated that different M1 cortical maps were associated with the pre- and postoperative period, namely, a more lateral region before the graft and a more superior medial region 6 months after the graft. Thus, changes observed in motor cortex hand and elbow representations were strongly correlated. Interestingly, in the presurgery period, the COG coordinates for elbow activations were similar to

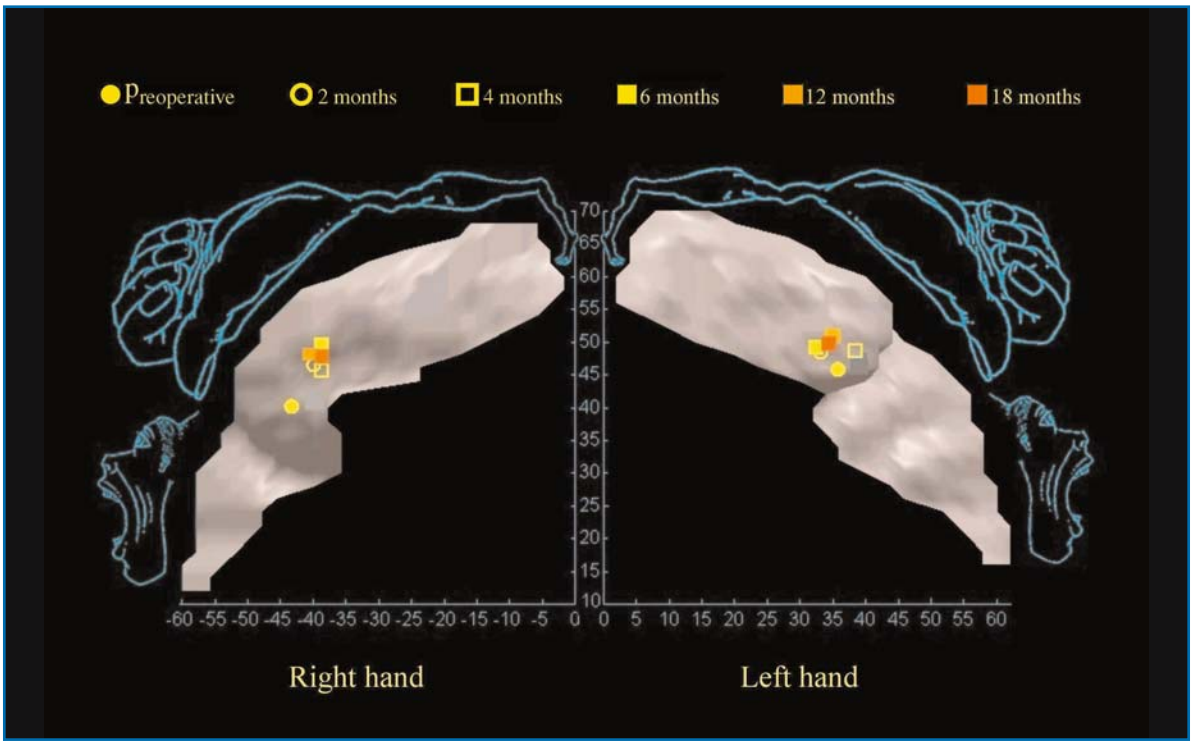

Fig. 2. Temporal displacement of the centres of gravity (COGs) for primary motor cortex (M1) hand activation from 2 to 18 months postgraft. Reconstructed coronal view of both right and left precentral sulci. Activations were obtained in the examinations before surgery (*yellow round* and *green square*) and 2, 4, 6, 12 and 18 months afterwards (*yellow*, *orange* and *red squares*)

those for the COG of hand movement at 6 months, suggesting that during that amputation period, the elbow representation had occupied the hand region. It should also be noted that hand and elbow activations showed a high degree of overlap. The extent of this overlap increased longitudinally from preoperative through postoperative exams (Fig. 3).

These results show that a bilateral hand allograft has a direct effect on hand and elbow representations in the sensorimotor cortex. The main finding is that the displacement of cortical activity from lateral to medial along the precentral gyrus is remarkably similar for both hand and elbow movements. These changes in these cortical maps covered similar distances in the same amount of time, as revealed by the temporal trajectories of COG coordinates. This suggests that hand transplantation resulted in global remodelling of the limb cortical map, reversing functional reorganisation induced by the amputation. The spatial trajectory of these activations in time further indicates that cortical rearrangement takes place in an orderly manner: hand and arm representations tend to return to their original cortical locus. Therefore, brain plasticity seems to be accomplished with reference to a preamputation body representation.

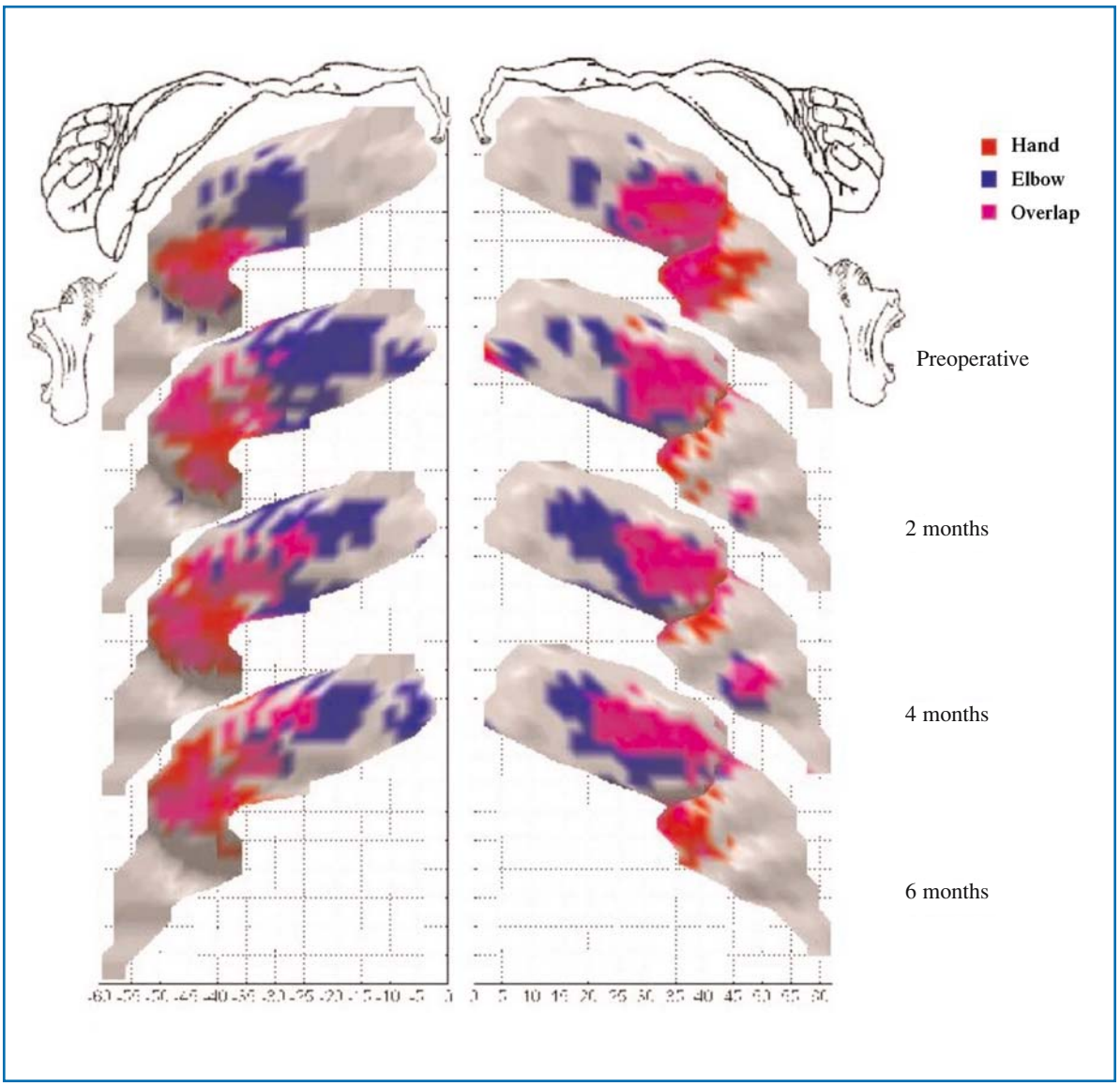

Fig. 3. Spatial overlap between hand and elbow activations. Increasing overlap between hand and elbow activations from presurgery to 6 months after the graft

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Section 9-i

The Sensor Glove in Preoperative Conditioning and Postoperative Rehabilitation

Göran Lundborg, Birgitta Rosén

Introduction

Hand transplantation represents a unique situation from the biological, clinical, psychological and cognitive point of view. The transplanted hand has to be accepted by the recipient, and the recipient's nerve fibres have to reinnervate nervous pathways, muscles and sensory receptor organs of the donor's hand. Various factors influencing the nerve regeneration process in such a situation has been discussed elsewhere [1]. However, the sensory motor functions of the transplanted hand are dependent not only on peripheral events in the transplanted body part, but establishment of central projections of the transplanted hand in the motor as well as somatosensory cortex is essential for the functional outcome. The original amputation injury has – in itself – induced extensive cortical reorganisations in the amputee's brain with disappearance of the hand representation, and functional recovery in the transplanted hand requires reestablishment of hand projectional areas in the motor and somatosensory cortex.

The purpose of this chapter is to provide an overview of what happens in the brain when a hand is traumatically amputated and when hand transplantation is performed. It also focuses on new principles to facilitate cortical integration of the new hand and a potential possibility for preoperative conditioning of the brain to facilitate this integration process.

Cortical Body Map

Afferent signals from all body parts reach the somatosensory brain cortex after passing the dorsal root ganglia, up the dorsal column of the spinal cord via the medial lemniscus pathway and intermediate relay stations situated in the cuneate nucleus in the brain stem and the ventroposterior nucleus in the thalamus. In this way, the various parts of the body are projected in the brain cortex in a specific order at cortical as well as subcortical levels [2–5]. In brain cortex, the hand and face are localised laterally and inferiorly. The somatosensory cortex, receiving sensory inputs, is localised posterior to the central sulcus while the motor cortex, showing an analogous architecture, is situated anterior to the central sulcus. Originally, the cortical body map was delineated by Penfield and Boldrey during open brain surgery on awake patients [6], but over the years, the cortical body map in humans has been analysed in detail by the use of modern brain imaging techniques. Signals elicited by touch primarily reach the contralateral hemisphere but to a lesser extent also the ipsilateral somatosensory cortex [7–10]. The hand and the face have very large projectional areas in the somatosensory and motor cortex, reflecting the unique sensory and motor functions of these body parts.

Effects of Amputation

It was long believed that the cortical body map was hard wired from birth and could not be altered, but it is now known that the functional organisation of these cortical projections can be rapidly changed as a result of changes in peripheral activity and sensory input [11–13]. There is constant, ongoing competition between the various body parts with regard to their representation in the brain cortex. Amputation of a body part represents a sudden arrest in sensory input resulting in rapid as well as long-term changes in cortical organisation. For instance, amputation of a finger induces a "silent area" in the corresponding cortical projectional area and a rapid expansion of adjacent cortical territories over the former finger projection [4, 14, 15]. Experimental studies on finger and limb amputations in primates have demonstrated that cortical changes in somatosensory cortex quickly become established and permanent [13, 14, 16, 17]. In chronic amputation, cortical reorganisations may occur over a distance of up to 14 mm in primates [17, 18], and analogous reorganisational changes may also occur at subcortical levels [13, 19]. More than 10 years after forearm amputation in primates, electrical stimulation of those part in the motor cortex that were formally devoted to the missing hand may evoke movements of the stump and the adjacent shoulder, indicating a substantial sustained reorganisation of the motor cortex.

In humans, cortical reorganisational changes after amputation of a hand or an arm have been studied by use of neuromagnetic and neuroelectric source imaging, as well as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Following amputation of a hand, nearby areas of the primary somatosensory cortex expand and become functionally reactivated by inputs from the face or arm stump [12]. There is a rapid displacement of the face representational area towards the hand representation, which may give rise to a strange clinical phenomenon as early as 24 h after an arm amputation: the missing hand can be mapped in the face so that touch of specific areas of the face can give rise to tactile sensations in individual fingers of the missing hand [20–24]. The functional reorganisations that occur after hand and arm amputation may give rise to troublesome phantom sensations, which may vary between patients. It seems that the extent of phantom pain is in proportion to the extent of cortical reorganisation and that phantom-limb pain occurs to an extent and correlates well with the extent of shift in cortical representation associated with the original amputation [22, 24–29].

Hand Transplantation: Cortical Effects

It is well known from clinical experience that replantation of an amputated hand may result in fairly good functional results [30, 31], and fMRI studies have shown that such a replanted hand may regain its cortical representation in the motor as well as the somatosensory cortex [31, 32]. Also, following transplantation of a homologous hand to an amputee, there is a continuous expansion of the corresponding projectional hand areas in the brain cortex, which occurs parallel to increased use of the transplanted hand [31, 33]. The motor representational area of the hand may be regained within months [31, 33, 34] although activation of the somatosensory cortex may require longer.

Can the Cortical Hand Projection Be Maintained After Hand Amputation?

An attractive concept is to maintain the cortical hand projection after an amputation injury in order to reduce the extensive cortical reorganisation. From a theoretical point of view, maintenance of the hand projection might be expected to facilitate functional recovery of a transplanted hand after surgery. "Preconditioning" of the brain *before* surgery, aimed at preoperative establishment of a cortical hand map, as well as training very early in the postoperative phase, may help facilitate the process. From experiments on normal primates and healthy control

persons, it is known that there are several ways to activate the hand representational areas without actively using the hand. For instance, the premotor cortex may be activated by *mere observation* of motor activities performed by others, a phenomenon based on the occurrence of so-called mirror neurons in the premotor cortex, which are activated by active motor performance as well as by *observation* of active motor performance [35, 36]. Neurons in the premotor cortex may be activated also by reading or listening to action words associated with movements of the hand [37]. In analogy, the somatosensory cortex may be activated by the observation of lower limbs [38] or hands [39] being touched. This latter principle is based on the multimodal capacity of the brain, making possible visuotactile interaction based on activation of neurons responding to visual as well as tactile stimuli [40, 41].

Another attractive principle is to use the brain's multimodal capacity for *audiotactile interaction* based on activation of neurons responding to tactile as well as auditory stimuli. For this purpose, we have utilised the Sensor Glove System (SGS).

The Sensor Glove System

The SGS is based on a principle in which an alternate sensory inflow is constituted by use of sense substitution using hearing as a substitute for sensibility [42]. With this principle, miniature microphones are mounted in a glove at fingertip level (Fig. 1). Signals from the microphones are processed in a miniature stereo processor at wrist level, and weighted signals are transmitted to earphones, thereby making possible a "three-

Fig. 1. The Sensor Glove equipped with stereo processor and ear phones. Miniature microphones are incorporated in the glove at fingertip levels

dimensional" (3D) perception of the friction sound, which is generated by the touch of various textures and structures. After a short period of training, a patient with a denervated hand using the SGS can easily discriminate between various textures and structures by listening to the elicited friction sounds. fMRI studies indicate that the tactile information, expressed in auditory signals, activate not only the auditory cortex but also the somatosensory cortex [43]. The purpose in this situation is to feed the somatosensory cortex with an alternate sensory inflow, and in this way maintain the cortical hand map – a "sensory bypass". Results from a pilot case [44] as well as a prospective randomised study on nerve-injured patients show an enhanced recovery of tactile gnosis after 6–12 months compared with control subjects [45].

Use of the Sensor Glove System in Hand Transplantation

We applied the SGS in one case after hand transplantation, showing encouraging result with regard to functional restitution in the transplanted hand as well as cortical integration of the transplanted hand. In a joint project with Lanzetta's group, the system was tested comparing the outcome with another hand transplantation case not using the system [34]. Both patients were selected in accordance with strict inclusion criteria adopted by the Italian hand transplantation programme. Both cases were subjected to traumatic amputation at the wrist level involving the dominant hand. The patients had previously been using various types of available prosthetic alternatives. fMRI investigation was performed 3 and 9 months postoperatively in both cases.

Case One

This patient was subjected to amputation injury at the age of 13 during a farming accident. At the age of 35, he was treated with a hand transplantation. The surgical procedure and postoperative immunosuppression regimen and rehabilitation followed the detailed protocol devised by the Italian hand transplantation group [34, 46, 47].

Case Two

This patient was a 32-year-old man subjected to a explosion injury resulting in amputation of his right dominant hand 4 years previous to hand transplantation. The postoperative immunosuppression regimen was identical to that used in case one [34, 46, 47]. During the intensive rehabilitation period following hand transplantation, the Sensor Glove was used in addition to the standard Italian programme following hand transplantation.

Starting during the early postoperative period, patient two used the Sensor Glove according to a well-defined protocol (Fig. 2). According to this protocol, the patient used the Sensor Glove for at least 2 hours twice a day with emphasis on learning to connect specific sounds with spatial localisation of fingers and texture identification. This alternative sensory relearning was performed according to traditional sensory re-educational principles [48, 49]. The patient was also encouraged to use the glove during daily activities. In both patients, fMRI investigations were performed preoperatively, and periodically from 3 to 9 months postoperatively. Specific assessment of sensory recovery was based on Semmes-Weinstein monofilament test [50], static two-point discrimination test (2PD) [51] and the shape texture identification (STI) test [52].

Functional Outcome

Functional assessment at 6 months showed in both patients return of some sensibility in the median innervated area while there was no sensory recovery in the ulnar nerve innervated territory in the case not using the SGS (Case 1). After 1 year, the patient using the SGS (case 2) presented with normal or only limited reduction of perception of touch in median and ulnar innervated areas, respectively (filament 2.83 and 3.61) while case 1 presented with some protective sensibility in median and ulnar nerve innervated areas (filament 4.31). In both cases, tactile gnosis measured with 2PD was still not measurable (>15 mm), but in case 2, there was measurable tactile gnosis in the STI test (score 1 of maximum 6) [34, 52].

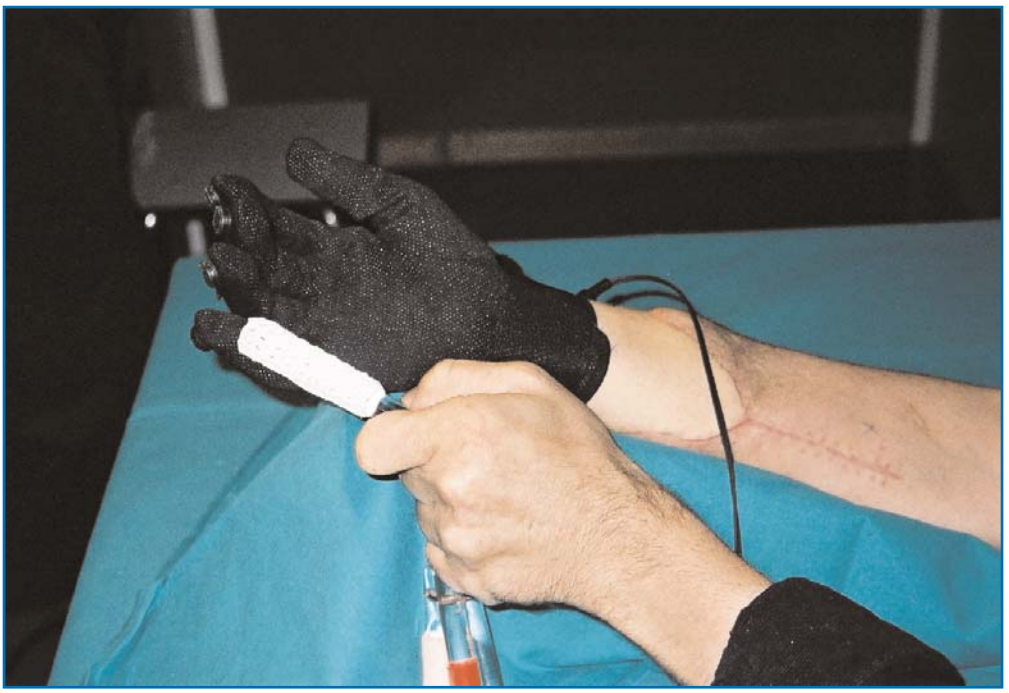

Fig. 2. Use of the Sensor Glove in the rehabilitation period following hand transplantation. Training to identify individual fingers

Functional Magnetic Resonance Imaging

The fMRI investigations were performed using a 1.5T General Electric Signa Horizon system (GE, Milwaukee, WI, USA) using a standard quadrate head coil [34, 35, 53]. fMRI investigation of case 2 showed a more rapid cortical integration of the transplanted hand compared with case 1. There was a reduction in the activation extent of the sensory motor map over the course of the follow-up in case 2, reflecting less recruitment than neural substrate and a reorganisation towards a typical cortical hand representation compared with case 1 at 5 months after transplantation (Fig. 3).

Our data indicate that the patient using the SGS had a more rapid cortical integration of and recovery of sensory functions in the transplanted hand compared with the patient not using the SGS. However, one must consider also other differences in the basic condition regarding these two patients. In case 2, the time laps between traumatic amputation and hand transplantation was only 4 years while the corresponding time laps in case 1 was 22 years. Thus, besides differences in use of the SGS, there may be have been differences in manifested cortical reorganisations over time, which may help to explain the differences in outcome.

Preconditioning of the Recipient's Brain Before Transplantation

Can the recipient's brain cortex be preconditioned to facilitate acceptance of a new hand and functional restitution? This is so far a hypothetical question although based on scientific evidence. It has been demonstrated that, in normals, acoustic signals elicited by use of the SGS may activate the somatosensory cortex [43], but we do not know if or to what extent this phenomenon occurs in amputees. Brain imaging studies are required to support or disprove this hypothesis. However, we strongly believe that the sensory by-pass principle will be applicable also to amputees so that acoustic signals under the right conditions may well activate the somatosensory cortex. If the amputee waiting for transplantation is using a prosthesis, the clinical setup would be to provide the prosthesis with a Sensor Glove. Alternatively, a cosmetic prosthesis provided with the same equipment and posi-

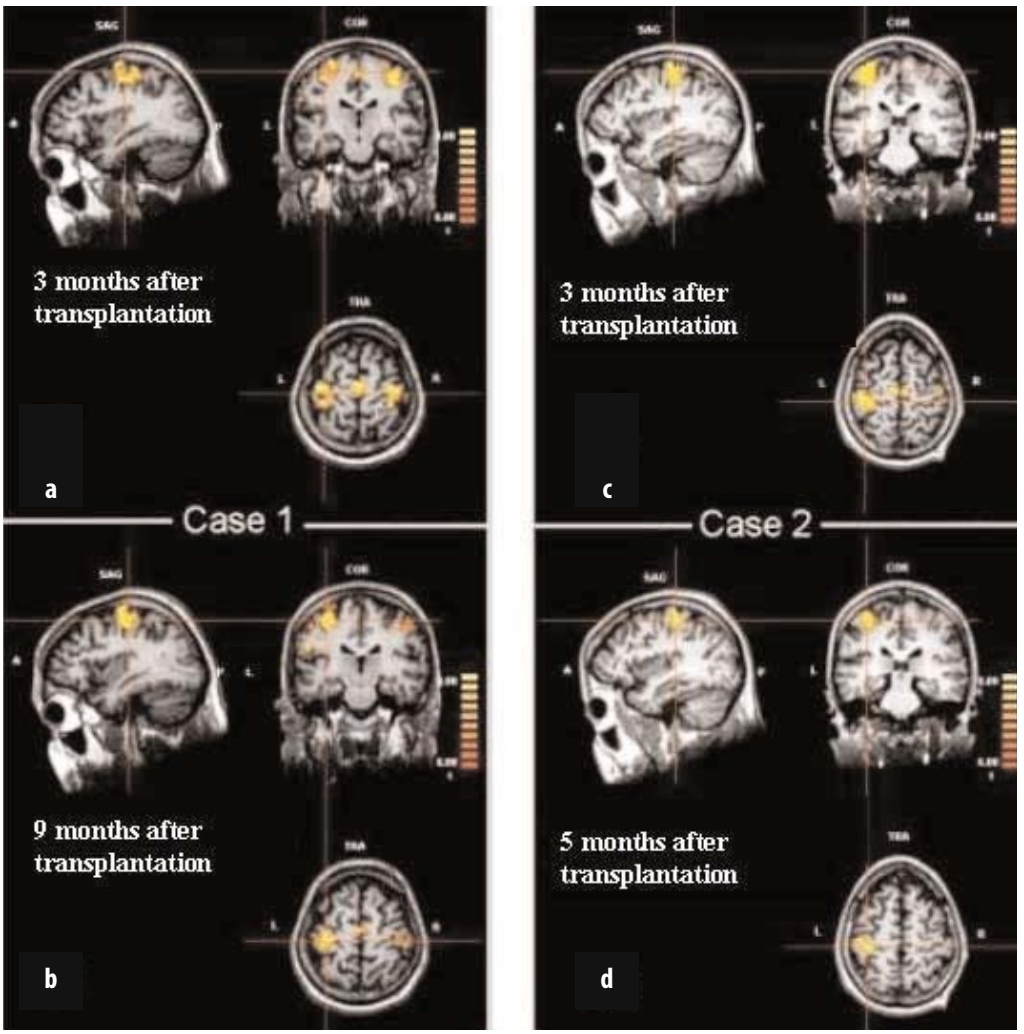

Fig. 3a-d. Functional magnetic resonance imaging (fMRI) showing cortical activation induced by movements in the right transplanted hand. Case 1 (not using the Sensor Glove). **a** Three months and **b** Nine months after transplantation. *Case 2 (using Sensor Glove)*. **c** Three months and **d** Five months after transplantation. There is a reduction of extent in sensory motor map activation as well as in the connected areas in case 2. From [34], used with permission

tioned in a realistic way can be used during training sessions. Our hypothesis is that such a principle, when applied during a concentrated training period preoperatively, will re-establish the projectional area of the hand in the somatosensory cortex, thereby facilitating a rapid functional restitution when the hand transplantation is performed.

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Section 9-j

A Comprehensive Functional Score System in Hand Transplantation

Marco Lanzetta, Palmina Petruzzo

Introduction

The first hand transplantation was performed in Lyon, France, on 23 September 1998 by an international team of surgeons. Since then, hand transplantation programmes have been launched in the United States, China, Italy, Austria and Belgium. Since 1998, 11 patients have received a single hand transplant, four a double hand transplant and two a double forearm transplant.

The first 7 years of clinical experience confirm beyond any doubt that hand transplantation is technically feasible. Nerve regeneration occurs in grafted hands, indeed even many years after the amputation. Sensibility and movement recovery have exceeded expectation in many cases. In particular, return of sensibility has been documented in all transplanted hands. The grade of sensory recovery paralleled or even exceeded results that are usually expected in autologous replantation after trauma [1]. In particular, protective sensation was achieved in all patients within 6–12 months and, as time progressed, 88% of them showed the onset of more subtle discriminative sensation. Recovery of motor function of both extrinsic and intrinsic muscles enabled patients to perform most daily activities, improving their quality of life in 83% of cases. When performed, functional magnetic resonance imaging (fMRI) demonstrated [2] that after transplantation, hand representation regained the cortical site that corresponds to the hand knob area in normal subjects. The beneficial psychological effects on the patients have been well documented and are one of the most important results of these procedures. Patients reported a clear improvement in their social and affective life.

In 2002, a worldwide registry [3] was created to provide a basis for cooperation by all teams performing hand transplantations: International Registry on Hand and Composite Tissue Transplantation (IRHCTT); www.handregistry.com. When gathering data for the presentation of a comprehensive report by the registry at the 2002 Hand and Composite Tissue Allograft meeting in Italy, it was evident that we needed to adopt a common functional score system, as none of the existing ones could be adapted due to the fact that they were used mainly for evaluating results of hand/limb replantation [4–6] or disability due to single or multiple disorders of the upper limb [7–9]. The unique nature of hand transplantation requires evaluation of a general and complex outcome. It must include specific parameters, such as cosmetic appearance, color, size and shape matching with the contralateral hand (in case of a single hand); psychological and social effects of the procedure; and the functional result as a whole. The main purpose of this score is to allow evaluation of cosmetic and functional results as well as to take into account "what really happened to the patient" following hand transplantation, assessing his or her psychological outcome, social behavior, work status, satisfaction, body image and well being (Table 1). The importance of body image must specifically be taken into consideration; usually,

the patient has been carrying and therefore exposing his or her own disability for some time, causing a severe adjustment to personality and ability to enter affective relationships. The score is based on the concept that the word "hand function" must be expanded to embrace aesthetic, psychological and socioeconomic factors.

The Hand Transplantation Score System (HTSS) as Adopted by the International Registry of Hand and Composite Tissue Transplantation

The score system is based on a value of 100 points, which involve six items with different weight: appearance (15), sensibility (20), movement (20), psychological and social acceptance (15), daily activities and work status (15), patient satisfaction and general well-being (15). A total result of 81–100 points is graded as an excellent outcome, 61–80 as good, 31–60 as fair and 0–30 as poor. The new scoring system is easy to use, and correlation with the Disabilities of the Arm, Shoulder and Hand (DASH) score [10], which was designed to measure upper-limb disability and symptoms, is excellent. This scoring system has good test–retest reliability and responsiveness. Furthermore, it allows measurement of the "ability and the performances" of the grafted patients instead of measuring the disabilities of proximal or distal parts of upper extremities (Table 1). In Table 2, the score of all European patients is reported. It is important to note that the recipients present a different follow-up, ranging from 2 to 6 years.

Table 1. Hand transplantation score system

APPEARANCE (max 15 points)	
SKIN COLOR AND VASCULARIZATION	
Normal	3 points
Abnormal	0 points
SKIN TEXTURE	
Normal	3 points
Abnormal	0 points
HAIR GROWTH	
Normal	3 points
Diminished	1.5 points
Abnormal	0 points
NAIL GROWTH	
Normal	3 points
Diminished	1.5 points
Abnormal	0 points
MATCHING WITH CONTRALATERAL HAND (monolateral Tx – size, color, texture)	
Excellent	3 points
Good	2 points
Fair	0.5 points
Poor	0 points
MATCHING WITH UPPER LIMB/BODY (bilateral Tx)	
Excellent	3 points
Good	2 points
Fair	0.5 point
Poor	0 points
TOTAL	points

SENSIBILITY (max 20 points)		
TACTILE SENSATION (Semmes-Weinstein monofilament testing)		
Median nerve	Green (1.65 – 2.83)	3 points
	Blue (3.22 – 3.61)	3 points
	Purple (3.84 – 4.31)	2 points
	Red (4.56)	1 point
	Red (6.65)	0 points
Ulnar nerve	Green (1.65 – 2.83)	3 points
	Blue (3.22 – 3.61)	3 points
	Purple (3.84 – 4.31)	2 points
	Red (4.56)	1 point
	Red (6.65)	0 points
PROTECTIVE SENSATION (hot-cold-pain)		
Yes (median – ulnar)		5 points
Yes (median)		2 points
Yes (ulnar)		1 point
No		0 points
Radial nerve		1 point
DISCRIMINATIVE SENSATION*		
Median nerve	S2PD – grade S4 (2–6 mm)	3 points
	S2PD – grade S3+ (7–12 mm)	2.5 points
	S2PD – grade S3 (>15 mm)	1.5 points
	S2PD – grade S2 (none)	0 points
Ulnar nerve	S2PD – grade S4 (2-6 mm)	3 points
	S2PD – grade S3+ (7-12 mm)	2.5 points
	S2PD – grade S3 (> 15 mm)	1.5 points
	S2PD – grade S2 (none)	0 points
* (Highet scale as modified by Dellon et al)		
SWEATING:		
Normal		2 point
Abnormal		0 points
TOTAL		points

MOVEMENT (max 20 points)		
ACTIVE RANGE OF MOTION		
Forearm (combined pronosupination):	>150°	2 points
	>120°	1 point
	>90°	0.5 points
Wrist (combined flexion/extension):	>90°	2 points
	>45°	1 points
	>25°	0.5 points
Thumb and long fingers [total digital range of motion (ROM) of contralateral or normal hand – %]:	>50%	2 points
	>25%	1 point
	>10%	0.5 points
STRENGTH (Jamar dynamometer)		
Grip:	>10 kg	2 points
	>5 kg	1 point
	>2.5 kg	0.5 points
Pinch:	>2 kg	2 points
	>1 kg	1 point
	>0.5 kg	0.5 points
INTRINSIC MUSCLES ACTIVITY		
Clinically useful		6 points
EMG detectable		3 points
None		0 points
CORTICAL REINTEGRATION OF THE HAND*		
Yes		4 points
No		0 points
TOTAL		points

* Based on a positive Functional Magnetic Resonance Imaging

PSYCHOLOGICAL AND SOCIAL ACCEPTANCE (max 15 points)*

SOCIAL BEHAVIOR (max 7 points – 1 point each aspect)
Holding/shacking hands
Feeling well in a group
Overcoming sense of embarrassment
Sense of being accepted
Ability to create new relationships
Being able to overcome handicap
Satisfactory global social acceptance

AFFECTIVENESS (max 5 points – 1 point each aspect)
Caressing
Hugging
Touching
Sense of intimacy with partner
Satisfactory global affectiveness

BODY IMAGE (max 3 points – 1 point each aspect)
Sensation of having a complete body
Self confidence in personal appearance
Use of jewellery, watch etc. on hand/s

TOTAL points

* Based on subjective improvement compared to preoperative status

DAILY ACTIVITIES AND WORK STATUS (max 15 points)

ACTIVITIES OF DAILY LIFE (max 9 points – 1 for each activity)
Driving/riding a bicycle
Combing hair/personal hygiene/shaving
Grasping glass
Pouring water from bottle
Using cutlery/chopsticks
Brush teeth
Holding hands
Writing
Symmetrical use of hands

WORK STATUS
Employed 6 points
Unemployed 0 points

TOTAL points

PATIENT SATISFACTION AND GENERAL WELL BEING (max 15 points)	
PATIENT SATISFACTION	
Very satisfied	5 points
Satisfied	3 points
Unsatisfied	0 points
WELL BEING	
Physically and mentally healthy	5 points
On pharmacological treatment for side-effects	0 points
Permanent side-effects/pathologies from drugs	-5 points
QUALITY OF LIFE	
Improved a lot	5 points
Improved	3 points
Same	0 points
Worsened	-3 points
Worsened a lot	-5 points
TOTAL	**points**

Appearance = 15 points; Sensibility = 20 points; Movement = 20 points; Psychological and social acceptance = 15 points; Daily activities and work status = 15 points; Patient satisfaction and general well-being = 15 points; Total = 100 points; 0–30 points = poor; 31–60 points = fair; 61–80 points = good; 81–100 points = excellent

Table 2. Outcome of all European patients (Total 8)

Patient	1		2		3		4		5	6	7	8
Side	Bil - R	Bil - L	Bil - R	Bil - L	Bil - R	Bil – L	Bil - R	Bil - L	R	R	R	R
Follow-up	6 yrs		4 yrs		6 yrs		3 yrs		4 yrs	3 yrs	2 yrs	2 yrs
Appearance	14	14	12.5	12.5	15	15	11	11	15	14	12.5	12.5
Sensibility	18	16	18	18	14	15	6	6	14	17	14	18
Movement	12.5	14	14.5	15	18	14.5	2	1.5	10	14	11.5	14.5
Psychological & social acceptance	14	14	7	7	14	14	9	9	15	13	13	7
Daily activities & work status	14	13	6	6	15	15	3	2	13	13	12	6
Satisfaction & general well being	11	11	11	11	15	15	2	2	11	11	6	11
Total	83.5	82	69	69.5	91	88.5	33	31.5	78	82	69	69
Grade	Excellent	Excellent	Good	Good	Excellent	Excellent	Fair	Fair	Good	Excellent	Good	Good

Bil, bilateral; *R*, right; *L*, left

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Section 9-k

Quality of Life in Hand Transplant Patients

Danièle Bachmann

Introduction

The concept of quality of life is above all a subjective notion, even if it can be made objective using general or dependence scales such as those used in physical therapy. It is also possible to evaluate quality of life before hand transplantation using psychological interviews in order to identify and discuss important issues with the future recipient. The issues involved in hand transplantation are nonetheless quite different from those concerning internal organs: the hands are a part of the body that is always visible to the transplant patient; the hand (sor hands) of a cadaver at the end of the recipient's arm is permanent evidence of the presence of another person, of a "stranger"; the recipient only recovers use of the hand(s) after several months, according to the progress he made during reeducation and the regeneration of the nerves; and finally, the hands are important on both a narcissistic and a relational level. Moreover, hand transplantation is not really essential for the survival of the patient though, as we shall see, it might seem to be in the minds of certain patients.

The first hand transplant, involving only one hand, took place in 1998. The experiment ended in failure, after about 2 years following the operation because of the recipient's intolerance of certain risks linked to taking anti-rejection drugs for life, namely, increased susceptibility to infection and cancer. This first transplant recipient thus found a surgeon willing to amputate the transplanted hand, which in fact had been presenting signs of rejection for several months on account of the patient's refusal to take the immunosuppressive drugs.

At the conclusion of this first transplant, the French National Ethics Committee, after consulting several members of the operating team, decided in favour of hand transplantation but only in the case of bilateral transplants. In the case of a unilateral transplant, the improvement in quality of life did not seem sufficient when weighed against the drawbacks of immunosuppressive drugs and the amount of reeducation required in order to recover motor functions and sensitivity. It was in this context that the first bilateral hand transplant took place on January 12, 2000.

In order to better evaluate posttransplant quality of life, we conducted a number of open-ended or semi-directed interviews both before and after the operation. We have grouped together the topics explored in these those interviews under four major headings: motivation; the mourning of the lost hands; the patient's personality; the patient's and family circle.

Motivation

What motivates the patient to seek a hand transplant, instead of making do with a prosthesis or the stumps? This question can be addressed in several ways.

Conscious Versus Unconscious Motivation

The patient's spontaneous discourse gives easy access to his conscious motivation. The following elements are typically mentioned: the desire to recover motor functions beyond the plierpliers function of the thumb and index finger; the wish to be able to modulate the force that is developed in the hands and fingers, which can be quite difficult with prostheses; the desire to perform ordinary daily activities without help (bathing, for example); recovering sensitivity for physical contact with family members; and driving a car, and working, etc. Yet despite all these good reasons advanced by the patient, we must not lose sight of certain ambiguities which that point to a darker component of the desire to undergo hand transplantation. Hence one candidate, for example, a well-known former mine clearer who had lost his hands while on assignment, complained that now he could only work as an instructor of younger mine clearers, and wished to have hands in order to come back to the service in a more concrete way! We can assume in this example that the destructive impulse is very much at work in the request the patient had formulated in a rather naïve way, but which that nonetheless attests to his attraction for situations of repeated risk.

Narcissistic and Functional Dimensions

The motivations mentioned in the preceding section refer to the functional dimension. Despite the improvement in prostheses, they cannot for the time being offer the same benefits as those of transplanted hands brought back to life by nerve regeneration. The possibilities offered by prostheses seem inferior in matters of motility, particularly in precise movements but also in the perception of muscular force, which is partly related to sensitivity: patients with both hands amputated of both hands regularly break glasses when getting themselves something to drink; they risk hurting their children at moments of everyday physical contact; they cannot perform certain movements related to bathing, particularly with regard to parts of the body which are hidden from sight. All these difficulties are part of the handicap experienced on a daily basis by these patients. Motivation may, however, be based on more narcissistic factors: the unbearable aspect of being seen by someone else, and of seeing one's own prostheses or stumps, which reactivate a feeling of incompleteness, or even of intense worthlessness. In this case, transplanted hands are wished for not to improve the quality of everyday life, but to restore a self-image damaged by the absence of hands. Here, the future transplant patient wants to become a complete person again.

The predominance of narcissistic over functional motivations, or even the near exclusivity of narcissistic motivations in the case of a moderate handicap (when the patient has the use of one hand, or has developed a great deal of skill with prostheses or the stumps), suggest that the improvement in quality of life brought about by the transplant will be minimal, and that the risks and drawbacks of taking immunosuppressive drugs are likely to take center centre stage after the operation.

Mourning Lost Hands

This is an important dimension to take into consideration, with the knowledge that the process of mourning lost hands, that is to say, the acceptance of having lost them, can never be complete or total, particularly because of the significant limitations encountered in daily life. If the mourning process had been perfectly completed, there would be no reason to ask for a transplant. One patient who lost his hands in 1996, and thus well before the first actual transplant operation, was in such denial about the loss that he was sure that one day medical science would allow him to have hands again, and that he would not spend the rest of his life with prostheses. Though future events proved him right, his unshakable conviction, which could have appeared to be the sign of madness in 1996, attested to the unbearable aspect of a life without hands, and to an insurmountable kind of grief. In this respect, and for this particular patient, the hand transplant did not merely signify the recovery of a manual function, but was a life-or-death matter at a psychological level, even though hands are not as vital for example as, for example, the heart or the liver.

Having hands again is one thing; imagining that this will resolve all of life's problems is quite another. It thus becomes important to understand as fully as possible what the patient expects from having functioning hands again. If, in fact, expectations are too far from what is possible in reality (never having relational or professional problems again, after the transplant, when, in fact, the individual has always had these kinds of problems), the candidate runs the risk of a posttransplant disappointment which that no surgical procedure can prevent. We should, nonetheless, mention that currently, transplant patients, because of their small number, have obtained a narcissistic benefit related to the exceptional nature of their status, which has allowed them to change certain things in their lives which are not directly related to functional recovery. Yet, the transplantation of hands changes the patient's body in a radical way; he does not get his own hands back (we say "he" because all transplanted patients thus far have been men), nor does he return to a previous state. The recipient has to make the donor's hands his own, and, even with the recovery of motor functions and sensitivity, these hands are forever present before the patient's eyes, and they retain morphological characteristics which that are not necessarily similar to his own (skin color, a potentially different pilosity, finger shape). We have been able to observed the re-emergence at difficult points in the patient's life, of issues related to the donor's hands, which the patient was unable to process completely, and which reactivate a feeling of strangeness or of the incomplete integration of the transplants. This can take the form of anxiety, or dissatisfaction about a morphological detail; most of the time, these feelings are not evoked in the interviews.

The Patient's Personality

In a more general way, the patient's personality impacts his posttransplant quality of life. Certain factors are favourable;, others are rather unfavourable. Thus, as in the case of organ transplants, compliance with the drug regimen is a predictor of the persistence of a satisfactory quality of life. This compliance is in part related to a kind of mental flexibility, which allows the patient to better accept the negative aspects of experiencing a high level of dependence during the first months. A rigid personality, on the other hand, is likely to have trouble tolerating the inevitable degree of uncertainty (the risk of rejecting the transplant rejection, for example) and the necessary period of regression during the first weeks following the transplant operation: not only are the transplanted hands not yet functional, but, moreover, all the skills acquired using the stumps or the prostheses have been lost. When it comes to eating, washing, or even scratching himself, the patient finds himself in a state of total dependence. It thus becomes quite important to talk with the preoperative patient about the period which that followed the loss of his hands, because the degree of dependence in the postoperative state is comparable. This, of course, revives the initial trauma, which the patient's psyche dealt with in a more or less satisfactory way.

The Patient's Family Circle

The hands are also highly charged with meaning in the human being's imagination: this came across more or less clearly in the discourse of the patients or of their families. What, for example, did the donor's hands do before his death, during moments of intimacy? The patient's ability to integrate the transplants is also dependent on the reaction of the close family circle, which could display feelings of rejection, of disgust or worry, or, on the other hand, could be quite happy for the patient and give him vital support in accepting the transplant. Postoperative quality of life thus also depends on the family circle's ability to accept the transplant.

Conclusion

In the end, as we have tried to show in this article, patients receiving hand transplants have a high level of satisfaction, once the critical period of dependence, with the initial absence of motor

functions and sensitivity, has passed. We must, nonetheless, keep in mind that these patients were particularly motivated for setting off on this kind of adventure. The deep feeling of satisfaction which that patients express several years after the transplant operation is not just that of having functioning hands again, but of having begun a new life.

10. Psychological Issues in Hand Transplantation

Section 10-a

Psychological Evaluation and Patient's Profile

Italo Carta, Ornella Convertino, Juliette Bagnasco, Serenella Fornara

Introduction

An examination of the psychological, psychiatric and, above all, anthropological literature underlines the importance of the hands in psychophysical development of human beings, representation of body image and structuring of identity [1–3]. From the anthropological point of view, and in an evolutionary phylogenetic perspective, the process of humanisation is strictly related to changes in the spatial position of the upper limbs, which, after having been released from the need to adhere to the ground, became free to explore the world. This fact gave the hands a symbolic value that has been enriched over the course of millennia and, in a certain sense, has paralleled the development of cultures and their pragmatic and artistic expressions. By means of exquisitely cultural processes of transformation, the impulsive and instinctive potentials rooted in human bodylines became expressive of affections and the various declinations of love, as well as of the broad paradigm of aggressiveness. In terms of the pragmatic expressive potential of humankind, the hands act as inseparable tools, but they are also material supports for the symbolic functions that define humans and distinguish them from all other species.

It is therefore not difficult to understand that the traumatic loss of one or both hands can evoke feelings of terror and lead to distortion of the body image and disturbances of the identity [4–6]. Within certain limits expressive of normal functioning of cortical activity, the phantom limb phenomenon can take on a psychopathological significance in subjects whose personality structure is predisposed to conferring on the experience of particular configurations that become inscribed in the general economy of an albeit partial posttraumatic change in identity [7].

Clinical Study

Given the symbolic importance and pragmatic value of the upper limb (particularly the hand), and on the basis of published data concerning normal and psychopathological phenomena associated with its possible amputation – phantom limb phenomenon, alterations in body image and schema, strengthening of denial mechanisms, hypercompensation of the disability [4, 8–10] – a protocol has been developed on the basis of validated psychological instruments with the aim of exploring personality traits of the transplant candidates.

Hand transplantation involves deep psychological, existential, ethical and social implications for the patient, the patient's family, the donor's family and the attending equipe. Therefore, it is necessary to study the psychic, psychopathological and personality aspects of patients as important factors to be considered in order to avoid underestimated aspects leading to psychic discomfort following transplantation

and compromising the effort in terms of pain, trouble and displaced resources. Surgical transplantation, on the one hand, represents the way to restore physiologic functioning. On the other hand, it is overburdened with symbolic meanings related to the subject's experience and requires cognitive and emotional integration with the anatomic component. Besides the surgical transplantation exists a "psychic transplantation", linked with the psychosomatic unit of the individual and the existence of an imaginary body as well as the real one. Psychic transplantation, however, is never as fast as the anatomic one; it is overburdened with meanings intimately linked with the subject's experience and requires cognitive and emotional integration of the organ alongside anatomic integration. Therefore, the transplantation experience presents itself, from the beginning, as an experience that deeply involves the patient's ego, recalling the necessity to find a meaning even through mobilisation of archaic defence mechanisms. Therefore, the ability to integrate the donated organ must be evaluated carefully with the transplantation proposal, following the patient through the heavy psychic burden it involves.

A transplant is never psychologically inert, but it involves at least partial identification of the recipient with the donor. When a person gets ill, the experience of pain is a fact of fundamental importance within the evolution and organisation of the body schema: it is an experience with strong regressive and narcissistic meanings in which attention is totally concentrated on bodily sensations and all the energy flows to the affected organ. The aim of the psychological-psychiatric evaluation consists of containement of anguish and attentive investigation of the psychological involvment related to the operation for patients and their families.

The waiting phase can be used as an extension of the patient's psychic evaluation phase, which consists of the verification "on field" of the capability to tolerate a complex and compelling experience, the transplantation, from both the psychological and affective points of view. The fact that the psychologist and psychiatrist present themselves to the patient as part of the team gives a human aspect to the treatment that the candidate receives before the transplantation and place the presuppositions for the beginning of a good relationship between the patient and the equipe. For these reasons, it was considered necessary to submit uni- or bilateral transplant candidates to a battery of tests aimed at defining their personality profile, measuring their intelligence quotient and establishing the efficiency with which they perform pragmatic tasks. Results of the tests are evaluated in light of an overall clinical assessment by means of clinical interviews conducted before and after performance of the tests.

On the basis of these considerations, patients who underwent transplantation were selected from a group of subjects whose suitability was evaluated by means of methods specially designed to achieve the preestablished objectives. These objectives are summarised as follows:

- Definition of the sociodemographic situation
- Verification of the integrity of psychic functions, with particular attention to the affective-emotive and cognitive structures
- Verification of the capacity for adaptability in relation to occupation and socioenvironmental settings within the family
- Evaluation of the capacity to confront stressors and the related threshold of frustration
- Definition of the nature of the reasons for transplantation
- Evaluation of the psychological attitude towards the dead donor
- Evaluation of the psychological attitude towards the prosthesis
- Evaluation of the neurofunctional organisation of the areas of cortical projection corresponding to the upper limb.

Once having selected the subjects suitable for transplantation and performed the transplant itself, the objective of the study moved to the development of psychological, clinical and psychotherapeutic procedures aimed at providing the transplanted subjects with adequate psychological care. These procedures have so far been applied to three subjects who have undergone transplantation. It was felt that these procedures were required and necessary to facilitate integration of the limb in the body schema [11] and

overcome the difficulties encountered during the course of a laborious process of physiotherapeutic rehabilitation. This procedure is easily justifiable on the grounds of the aims of an intervention intended to restore manual functions and their related skills. These skills should be considered as emerging manifestations of a highly organised and complex structure, such as the identity. On the basis of these considerations, it was agreed to use the following instruments:

- Clinical and psychodynamic anamnesis
- Clinical psychological interview
- Rorschach's test [12]
- Cattell's 16 Pf test [13]
- Raven's 38 Matrices [14]
- Somatic inkblot series (SIS) [15].

Evaluation criteria were agreed upon on the basis of an analytical examination of the proposed objectives, which are:

- Sociodemographic characteristics
- Patient's location within the context of the family and characteristics of the family's interactive dynamics
- Level of maturity of the personality
- Development of ego functions (capacities of concentration, attention, memory, judgement and examination of reality, impulse control and adjustment to the principle of reality and tolerance of frustrations)
- Characteristics of defence mechanisms mainly used by the subject
- Affective polarity and emotional control
- Nature of the reasons for the transplant
- Attitude towards the donor (bearing in mind that the donor is a dead person)
- Attitude towards the prosthesis: adjustment or rejection.

Psychoclinical Profile of the First Transplanted Subject, V. V.

The patient's family originally conformed with the patriarchal structural model. At the age of 14, V. V. suffered the amputation of his right upper limb following an agricultural accident, an event that significantly affected family dynamics because of its economic consequences. The economic compensation was handled by his father, and this decision was the origin of conflictual tensions that became protracted over time. The patient assumed a marginal family role. He was designated the "black sheep" of the family, and therefore felt little esteemed and valued by his parents; this led him to definitively break off all relationships with them after his marriage. His nuclear family consists of the married couple and an 8-year-old daughter. The family is adequately structured and has good adaptive capacities; within it, there is a good level of affective, psychological and protective support. (It is important to highlight that the conflict with his family of origin has significantly changed since the transplantation).

During the postoperative period, the patient's parents attempted an approach of reconciliation that he initially saw with diffidence and suspicion. Subsequently, he accepted his father's offer to support him during the rehabilitative treatment, and this allowed a modification in the role that the patient had been structuring in the recent past. The social process of the acquisition of a new identity by means of the support and recovery of self-esteem and self-image, reinforced by the recognition that the transplantation is a scientifically important event, led to the reorganisation of relationships within the family and redefinition of his role within his family of origin. This new position has meant to him a sort of reward and indemnity for the marginalisation he suffered.

The patient's personality structure is harmonious and well functional, both in cognitive and affective terms. He is capable of adjusting to everyday events using evolved mechanisms of defence such as humour, anticipation, affiliation and altruism. However, the amputation clearly caused a traumatic effect on the perception of his body. The patient has a normal intellectual level. He has a simple cognitive style oriented towards the global perception of situations, and the effort made to integrate the elements of reality can be perceived. Regarding ego functions, his attention, concentration and memory are normal. His critical judgement and examination of reality are well developed; his readiness to use his imagination is an important factor for the elaboration of affective content.

The patient needs stable and repetitive daily activity in order to express sufficient adjustment and avoid the manifestation of anxiety states. The type of affectiveness is sufficiently evolved, and impulsive instances are well controlled. He has good self-esteem although sometimes needs external narcissistic reinforcement in order to integrate at a social level: it is understandable that he has needed and still needs confirmation and reassurance given the serious impairment suffered in adolescence and the consequent narcissistic wound.

The reasons for the transplant are, firstly, restoration of self-image [16] – the patient never became resigned to the loss of his hand – and secondly, recovery of motor skills and sensitivity in order to reacquire autonomy and improve social relations and general openness towards the world.

The patient has a neutral attitude towards the donor, and rejected the prosthesis, which corresponds to an experience of loss and impairment that is more frustrating than the sight of the stump.

Psychoclinical Profile of the Second Transplanted Subject, G. D.

The second candidate chosen for hand transplantation is a 33-year-old man from Abruzzo, southern Italy. He comes from a large family, being the last of seven siblings, and grew up in a patriarchal family environment in which his father worked as farmer. The patient describes his family as a very united nucleus of members. His father, who died 11 years ago, is described as a strict man though good and tolerant; he did not give impositions. G. D. declares he received values from his father, which he considers fundamental, such as honesty, and work as the source of gain and main aim of life.

The patient lost his hand during the night of the New Year's day Eve in 1997 due to the explosion of a defective petard. After this event, G. D. had to significantly change his life style. He could not carry on with his job in his brother butcher's shop and began helping out on his family's small farm, which was managed mainly by his sister, performing activities such as ploughing, sowing the fields, harvesting and attending a few head of cattle. During their spare time, his brothers also attended the farm. However, the patient was not happy with this situation. In fact, he declares that he appreciates his job only when he "receives his salary at the end of the month". Therefore, he found a job as a fitter in a factory manufacturing hospital products. This job takes up half of his working day so that the rest of the time he can attend the farm. He met his girlfriend four years ago and describes this relationship as stable. He has expressed the desire to create a new family with his girlfriend based on the same model of his family of origin.

When the patient learned, thanks to the mass media, about the possibility of undergoing a hand transplantation, he became very enthusiastic and did all he could to contact San Gerardo Hospital in Monza. He is the only one in his family and within his group of friends to be so open and eager to the possibility. He does not feel abandoned or misunderstood by the lack of optimism shown by other people and, in spite of the disagreement openly expressed by them, is aware he may have to rely on them for all the help he may need. The patient lives in a cohesive environment that creates a kind of safety net, where he feels he can let himself fall. Lack of conflictual relationships, expressed or unexpressed, within his family, as well as his emotional environment, allow him to focus all his attention and energy on the therapeutic-rehabilitative programme.

The patient has a normal intellectual level, oriented to abstraction, and this prevents him from considering the practical and concrete aspects of everyday life. His method of comprehension reveals concise and integrative thought processes, with a global approach towards the administered tests. The nature of the global answers (which emerged from the Rorschach test) is mainly banal, which indicates a cognitive

laziness. His capacities of concentration, attention and memory are poor. His thoughts seem to be adaptable but only slightly sociable, characterised by a stereotyped and reproductive intelligence, with reduced capacities of internalisation, mentalisation and creativity.

His affect seems to be flat, with a small empathic capacity. These elements let us suppose a certain level of difficulty in his social relationships. From the Rorschach protocol, it appears the subject has great reactivity and sensibility towards stimuli and stressors; a floating anxiety is present, which is kept under control by attachment to reality and his needs to outdistance from it. His defence mechanisms are mainly negation, rationalisation, devaluation and inhibition, and these are strategies placed at the upper levels of Vaillant's hierarchy of ego functions. His body image does not appear solid although this aspect has to be correlated to the mutilation he suffered.

The appearances shown by the subject during the clinical interview compared with the answers given during the tests examination. We can suppose that the subject displaced defensive strategies during the interview, even if unconsciously, in order to hide his deep uncertainty and difficulty in controlling his impulsive tendencies. On the other hand, his biographic profile gave evidence in favour of personal assets in which there are good adaptive resources. The strong desire for a "new" hand came from a deep desire get well. The mutilation deprived G. D. of the ability to value himself as a self-determining individual and contributing member of a social community. His autonomy was damaged, and he developed a feeling of inferiority in comparison with the others from a physical and functional point of view.

Therefore, as in the first case, the goals of transplantation were restoration of the patient's self-image (both the physical and psychological experience of his body) and recovery of his autonomy in order to improve his attitude towards others. G. D. dreams of being able to hunt again, a passion he has denied himself primarily because of his feelings of defeat rather than his real handicap after the loss of his hand.

During the initial phase of the psychological-clinical evaluation, this patient, as did the first candidate, demonstrated an attitude of indifference towards the donor. However, this attitude changed a few months after the operation, and he now views the donated limb as a gift. He declares he often thinks about his donor's family and would like to be able to thank them even though he is aware of the conflictual dynamics that could emerge during this meeting.

Regarding his attitude towards the transplanted limb, during clinical interviews, it emerged that he considers it as any other internal organ and not evident to others. The pragmatism of this thought allowed the patient to consider the transplanted limb as his own. G. D. did not accept the prosthesis as a substitute for his upper limb for the same reasons as the first candidate, V. V. He felt that the prosthesis, besides being uncomfortable and heavy to wear, emphasised the mutilation in other people's eyes.

Conclusions

The subjects who met the criteria for suitability underwent the transplantation and, following the operation, a sequence of procedures was developed, as summarised below:

- An adequate setting for periods of exquisitely clinical care and periods of psychoclinical and psychotherapeutic interrelations
- Tests to be performed during pharmacological and psychotherapeutic treatment
- Techniques to observe and monitor the events characterising relationships between patient and nurses and psychotherapeutic team
- Elaboration of events and experiences specifically related to the psychoclinical and psychotherapeutic activities
- In the Section 10-c, we will carry on with our conclusions, considering the entire study.

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Section 10-b

Hand Transplant and Body Image

Gabriel Burloux

Introduction

The 1960s and 1970s were the beginning of a new era – the era of transplants. Some of these transplants were more impressive than others; for instance, the first heart transplant (1968) was a real scoop, probably because the heart has a special place in the collective imagination. Of course, it is the organ of life, but besides its somatic functions, it also has other virtues for the "person in the street", such as courage, will or intelligence. So, people would think that having a heart grafted could do much more than improve health and save life: mysterious and unknown things that come from the donor could be inherited by the recipient, such as ideas, impulses, and emotions, whereas kidney, pancreas, liver, lungs and all the combined grafts make less of an impression in the public imagination.

What about hands? They also carry a lot of fantasies. Languages often refer to hands to convey the idea of cleverness, friendship or other human qualities. A skillful person is "handy", and the number of locutions and proverbs around the word hand is endless. The reason is that hands are at the boundary between symbols and reality. As a matter of fact, hands would not be worth anything without the brain and, conversely, what could the latter do without the motricity provided by the hands? Only "brain-plus-hands" working as one are efficient: this combination, with the help of genes, allowed the evolution of humankind and its mastery over the world. The brain is an organ that is unable to act on its own but gives orders to other organs, which in turn are capable to do what it cannot do. There is complicity between hands and brain that is much more than the addition of two abilities working together. It is as though the brain-plus-hands was a third organ born from their alliance and specific to human beings. Consequently, losing one's hands is a tremendous "handicap". Through the centuries, hands have enhanced the brain and vice versa. The brain has learnt a lot of things, above all, to think and then to fantasize, to make mental images. One of these images is a certain representation of one's own body.

The Body Image

Paul Schilder [1] defined body image as "the picture of the body which we form in our mind". This definition does not add much to the term itself. Most likely, if somebody is asked: "Do you understand what 'body image' means?" he or she would answer: "Yes." But less certain is whether they could provide a precise and clear explanation of the notion of body image they have in their minds. This is because the notion encompasses several different things. Cash and Pruzensky, quoted in *Enabling Technologies* [2], identified seven integrative themes from body-image literature. Body image experiences:

- Are multifaceted;
- Refer to perceptions, thoughts, and feelings about the body and bodily processes;
- Are intertwined with feeling about the self;
- Are socially determined;
- Are not entirely fixed or static;
- Influence information processing;
- Influence behaviour.

This looks rather scientific but has been common wisdom and, indeed, common sense, for a long time.

The stream of consciousness is like a river, always on the move but always looking the same, so that we recognise it at first glance. It is always fed by the same springs. The image of our body, which is part of that stream of consciousness, becomes more precise when we think of it but always remains in our minds, albeit in a fuzzy way, when we do not. Our body is always more or less present, active or passive, and the hands play their part in this continuous process. When we communicate with another person, the hands play an essential role. Thanks to them, our language is more colourful, more expressive. With our hands tied behind our backs, we would not say the same things in the same way. The gestures of our whole body *go with* our words whereas every subtlety of thought is *embedded* in our hand movements. The representations we have of our body are not static; they are living images because we want our body alive and in good health.

This is what is going on with two-handed people. Life is easier when we have a good relationship with our body image, whatever it is. However, some people are not happy with their image even though it seems good enough to others, even fine. Some men would like another nose, some women, and teenagers are often dysmorphophobic. They may ask for surgery but, once it is done, do not always like the results. They remain unsatisfied. What did they actually want? This introduces the notion of narcissism, of a narcissistic imago. The best option is to make do with what we have. Later, we shall address the requirements and demands of some people whose psychic organisation is narcissistic; however, no one would be surprised that a human being may want to be intact and complete.

What happens when a human being suffers an amputation? Obviously, losing one hand has no comparison with the loss of both hands. I remember the interviews I had before transplant surgery with a would-be patient who was to have another man's hand grafted. He agreed to help medical science through his experience. I learnt a great deal from him. He told me: "With only one hand I can do almost 80%–90% of what I could do normally, but in some cases I am slower". So I wondered why he wanted the graft. Generally speaking, he indicated two basic reasons. First, he needed use of both hands to perform certain tasks, for instance, piloting a plane or playing the piano. Second, and less important to him, was that he could hardly bear and was not at ease with "how other people looked at him" and did not want to be looked upon as a disabled person. I understood then that the grounds for requesting this type of graft could be functional (which are understandable) but also narcissistic. Concerning the latter, why not, then, try to understand? When the body is injured, the body image is injured as well; there are narcissistic wounds. Remember that Narcissus loved seeing his own image in the water (he died from it).

The boundaries between those two kinds of wounds, somatic and narcissistic, are not precise. They overlap because the self may also be proud of its functional abilities. Therefore, the handicap caused by the loss of both hands is much greater, and the body image far more damaged. This is mainly because the patient becomes dependent on others for almost all the simple acts of everyday life, and the help of a prosthesis allows only very limited autonomy. At least in the beginning, the patients are depressed and depreciated, as is their body image. Moreover, they may feel ashamed, sometimes guilty, and cannot bear or face other people looking at them. And this is worsened by their functional incapacity. Before the era of the graft, hand-amputated patients had to go on living under these conditions. They could not be given the possibility of a transplant. They could not choose to say yes or no. Even now, although such a choice exists, some of those patients would not agree to be grafted, mainly because of the need to take anti-

rejection drugs forever. But that is not the only reason: they prefer to make do. Their decision depends on what is depicted in their minds, i.e whether the idea of a graft is anticipated or not. We must remember that grafts are not common (nor is losing both hands). If nothing is anticipated, then the patient goes through a period of bereavement and mourning and followed by healing and resignation.

Which Body Image Before the Transplant?

Before a scheduled graft, the period of mourning is over, which is followed by the period of hope. Now the patient may forget the past and begin to look forward with desire instead of depression. This is a period of great change in their lives. At that time it is very important that they be followed by a psychiatrist who is used to the problems associated with grafts. This is because whatever the patient is told may be "translated" by the patient into what is significant to him or her. The intellectual mind understands what is said, but the need to have hands, to use them, to do what could not be done before makes the patient's imagination create a mental and emotional world that takes little or no account of things that need to be considered, such as the long wait (1 year) before using the hands properly (nerves grow only about 1 mm per day) or the difficulties and constraints of everyday rehabilitation and physiotherapy, which are tedious. Also, there is the long period spent in hospital. These facts tend to be ignored. Furthermore, there is a risk that the p atient might want to recover mastery of a musical instrument or the precision required for a professional skill whereas the doctors are aiming for recovery of basic movements. A certain level of idealisation cannot help but be linked with the desire to have hands again. Too much enthusiasm can lead to disappointment inasmuch as the request for the graft can be made to deny the accident that caused the loss of the hands. This may add a grandiose, omnipotent dimension to the surgical act *per se*.

Before a graft, the patient should be "vetted", said a hand-grafted patient to me. "Assessment is not enough". He meant that such a big gap should not exist between reality and fancy even though there always is one. The doctors have to thoroughly inform the patient, and the psychiatrists have to make sure the patient has understood the information. But this is not enough. Psychiatrists should not only assess or vet the patient but emphasise what is in store in order to combat idealisation, which is always misleading and deceptive. Patients do not forget.

After the Graft

Any graft is different, but the hand graft poses specific problems because it is visible, comes from a cadaver and is not immediately functional. A grafted heart works immediately, a kidney or a liver, very quickly, but hands are made of a composite tissue and are motor and sensory organs and, as noted above, a hand-grafted person has to wait a long time before being able to use the new hands. One year is necessary for the patients to do what could be done with a myoelectric prosthesis.

Usually, transplanted organs come from a cadaver. However, when hands have just been grafted, it is *obvious* that they come from a dead body. They look dead and they *are* dead. They have to come to life. When the patient regains consciousness after the graft, what he sees (I say *he* because I never saw a hand-amputated woman) is a huge dressing at the tip of his wrist(s) or forehand(s). He is in the same condition as he was after his amputation with a noticeable difference: he has hands but cannot use them. What follows is the story of a conquest. Many points are to be considered:

1. Anxiety, which in this case takes the form of fear of the risk of depersonalisation. It is linked to the alien nature of the grafted hand, its cadaveric aspect, but also to the apparent boundary between the receiver's arm and the donor's hand, symbolic of the troublesome coexistence of the living and the dead, between the familiar and the unfamiliar. This

anxiety surges with the first dressing change but fails to be expressed because there are no words to qualify that vision. The patient sees he is mortal.

2. Regression, which is necessary and desirable in the immediate aftermath of the graft, because the patient is entirely dependent on others. It should not be long enough to prevent the mind becoming active again.
3. The risk of depression depends on the fragility of the patient's personality and on the gap between what the patient had imagined and what he is capable of.
4. Postoperative shock, steroid treatment, serum reaction or reaction of the grafted hand against the host may induce confusion and even a confusional type of delirium. For instance, one patient was seeing hands on the wall making signs to him. Obviously, this was an external projection of what threatened him internally: the hands.
5. The initial trauma is reactivated under the form of visual flashes of the initial accident that caused the loss of hands and the surgery that followed. The patient can see his hands cut to pieces or blown up and blood everywhere. He has bad day and night dreams and nightmares. All this comes with the state of regression but does not last long. Of course, body image just after the graft is much disturbed and must be rebuilt.

Appropriating the Hands

When the somatic graft is done, the psychic graft is only beginning and has yet to occur. Psychological reactions to the hand graft and the fantasies of the patients are linked to the visible aspect and the very long nonfunctionality of the grafted hands. Very soon and in the midst of what is described just above, the patient recovers both his enthusiasm and desire. "The dream came true" one patient told me. A new period begins. I explained to the patient that he will have to "tame" his new hands. "Like a squirrel or a tiger?" he asked. "Both!" I answered. Another image comes to mind that I communicate to patients: Imagine a teenager. He (and even more if she is a she) has to discover and appropriate a new body and a new body image. So do you. The same patient told me later: "I touched my hand and it was like an old friend." By this time, *the* hands had become *his* hands.

This time of appropriation needs to be assisted by the psychiatrist because of the double phenomenon of *denial* and *splitting*. Denial consists of the patient saying – or rather thinking – at the same time: "I know the hands come from a corpse and I do not want to know anything about that." Therefore, the mind splits into two different parts, which is the best way to do away with any contradiction. This is a well-known defence mechanism in psychology. It is very efficient but cannot last long because reality always has the final word. It is used when something is unbearable. Sometimes, such defence is not possible due to the pressure of internal impulses and emotions. Then a patient may come back through regression to other means of defence, for instance, daydreams or old memories.

One very strong-minded patient said to me once that a spirit, or rather an evil spirit, that was well known in the region he was living in, tried to suffocate him by pushing down his hand onto his mouth as he was asleep. This happened twice. The first time he had time enough to turn over and escape. Another time, it was a male spirit with a hole in his hand, so he could breathe through the hole.

What is Unbearable: The Frankenstein Aspect

We know that Mary Shelley's hero, Frankenstein, was made of pieces of human bodies sewn together. The first time a patient sees his newly grafted hand, the sight is rather awful. Stitches, threads, the swelling of the hands and the additions, the possible different colours of the skin – all that makes us understand why the patient denies what he is seeing. And from that moment on, the fantasies burst out. The unnamable is there. The perception is traumatic and revives the idea of the lifeless coming back to life. Will

the hand, that is still the "the Other Person's hand", become autonomous again? Will the grafted hand take control over me, when I am "hand-less"? What is it going to do? Will it do something unmentionable, which could be a secret desire?

The fantasy of inheriting the donor's qualities or shortcomings or to be inhabited by him or her is frequent in all types of grafts but, in the case of a hand graft – because of the visibility and because they are hands – it is much more conscious. But even in nonvisible grafts, the fantasy may appear suddenly. I remember a young boy whom I saw in a television programme. He was about to receive a bone marrow transplant. He said: "If the donor is a girl will I be a girl? If he does not like to go to school, will I be the same? But I think he is American so I will be able to speak American!" The fantasy of being controlled by the donor disrupts the reassuring sensation that we have of our own body, of our body image. That image had already been much affected by the loss of hands. The graft that should repair the image makes it even worse in the beginning, and so does the idea of a debt towards the donor. Then there is the sight of strange hands that have to become the patient's hands. The whole process needs mental elaboration and the help of the psychiatrist.

The hand transplant is a great step in the history of grafts. I will not conclude myself; I will let a patient do it. He told me: "This graft is not life saving but is much more than that, it's life giving."

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Section 10-c

Psychological Effects of Hand Transplantation

Italo Carta, Ornella Convertino, Juliette Bagnasco, Serenella Fornara

Introduction

This chapter describes observations during the treatment of two transplanted subjects after the onset of a psychotherapeutic relationship and after having built an adequate setting in which to explore the internal subjective dynamics. The remarks are not only limited to the simple illustration of their psychological manifestations as a result of the transplantation, but they are also extended to their experience and the counter tranference of the staff members. An ad hoc setting was created to contain nursing and rehabilitative activities of the various postoperative phases. Particular care was taken to define a flexible rehabilitative therapeutic context that could be modulated over time. The functions of the clinical psychologist were differentiated into three parts:

1. Testing investigations
2. Observation of what happened in terms of relationships with the nursing and physiotherapeutic teams
3. Active psychological, clinical and psychotherapeutic intervention.

Two weeks after the surgical operation, it was possible to establish a psychotherapeutic contract that foresaw 2-weekly sessions consisting of a clinical interview and a period of relaxation in accordance with Jacobson's systematic desensitisation method [1]. The same tests as those administered previously were also used after the operation [2–5]. The importance of clinical psychological intervention during different phases of transplantation can be described in three objectives:

1. Complexity of the intervention is evaluated not only from the surgical point of view but, above all, from the point of view of the sequence of actions involving staff with different medical and nonmedical professional backgrounds, including physiotherapists involved rehabilitation and technicians engaged in monitoring biological parameters related to antirejection therapy [6]. Despite using different methods, personnel pursuing the same goal make up a team in which development of active interpersonal dynamics need to be followed to detect possible moments of conflict and ensure the greatest possible integration. The aim is to reach the fundamental objective of guaranteeing adequate responses to the patient's needs.
2. Promotion of situational and relational contexts to facilitate the greatest possible integration of bodily self-image corresponding to redefinition of the subject's identity. With time, the transplanted limb regains its sensibility and mobility, and even though limited initially, afferent nerve pulses to sensitive motor areas originate from this. So gradually, diffused neuronal networks of body schema undergo further modifications (different and opposite to those following amputation) in their synaptic connections so that integration of the hand within the body schema is possi-

ble. We believe that psychic integration, the creation of a mental image of the transplanted hand, can help neurological integration. Here the words "psychic integration" mean the patient's ability to imagine himself with a new hand and to cast himself, in an imaginary way, into activities that require its use. These abilities developed over time progressively and spontaneously and can be improved by the patient focusing thoughts on the new limb. For this purpose, Jacobson's relaxation techniques are ideal, as they promote development of concentration and imagination. It is the responsibility of clinical psychologists to ensure full awareness that the subject treated by different staff members is, above all, a person who, in an essentially unknowing manner, has activated a process of integrating a highly significant body part that also involves changes in various declinations of "being in the world" (family, social and working relationships). In other words, a subject who has undergone hand transplantation is no longer the same person as before, and it is the job of the clinical psychologist to promote and encourage mentalisation of this new identity.

3. The third objective consists of observing the patient's modifications in psychological dynamics regarding the transplantation. This observation is very important, as it allows verification of the appropriateness of the assessment tools used during the patient's preoperative evaluation and in the choices following the results obtained. Analysing, in critical terms, the way followed by patients different intra-psychic and relational dynamics have been underlined.

One observation concerns the phenomenon that we will call "transitional identity". This consists of a phase in which the patient does the largely unconscious work of integrating the donor's hand with the physical self, the representation of which is modified as a results of the process. By analogy, this work can be defined as being the opposite of mourning, which is elaboration of the loss of a loved object. In the patient's case, instead of loss, it is the elaboration of the restoration of bodily integrity by means of acquisition of a limb that belonged to somebody else. On the basis of our observations, it seems that patients use the medical staff as prosthetic objects having the function of supporting a partially new identity and therefore contributing towards configuration of its psychic representation. Physiotherapists have the function of a prosthesis insofar as they manipulate the transplanted limb as if it were fully integrated with the totality of the patient's personality and, to different extents, all members of the team function in the same way by supporting and reinforcing the process of integration by the recipient.

Furthermore, the feeling of extraneity of the transplanted hand changes in the direction of integration and is coloured by affective tones corresponding to the experience the subject gains from physiotherapeutic and rehabilitative manipulation. The aim is assumption and stability of a new bodily identity, which is implemented by means of the process of identification-separation. During this process, it is possible to recognise movements that have connotations of ambivalence and that, in our cases, are attributable to relationships with the medical staff. It is the task of the clinical psychologist to recognise these ambivalences and encourage awareness and elaboration of them by both the patient and staff.

During psychotherapeutic activities, it was possible to observe that patients make a "hyperinvestment" in the new organ by organising new plans in a climate of euphoria. The patient has the sensation of being reborn, and this thought has a double aspect: on the one hand, being in possession of a new hand increases the strength of the desire for a new life; on the other, it arouses unconscious persecutory phantoms that generate anguish and hypochondriac polarisation. The idea of rebirth emerges in the transition from the sensation of death experienced by the patient in the immediate postoperative period (during which the patient is more vulnerable) to the reassuring experience that comes from placing oneself in the hands of the other – the staff.

The patient's mind contains ideas, the contents of which can be attributed to the acquisition of greater power by means of the transplant. The highly fusional connotation of identification

with the therapeutic–rehabilitative team is a factor that increases this sensation of greater power. The counterpart of this feeling of greater power consists of sensations of dependence that become clear from observing the patients when they recognise their own fragility. During the phase in which they are the object of rehabilitative and therapeutic interventions, their life is almost totally dependent on the staff: the physiotherapist who manipulates; the physicians who practice clinical therapies and different kinds of examinations and administer medication.

We are therefore confronted with a psychologically complex situation in which periods of maniacal omnipotence alternate with periods characterised by acute awareness of personal dependence. Feelings and attitudes of hostility emerge from this ambivalence; they are connected with dependence that is prevalently aimed at the staff but are also directed against the foreign body (the transplanted hand) that invokes unconscious phantoms of invasion accompanied by aggressive fantasies aimed at controlling them.

Results of Evaluation with the Questionnaire

We developed a questionnaire, divided into different sections, to quantify the modifications in different behavioural areas:

1. Daily activities
2. Social behaviour
3. Affectivity
4. Body schema
5. Personal health conditions.

Tables 1–5 document quantitatively differences that emerged before and after transplantation. Results shown in these charts underline a relevant improvements in subjects in every explored area that, although indicate different degrees, are substantially uniform. Nevertheless, we must note that the area regarding "personal health conditions", meaning subjective experience, indicates a negative modification compared with the remembrance of experience before transplantation. This is confirmation of previously explained hypochondriacal polarisation during the integration process of the transplanted limb.

Qualitative Analysis of SIS-I Test: Comparison Between Transplanted Patients

Symmetry of body schema after operation has been investigated by the somatic inkblot series (SIS)-I test [5], which is a projective technique to evaluate perception and body awareness. Images

Table 1. Quantitative differences in daily activities before and after transplantation

	V. V.			G. D.		
Daily activities	**Before transplantation**	**18 months later**	**3 years later**	**Before transplantation**	**6 months later**	**3 years later**
To hold big and medium objects	1	4	4	1	3	3
Hold light objects	1	4	2	2	3	3
Wash the face with hands	1	4	4	1	4	4
Massage	1	4	4	1	4	4
Drink and eat	1	4	3	1	3	4
Write	1	1	3	1	3	3
Drive a car	1	4	5	2	4	4
Ride a bicycle	1	3	3	1	4	3
Personal satisfaction	1	4	3	1	4	2

Satisfaction level: *1*, inadequate; *2*, poor; *3*, adequate; *4*, good; *5*, very good

Table 2. Quantitative differences in social behaviour before and after transplantation

	V. V.			G. D.		
Social behaviour	**Before transplantation**	**18 months later**	**3 years later**	**Before transplantation**	**6 months later**	**3 years later**
Shake the hand of another person	1	4	4	1	3	4
Relationship with others	1	5	4	1	4	4
Group acceptance	1	5	3	1	4	5

Satisfaction level: *1*, inadequate; *2*, poor; *3*, adequate; *4*, good; *5*, very good

Table 3. Quantitative differences in affectivity before and after transplantation

	V. V.			G. D.		
Affectivity	**Before transplantation**	**18 months later**	**3 years later**	**Before transplantation**	**6 months later**	**3 years later**
Caress	1	4	4	1	4	4
Hug	1	4	4	2	4	4
Touch	1	4	4	1	4	4
Intimacy with the partner	1	3	4	1	4	4
Affectivity satisfaction	1	4	3	1	4	4

Satisfaction level: *1*, inadequate; *2*, poor; *3*, adequate; *4*, good; *5*, very good

Table 4. Quantitative differences in body schema before and after transplantation

	V. V.			G. D.		
Body schema	**Before transplantation**	**18 months later**	**3 years later**	**Before transplantation**	**6 months later**	**3 years later**
Hand agility in walking	1	2	4	4	3	4
Agility in gesture during a conversation	1	1	4	3	3	4
Hand use in nonverbal communication	1	1	3	3	3	3
Sensation of a complete body	2	5	3	5	4	5
Hand acceptance	5	5	4	5	4	5

Satisfaction level: *1*, inadequate; *2*, poor; *3*, adequate; *4*, good; *5*, very good

represented on the tables are not all symmetric. Almost with the same distribution as the human body, the somatic spots are both asymmetric (heart and gastrointestinal) and symmetric (lungs, kidneys, hands, etc.). Data collected from the SIS test can be presented as the conclusion of this chapter. Indeed, the core of our interest consisted precisely in forecasting good integration of the transplanted limb in body schema and image. The SIS test is a suitable tool to realise that aim.

The generic meaning of the word symmetry is "harmony of the structure". Important observations can be made from the study of symmetry and asymmetry in investigating the structure of the human organism. Bilateral symmetry, which is found in living beings, presents essential advantages in the phylogenetic evolution. From

Table 5. Quantitative differences in personal health conditions before and after transplantation

Personal health conditions	V. V.			G. D.		
	Before transplantation	18 months later	3 years later	Before transplantation	6 months later	3 years later
What do you think about your health?	4	3	2	4	2	4
What about your strength?	4	4	3	5	2	3
Do you feel comfortable with yourself?	4	2	2	3	3	5
What about your mood?	3	3	3	3	3	5
What about the results of your daily activities?	4	4	3	4	2	5

Satisfaction level: *1*, inadequate; *2*, poor; *3*, adequate; *4*, good; *5*, very good

the psychological point of view, J. Helmcke [7] notes: "we could say that our sensibility towards spatial and temporal symmetry is inside and programmed in our hereditary characteristics". The possibility of understanding the existence of spatial symmetry allows the possibility of being conscious about the fundamental separation between self and other, as this makes possible the spatial representation of it. Nevertheless, natural phenomena around humans are not symmetric in a perfect way: whatever comes from absolute emptiness creates a certain asymmetry. Human consciousness, during the initial phase of development, observes with deep distress the existence of this asymmetry. Therefore, in this phase, rudimentary attempts are displaced in order to recreate a simple bilateral symmetry to allow integration of body perception and the correlated experience.

Figure 1 explains one of the fundamental reasons we chose this test, which is the possibility of patients projecting the representation of their own hands. The subjects experienced mutilation, and their body schema underwent an important alteration – an asymmetric alteration. As a consequence, we noticed that the problematic aspects in both patients we examined occurred in the following areas: (1) difficulty perceiving their own body as a unified element, (2) perception of the traumatic experience of mutilation as an aggressive act and (3) difficulty perceiving the symmetric aspects of their body schema. The anguish linked to these difficulties can be connected with the feelings of bodily catastrophe. The experience of mutilation caused doubt about the course of life so that an experience of aggression connected with death anguish emerges.

The SIS protocol was given to the first transplanted patient, V. V., 1 year after the operation. From thematic analysis of answers following exposure to the tables regarding body awareness, it emerged that this patient had some difficulties perceiving a gestalt of his body, as he was not able to organise and integrate the different physical components into a complete human figure, underlining limited awareness of his own

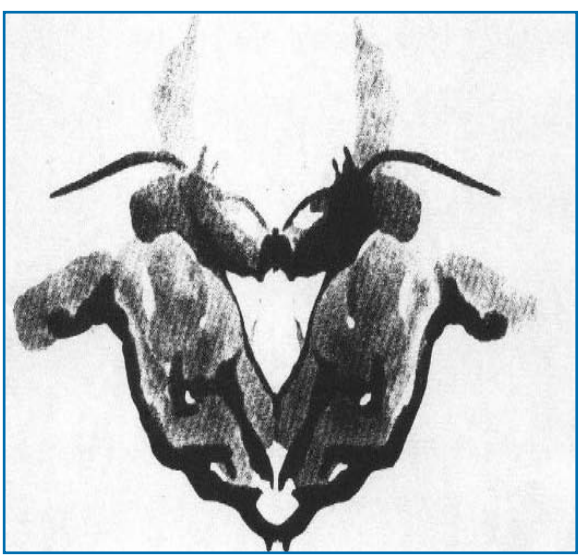

Fig. 1. Table XIX from [5]. Used with permission

body. Regarding table number XIX, the patient gave an answer in which it is possible to find the aspect of "catching". He uses the word "tentacles" regarding different table contents, a term that resembles fantasies of possession and mobility of the lost limb, which are threatened with the unconscious fear of bodily laceration. The action of catching was expressed throughout the entire protocol, indicating a desire to create a relationship with the world through the act of touching and exploring. Regarding the quality of relationships with the others, we observed difficulties in affective contact, which may have risen from a need to concentrate on himself (mind and body), as the patient found himself in a phase of reacquisition of his own motor abilities that were lost after mutilation.

In the second subject, G. D., we observed difficulties in elaborating the subjective experience of his own ability in cognitive perception and, in particular, adequately using thought processes linked with affectivity and corporeity. We also found inability to perceive a gestalt of his body, as did not "feel" and integrate the different psychophysical components of the whole human figure, which underscored limited awareness of his body. We also observed "irritation" when confronted with evidence of symmetry, which could be linked to the alteration of his body schema generated before mutilation and after transplantation. Afterwards, the patient tried to perceive the hands with a symmetric modality, showing slow recovery of body schema and ability to integrate sensations, perceptions and familiar movements, thanks to the transplanted hand. Eventually, it emerged that he had difficulty perceiving others in relationships, especially those of the opposite sex.

We emphasise that present in both protocols is the anguish and trauma linked with mutilation. Therefore, we cannot make the mistake of thinking that hand transplantation annuls, by a magic equation, the memory of the experience of loss. In fact, in the protocol, the ideation contents in connection with that anguish are also in connection with the fear that the event could happen again. Undergoing mutilation creates loss of self-identity as well as social identity as a whole body. Feelings of shame were expressed in the protocols through the difficulty of viewing themselves as active subjects, which is a characteristics of physical disablement. This is after transplantation, is the object of patients' psycological elevation.

Conclusions

In our experience, the balance of the economy of the subjects' psychological life was fundamentally positive. They maintained an attitude towards the team and the transplanted hand in which there is a prevalence of integration of the opposing affective polarities described above. The psychotherapeutic assistance encouraged this integration by promoting awareness of fantasies of maniacal omnipotence and fusion with the team, as well as the opposite signs of hostility and rejection of the team and the transplanted hand.

It is interesting to note a sort of isomorphism between phenomena of an exquisitely biological nature (integration-rejection) and the psychic dynamics poised between the opposed polarities of omnipotent fusion opening towards horizons of self-affirmation and independence and aggressive dependence characterised by fantasies of hostility and rejection.

We can therefore say that the success of a transplant comes from establishment of a good equilibrium in both the somatobiological sphere and the psychic processes.

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11. Other Composite Tissue Transplant

Section 11-a

Allogeneic Vascularised Knee Transplantation

Gunther Hofmann

Introduction

Major injuries of the thigh and knee joint, especially after high-energy trauma, often result in extended osseous and soft tissue defects. Despite optimal surgical management, there are no alternatives for resecting large parts of bone and sometimes the entire joint. Primary above-knee amputation and orthesis should be considered only as the last line of defence. The synthesis of transplant surgery and traumatology created new approaches in these situations. Vascularised allogeneic transplantation of bone and joints may help restore long-lasting integrity, stability and mobility of the mangled lower leg. This topic has been a matter of discussion for decades. Until 1996, no clinical attempts were made to perform such transplantations.

In 1908, the first pioneering whole-joint transplantations were performed. Judet [1] reported an experimental approach while at the same time, Lexer [2, 3] reported the clinical application of the approach in humans. These graftings of human bone and joint tissue were performed without organ preservation techniques without vascular pedicles and graft reperfusion. Transplantation immunology and the phenomenon of acute and chronic graft rejection were unknown, as were immunosuppressive drugs and antibiotics. Therefore, Lexer's attempts were doomed to fail.

During the past 30 years, various groups have demonstrated, in experimental settings, that vascularised hole-joint transplantation is technically feasible [4–15]. Up until 1996, all reported clinical knee-joint transplants were performed using nonvascularised bone grafts. In all these cases, the outcomes were disastrous, with incomplete vascularisation and subsequent microfractures, followed by graft disintegration. The experience emphasised that nonvascularised grafting was a poor solution [16]. In 1990, Chiron et al. [17] was the first to report a vascularised allotransplantation of a human femoral diaphysis. In 1994, Doi et al. [18] reported a single case of an allogeneic fibula transplanted from a mother to her 2-year-old son. In both procedures, no immunosuppression was performed and consequently no vascularisation could be detected in the allografted bone. In 1995, our group [19] performed the first clinical vascularised femoral diaphysis allotransplantation employing drug immunosuppression, and 1996, the first allogeneic vascularised knee joint transplantations followed [20–26].

Indications for Transplantation

The indication for transplantation of human knee joints may be considered in severe traumatic destruction of bone and soft tissue around the knee. Complete loss of the extensor apparatus makes the implantation of a total knee arthroplasty (TKA) impossible (Fig. 1). Primary above-

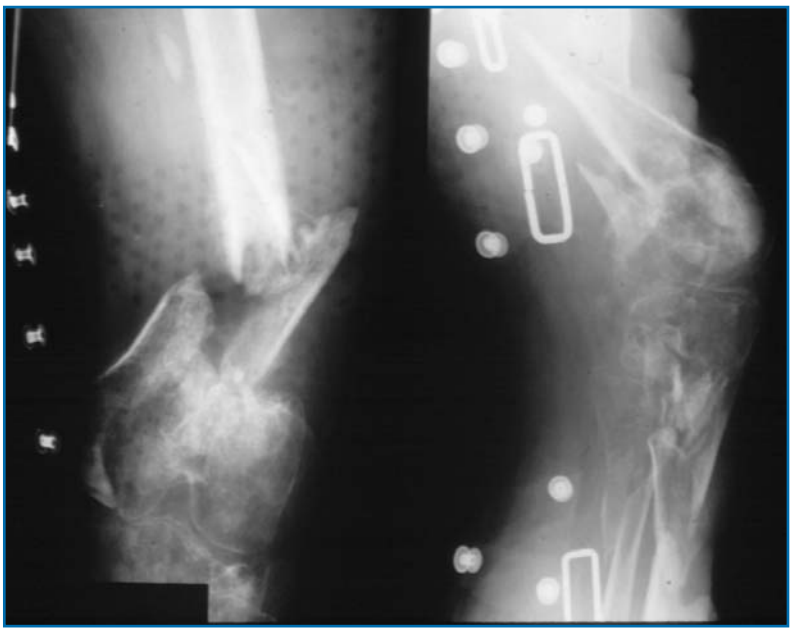

Fig. 1. A 35-year-old female motor vehicle accident victim: third-degree open avulsion fracture of the left knee joint; complete destruction of the joint and loss of extensor apparatus

knee amputation and orthesis is certainly the quickest rehabilitation but should be considered only as the very last line of defence. Primary arthrodesis requires subsequent bone-lengthening (Ilizarov manœuvre) to restore the original lengths of the lower extremity. This procedure is time consuming, accompanied by a high morbidity and results in various degrees of permanent disability with a stiff leg.

Contraindications

Allogenic vascularised transplantation of a human joint should never replaces established treatment procedures in orthopaedic surgery. Defect situations, where eradication of infection and restoration of soft tissue coverage have not been achieved successfully, are not suitable for transplantation because of postoperative immunosuppression of the recipient.

Donor Acquisition

From April 1996 until now, we have performed vascularised knee joint allotransplantation in 6 patients. All knee joints were harvested in accordance with standard organ procurement guidelines used in multiorgan donation (MOD). Therefore, fundamental criteria for postmortem MOD are to be respected:

1. Cerebral death
2. Unrestricted agreement to explantation
3. No exclusion due to risk factors
4. Negative serological tests [human immunodeficiency virus (HIV)-I and II, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV)].

MODs older than 45 years and those who had an accident involving the same leg where excluded from the pool of donors. Further grafts taken from MODs who had received blood substitutes or fresh frozen plasma during therapy before brain death and organ donation where also excluded. All allotransplantations were performed under ABO compatibility without human leukocyte antigen (HLA) status. Crossmatching was performed before transplantation and was found to be negative. An additional criterion was the geometrical compatibility between donor and recipient knee joints. To avoid soft tissue problems, donor's knee joint should be a little smaller (about 90% in both planes) than the recipient's contralateral side.

Allograft Procurement

After preparation of the abdominal organs in an MOD, the actual harvesting procedure consisted of cannulating the corresponding external iliac artery with a 14 fr catheter. The leg was perfused separately with 4 l of University of Wisconsin solution (UW) at 4°C. Femoral artery and vein were dissected distally to the proximal level of the adductor channel. The muscles were divided, and the femur and tibia were sawed, leaving approximately 5 cm of extra length. Particular care was taken not to endanger the vascular pedicles. The harvested graft was stored in sterile conditions in 3 layers of plastic bags at 4°C. Cold ischaemia time ranged from 18–24 h.

Preparation of Recipient and Transplantation

Recipient

A standardised preparation procedure was established and used in all our transplant cases. The strategy of treatment was subdivided into 4 steps:

Step 1: Eradication of infection
Step 2: Restoration of soft tissue coverage
Step 3: Preparation for transplantation
Step 4: Transplantation.

Eradication of Infection

As all patients with these disorders suffered from acute contamination or infection of the injured knee joint, radical *débridement* had to be performed first. Nonviable bone and all necrotic soft tissue had to be debrided surgically. Jet irrigation and vacuum wound dressings were employed until 3 consecutive microbiological cultures demonstrated that the defect was free of bacterial contamination. Open reduction and stabilisation of upper and lower leg was performed using external fixators.

Restoration of Soft Tissue Coverage

Local (gastrocnemius) and free pedicel flaps (latissimus dorsi) were than used to achieve soft tissue coverage, converting the defects into a closed, aseptic cavity. This procedure was combined with a switch in the osteosynthesis technique. Two interlocking compression nails were inserted anterograde into the femur and retrograde into the tibia. A simple polyethylene temporary hinged arthroplasty device was attached at the exposed tops of the nails and placed inside the knee-joint cavity (Fig. 2). This arrangement maintained assisted passive motion using a continuous passive motion (CPM) device during time on the waiting list to prevent contracture of soft tissue. Angiograms and phlebograms were performed to verify the presence of patent femoral vessels for revascularisation of the transplanted bone allografts.

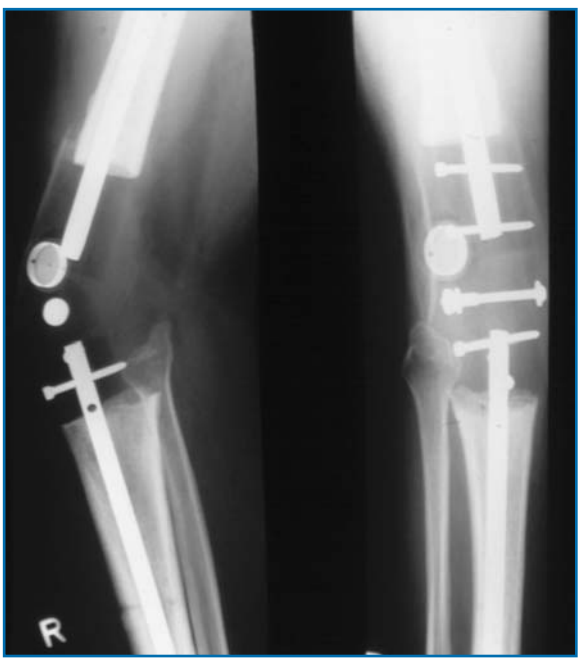

Fig. 2. Preparation for transplantation: at the tip of the implanted intramedullary nails in femur and tibia, a polyethylene hinge arthroplasty is fixed. Additional silicon expander is placed into the defect to prevent contracture of soft tissues over the defect

Back-Table Allograft Preparation

Bone allografts were carefully dissected free from their surrounding muscles and connective tissue so as not to damage their periosteal blood supply. Particular care was taken to keep the quadriceps tendon and articular capsule intact. All vessels penetrating the muscles were ligated while the vessels to bone were carefully preserved. Finally, the graft's arterial pedicel was perfused with methylene blue in order to verify the presence of adequate perfusion of the transplanted bone. Perfusion was considered adequate when dye freely perfused the graft and exited through the femoral vein. During the last transplantation, a piece of donor skin in subcutaneous tissue was transplanted with the graft an inserted into the skin of the recipient to monitor early signs of graft rejection or perfusion dysfunction (sentinel skin graft).

Surgical Transplantation Procedure

All transplantations were performed fresh and within a cold ischaemia time of 24 h. A negative serological cross-match should be performed to exclude cytotoxic antibodies to avoid the danger of hyperacute rejection. The surgical procedure of the transplantation consisted of 5 steps:

1. A 40-cm-long S-shaped skin incision was used to expose the recipient site. The temporary hinge arthroplasty device was removed. Superficial femoral artery and vein were prepared in the adductor channel and wound with vessel loops.
2. Intramedullary nails were withdrawn, and another *débridement* and lavage of the joint cavity was preformed. Under radiographic control, the grafts were cut to the precise size of the defect and put in place.
3. Osteosyntheses were performed with intramedullary devices using interlocking compression nails (Fig. 3).
4. Anastomosis between the graft's vascular pedicle and the recipient's superficial femoral vessels were performed in end-to-site technique employing 6 nonabsorbable sutures. Graft reperfusion was started immediately.
5. The bone graft's quadriceps tendon was inserted into the quadriceps muscle of the recipient to reestablish mobility of the grafted joint.

Follow-Up of Recipients

Early Postoperative Management and Immunosuppression

Due to the fact that all grafted tissue, such as bone, bone marrow, cartilage, synovial membrane, ligaments and menisci, have been proven to be rather immunogenic structures [8, 9, 22], immunosuppression was started immediately

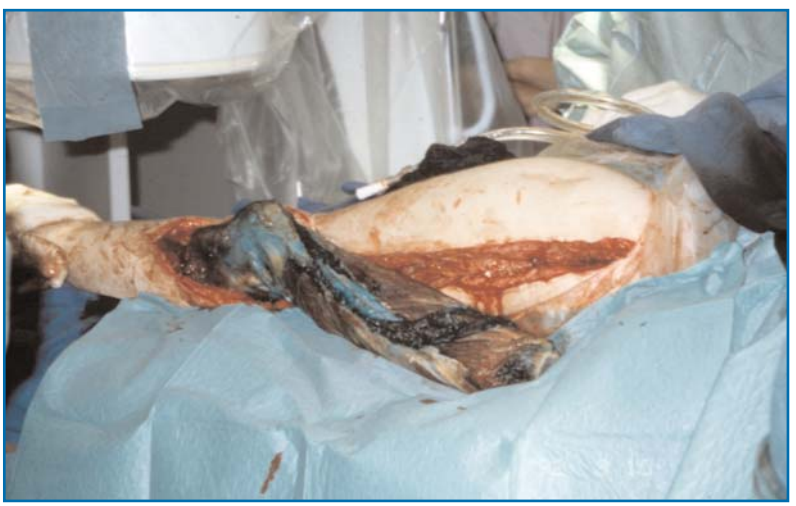

Fig. 3. Bridging of a 35-cm femur and 10-cm tibia defect with a 44-cm-long allogeneic vascularised combined femur diaphyseal and knee-joint graft. The tibial osteosynthesis is already performed; this is the situation before implantation of the femoral nail. The blue staining of the transplant results from the vital blue perfusion during back-table preparation

following reperfusion of the graft respecting defined immunosuppressive protocols. For the first 5 transplantations, cyclosporine A and azathioprine were the main immunosuppressive drugs [27]. For the last transplantation, we switched the immunosuppressive regimen to FK506 and mycophenolate mofetil (MMF) [28].

During the first 3 days, i.v. quadruple induction therapy with cyclosporine A (1.5 mg/kg body weight), azathioprine (1.5 mg/kg body weight), antithymocyte globulin (ATG) (4 mg/kg body weight) and methylprednisolone (250 mg) was administered. After the first 3 days, the immunosuppressive protocol was switched to an oral double-drug maintenance therapy that lasted for the subsequent 6 months: cyclosporine A (6.0 mg/kg body weight) and azathioprine (1.0–3.0 mg/kg body weight). This was the immunosuppressive regimen for the first five transplantations. For the last transplantation, the protocol was changed. Immunosuppression was again started with a quadruple induction therapy for the first 7 days: ATG (4 mg/kg body weight i.v.), methylprednisolone (250 mg i.v.), FK506 (tacrolimus 10 mg p.o.) and MMF (2 g p.o.). From the beginning of the second week, immunosuppression was reduced to an oral triple therapy with FK506, MMF and methylprednisolone (10 mg p.o).

Heparin was administered i.v. for the first 3 days and continued using subcutaneous administration to maintained partial thromboplastin times (PTT) within a range of 60–80 s. Two patients received phenprocoumon, and all received aspirin anticoagulation for 1 year after transplantation.

The interlocking compression nails provided active compression for the osteotomy and a highly stable connection between the transplanted graft and recipient's bone. Recipients were not allowed weight bearing on the transplants during the first 3 postoperative days. Physiotherapy consisted of continuous passive motion and active exercise and was started on the first postoperative day. Weight bearing increased subsequently, and full weight bearing was achieved between 6 and 15 weeks after transplantation. All patients were discharged from the hospital between 3 and 8 weeks after transplantation.

Monitoring of Graft Function

Postoperative monitoring of the transplantation consisted of clinical controls (signs of local inflammation, fever) and daily laboratory controls of white blood cell counts, C-reactive proteins, procalcitonin, trough levels of cyclosporin A and FK506 and PTT. In addition, different technical methods were employed for the follow-up:

1. Osseous consolidation of the osteotomy, nail and screw positions and bone healing were followed by conventional radiographs.
2. Digital substraction angiograms (DSA) were used during the first week following transplantation to monitor macroscopic circulation in the graft pedicle.
3. Additionally, duplex sonograms were taken whenever possible during the follow-up.
4. We used 99m-Tc-DPD scintigraphy to assess microcirculatory perfusion and cellular metabolism of the transplanted bone.
5. Single photon emission computed tomography (SPECT) was used to exclude tracer uptake from overlying tissue.
6. When clinical signs indicated the possibility of a rejection episode, arthroscopy was performed to take biopsies for histologic monitoring of cellular perivascular infiltration and viability of cells in synovial tissue. Capillary blood flow in the synovial membrane and signs of inflammation could be evaluated by an additional micro-arthroscopy.

After patients were discharged, they were seen weekly by an experienced physician near their homes and every 2 months in our surgical outpatient department. Our first five transplantation cases revealed a defined immunologic cellular and humeral response of the recipient against the graft. The problem we encountered was an inability to easily monitor allograft rejection. To improve monitoring, we decided to harvest a block of skin and subcutaneous tissue with a vascular pedicle together with the graft from the donor and integrate the allogeneic skin in the recipient skin. The same technique had previously been reported by Lanzetta et al. in their hand transplantation project where an additional full-thickness skin graft was transplanted onto the left hip area as an additional rejection monitor (distant sentinel skin graft [29]).

Results and Complications

In 5 of the 6 patients, early callus formation and consolidation of the osteotomies could be demonstrated on X-ray. These 5 patients were discharged from hospitals 3–8 weeks after transplantation. At that time, they were mobile. and full weight bearing was achieved 2–4 weeks later. Movement ranged from full extension to 120° flexion. In 1 patient, we lost the allograft within the first posttransplant weeks due to reinfection under immunosuppressive therapy. Two patients developed what we interpreted to be clinical signs of chronic rejection. Biopsies of the synovial membrane revealed viable and perfused tissue with significant perivascular infiltration of lymphocytes. Subsequently, these patients developed an occlusion of the allograft vascular pedicles and transplant failure. Meanwhile, 4 of the 6 patients have received an additional TKA because of chronic rejection of the cartilage in the transplanted knee joint. One patient received the additional knee arthroplasty 15 months posttransplantation, 1 patient 35 month posttransplant and 2 patients more than 50 month posttransplant. Two years after transplantation, possibly due to lack of proprioception in his allograft, 1 patient developed a fatigue fracture of the tibial head of his transplanted knee joint while running downstairs. Also in this case, we decided to perform an additional knee arthroplasty using part of the remaining transplanted bone as base. Unfortunately 3 of these 4 patients developed a deep infection in the operated knee joint within the following 2 years. At that time, due to social and private reasons, these patients decided not to continue limb-salvaging procedures and preferred thigh amputation. The last patient with a vascularised knee-joint allograft under postoperative immunosuppression with FK506 and MMF was still doing well with the transplanted knee joint four years posttransplantation (Figs. 4, 5).

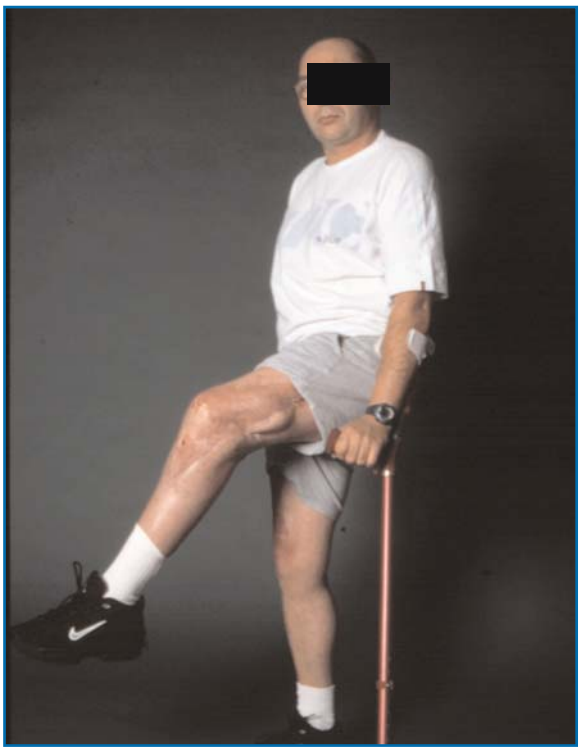

Fig. 4. 41-year-old man with combined femoral diaphysis and knee joint transplantation; 40 months after transplantation of Figure 3

Fig. 5. Same patient as in Fig. 4. X-ray of the left leg in sagittal direction; both intramedullary nails still in place

Future Perspectives

With the introduction of vascularised knee-joint allotransplantation, a new treatment option was made available to the field of orthopaedic surgery. This new treatment option is not intended

as a therapy that replaces treatments such a total joint arthroplasty. Therefore, the indication should be limited to the following situations:

1. Patients younger than 45 years
2. Completely destroyed joint
3. Large bone defects
4. Deficient extensor mechanism

Patients fulfilling these criteria cannot be treated with TKA in order to reestablish a mobile weight-bearing extremity. All other indications, such as osteoarthritis, rheumatoid arthritis and malignant bone tumours, should be treated by TKA. The cost of transplantation is the risk of immunosuppression. Most complications in our 6 patients where associated with immunosuppression therapy. For example, 4 patients experienced reactivation of deep bacterial wound infections. In addition to causing several infection-related complications in our first 5 patients, the cyclosporin A and azathioprine immunosuppressive drug regimen did not seem to effectively prevent rejection. Conversely, the different immunosuppressive regimen employed in the last patient eliminated many of the problems. The FK506-based immunotherapy demonstrated only minimal complications and effective protection against rejection for 4 years. All forms of drug immunosuppression appear to have no adverse effect on bone healing and osseous consolidation of the osteotomies.

Vascularised bone allografts are low-flow organs. This, in combination with the antigenicity of the accompanying vascular tissues, may pose an increased risk of graft thrombosis leading to necrosis. Whether or not anticoagulation is mandatory and for how long is open for discussion.

Finally, we would like to mention the problem of the knee joint transplant being denervated during harvest. This could potentially lead to neuropathic arthropathy. Proprioception of the joint allograft appears to be absent due to bone and joint denervation. This could lead to repeated unperceived microtraumatisation and also transplant fractures.

Further investigations are necessary before vascularised bone and joint transplantation have the potential to become established alternatives in orthopaedic surgery. Nevertheless, allogeneic vascularised transplantation of complete human joints is a challenging new procedure. For the immediate future, the procedure should be limited to clinical study designs. An open and serious discussion and informed consent with the patient is mandatory because of the necessary immunosuppressive therapy.

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Section 11-b

Laryngeal Transplantation

Robert R. Lorenz, Marshall Strome

Introduction

Human laryngeal transplantation was first introduced into the literature in the 1960s with experiments using dog models by Boles [1], Ogura et al. [2], and Silver et al [3]. In 1969, Kluyskens and Ringoir attempted to reconstruct the aerodigestive tract after laryngectomy for cancer with a cadaveric transplantation [4]. This transplant was subtotal, preserving recipient perichondrium to revascularize the donor organ without the use of vascular or neural anastomoses. Rapid recurrence of the tumor quashed interest in the procedure for nearly two decades

In 1987, the senior Author initiated a program to explore the potential of a total larynx transplant. The program focused on four issues crucial to successful transplantation: revascularization, reinnervation, rejection, and the ethical issues of transplanting an organ that some consider nonvital. Utilizing the rat as a model for laryngeal transplantation, the maximum tolerated ischemia time was determined [5], preservative solutions were investigated, stages of histologic rejection were defined [6], and immunosuppressive regimens were evaluated [7]. On 4 January 1998, a team led by the senior Author performed a total laryngeal transplantation in a man who had sustained severe laryngeal trauma in a motor vehicle accident [8].

The First Successful Composite Human Laryngeal Transplant

The recipient was a 40-year-old man who had suffered a crush injury to his larynx and pharynx during a motorcycle accident 20 years earlier. Despite multiple attempts at another institution to reconstruct his larynx, he remained aphonic and tracheotomy dependent. The patient underwent extensive pretransplant counseling, including psychiatric evaluation, speech pathology testing, and four interviews with members of the surgical team. All involved agreed that the patient understood the risks, and his motivation was appropriate. The procedure was approved by the Institutional Review Board of the Cleveland Clinic Foundation. After a 6-month search, a 40-year-old man who was brain dead from a ruptured cerebral aneurysm was identified as a suitable donor. He met all predetermined criteria for acceptance in regards to human leukocyte antigen (HLA) matching (4 of 5) and serum virology.

During donor-organ harvest, the entire pharyngolaryngeal complex, including six tracheal rings and the thyroid and parathyroid glands were removed (Fig. 1). The organ complex was stored in University of Wisconsin (UW) solution during transport until revascularization 10 h later. Prior to surgery, the recipient received cyclosporine A (CSA), azathioprine, and methyl-

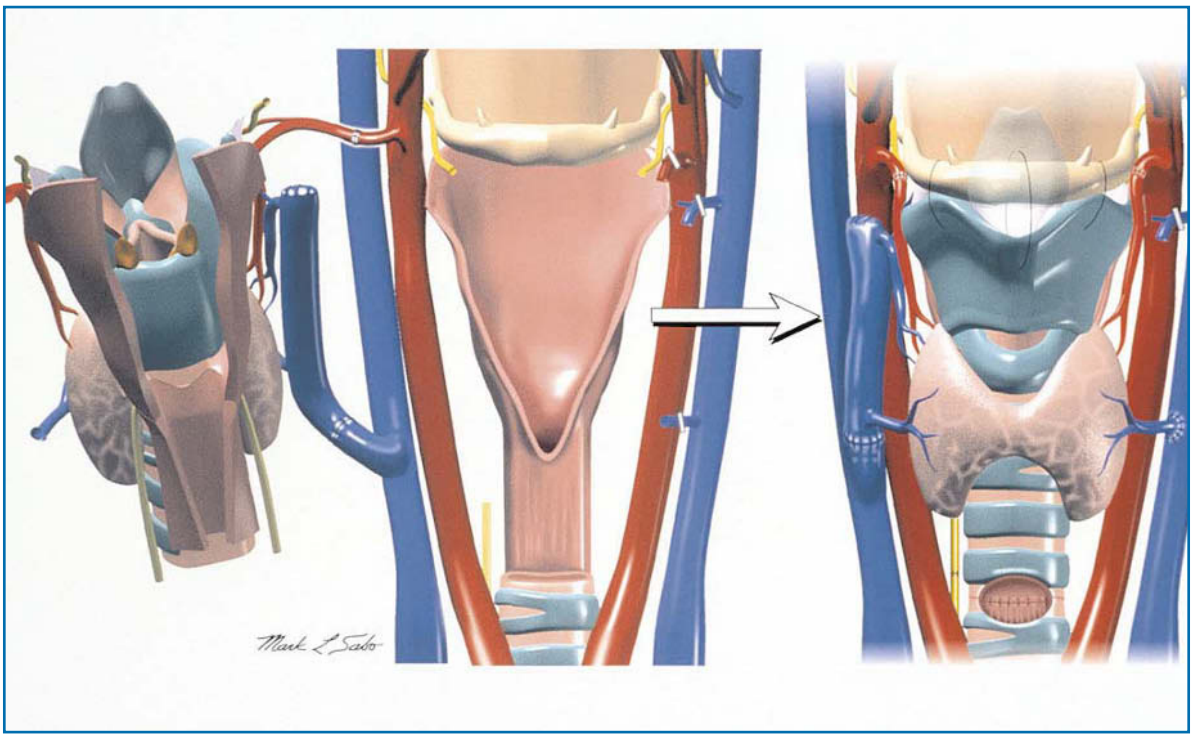


Fig. 1. The 1998 surgical technique of the first successful composite laryngeal transplantation. Anastomoses included the donor right internal jugular vein to recipient right facial vein, donor superior thyroid arteries to recipient superior thyroid arteries, and donor left middle thyroid vein to recipient left internal jugular vein. Note that both superior laryngeal nerves were anastomosed while only the patient's right recurrent laryngeal nerve (RLN) could be located for anastomosis to the donor organ's right RLN. Courtesy of Mark Sabo

prednisolone. After surgical exposure of the patient's severely deformed laryngeal structures but prior to their removal, perfusion to the donor organ was reestablished. The donor's right superior thyroid artery was anastomosed to that of the patient while the proximal end of the donor's right internal jugular vein was anastomosed to the patient's right common facial vein. Blood flow through the transplanted thyroid gland, six tracheal rings, larynx, and pharynx was observed within 30 min of clamp release.

A narrow-field laryngectomy was performed leaving the recipient's thyroid lobes lateralized and the hyoid bone in place. Seventy-five percent of the donor's pharynx was used to widen the patient's stenotic pharyngeal–upper esophageal complex. The donor laryngeal cartilage was sutured to the hyoid bone for laryngeal elevation. Five tracheal rings were needed to reach the patient's tracheostoma. The left-sided anastomoses, which included the donor superior thyroid artery to the recipient superior thyroid artery and the donor middle thyroid vein to the recipient internal jugular vein, were then completed. Both superior laryngeal nerves were located and reanastomosed, but only the recipient's right recurrent laryngeal nerve (RLN) could be located for reinnervation.

In the immediate postoperative period, the patient was maintained on muromonab-CD3, CSA, methylprednisolone, and mycophenolate mofetil (MMF). Initial aspiration was controlled with glycopyrrolate and atropine, which were later discontinued. At the end of a 1-month period of observation in the hospital, the patient's transplanted trachea was slightly edematous on endoscopy and showed no signs of rejection on biopsy. Fifteen months posttransplant, the patient experienced an episode of rejection that presented as a decrease in voice quality. After 3 daily doses of methylprednisolone 1 gm/day, his larynx returned to normal. The patient is now over 8 years posttransplant and is maintained on 7.5 mg of prednisone, 1 g of MMF, , and 3 mg of tacrolimus (FK506) per day and has stable blood pressure and renal function. A second episode of

rejection occurred 6 years after transplant due to laboratory error in (FK506) values measuring levels falsely high, which resulted in decreasing the patient's medication below therapeutic levels. Laryngeal edema was observed during the rejection episode but quickly resolved once medication levels returned to the therapeutic range.

Three months after transplant, the supraglottis and vocal folds were sensitive to touch and purposeful swallowing returned. Subsequent barium swallows revealed no aspiration, and the patient's sense of taste and smell returned. The patient did experience three early episodes of tracheobronchitis that were successfully treated with oral amoxicillin-clavulanate. At 16 weeks posttransplant, the patient inadvertently stopped his trimethoprim-sulfamethoxazole and developed *Pneumocystis carinii* pneumonia, which cleared rapidly with intravenous antibiotics. To evaluate thyroid function, a 4-h uptake of iodine-123 demonstrated 83% activity in the transplanted thyroid lobes as well as 17% in the patient's native thyroid. Thyroid function tests, serum calcium, and phosphate all remain within normal ranges

The patient's first posttransplant voicing was on postoperative day 3 (Fig. 2). At 1 month, both true vocal folds were lateral, creating a breathy voice. By 4 months, the right fold (the side of the recurrent nerve anastomosis) was midline, and at 6 months, the left had medialized. Recent electromyographic (EMG) measurements confirmed reinnervation of both folds [9]; we believe that the left thyroarytenoid muscle is supplied by surrounding motor nerves or has achieved "field-reinnervation." Bilateral volitional cricothyroid function was confirmed by EMG as well (Fig. 3a, b). Subjective and objective measures of phonation, including pitch, jitter, intensity, and maximal phonation time were within the normal range at 36 months posttransplant. The patient became a motivational speaker and reports that his quality of life has improved "immeasurably" over the 8 years posttransplantation. Laser cordotomy or "sling-tracheoplasty" remain options for either stomal closure or avoidance of finger occlusion of the stoma, both of which the patient continues to decline.

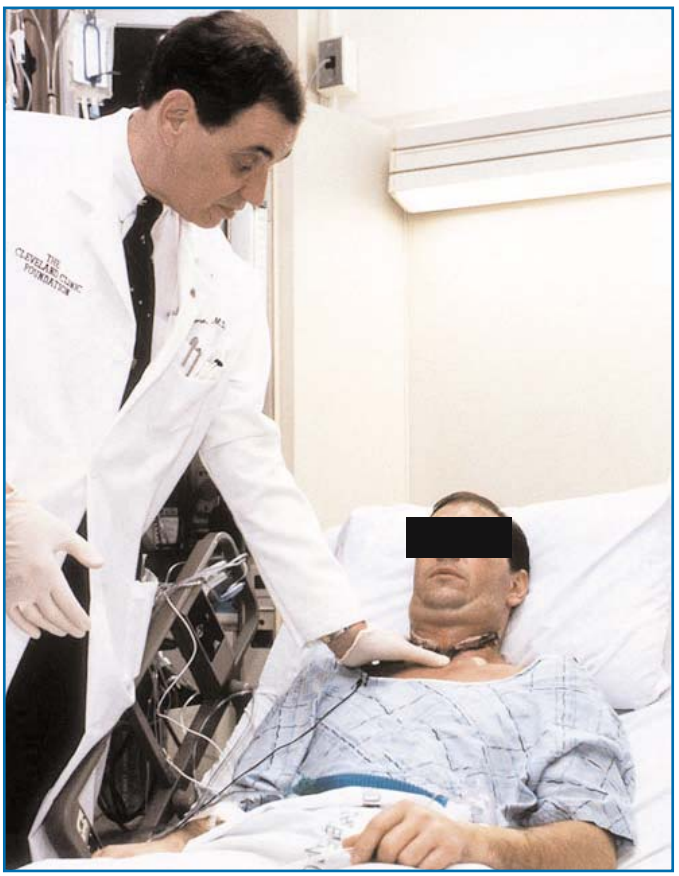


Fig. 2. The senior Author with the first successful laryngeal transplant patient. The patient uttered his first words on postoperative day 3 after 20 years of aphonia

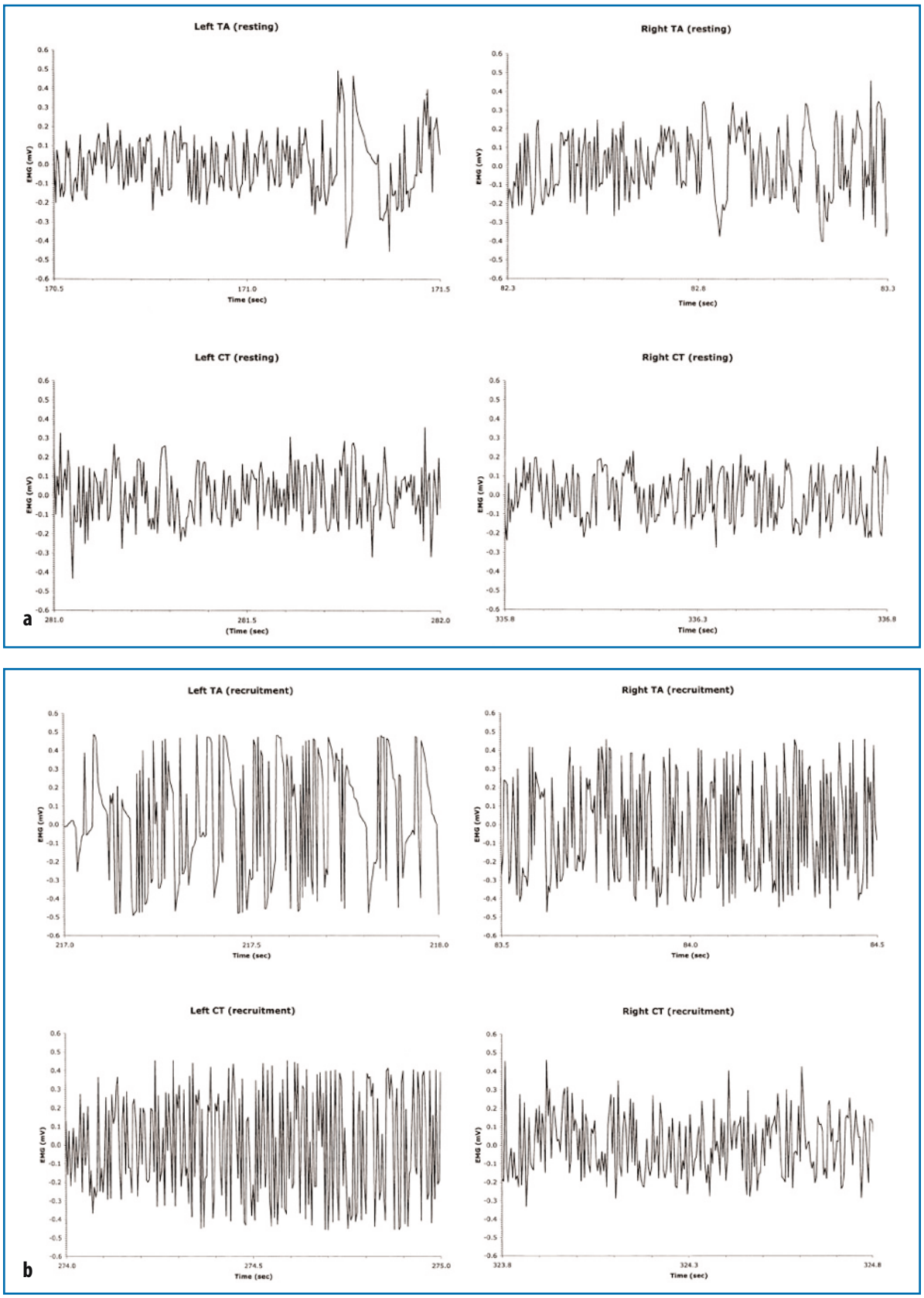


Fig. 3a, b. a Resting laryngeal electromyogram (EMG) tracings of 4 phonatory muscles 4 years after transplantation (*TA* thyroarytenoid muscle, *CT* cricothyroid muscle). **b** Laryngeal EMG tracings on phonation with "ee" (*TA* thyroarytenoid muscle) and raising and lowering of pitch (*CT* cricothyroid muscle)

Animal Models of Laryngeal Transplantation

Early experiments into laryngeal transplantation in the 1960s by Boles [1], Ogura et al [2], and Silver et al. [3] used the dog model. Berke's group applied modern microvascular techniques to continue investigating this orthotopic model in the mid-1990s [10]. Genden et al.'s mouse model of tracheal transplantation more recently examined the role of reepithelialization of the transplanted trachea that may be extrapolated to laryngeal work [11]. Birchall's group developed an orthotopic pig model that is robust and allows detailed assessment of immunology in an open airway coupled with functional reinnervation [12]. However, as with the dog model, this model is expensive in regards to time and labor and requires tracheostomy and gastrostomy, at least in the short term. These models all offer complementary information, forming the basis on which to build further understanding into laryngeal transplantation

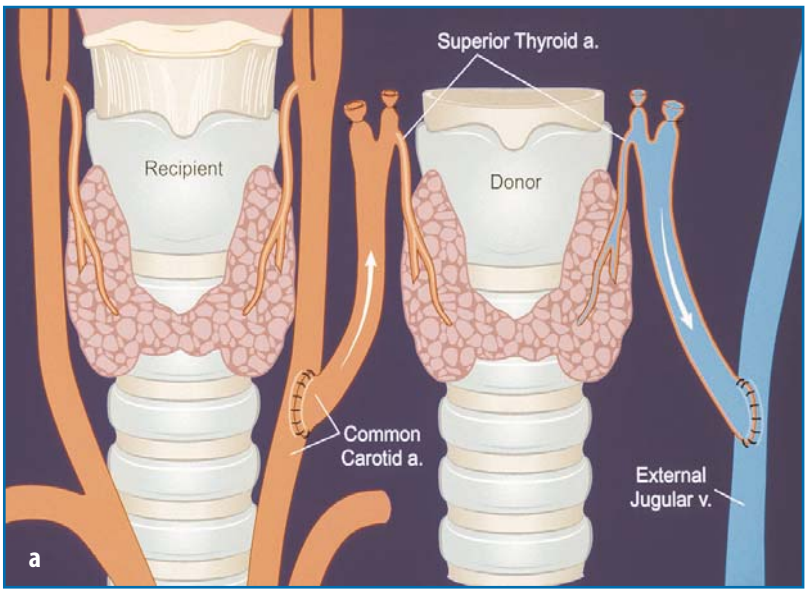
In our own laboratory, the rat model utilizes an arteriovenous shunt with venous outflow through the superior thyroid artery with the transplanted organ in tandem with the native airway (Fig. 4a-c). In 2002, the model's revisions were published, as well as a revised grading scale of rejection [13] (Fig. 5a-c). With the low cost, a near 100% survivability, and greater than 90% graft evaluability, more than 2,000 rat transplants have been successfully performed to date. Furthermore, recent experiments have shown how performing a total parathyroidectomy on the recipient during transplantation can utilize the production of parathormone from the transplanted larynx as a marker of graft viability rather than having to sacrifice the animal for histologic evaluation [14]. This has allowed for a new generation of studies examining the ability to "pulse" immunosuppressives and "salvage" the organ if parathormone levels drop.

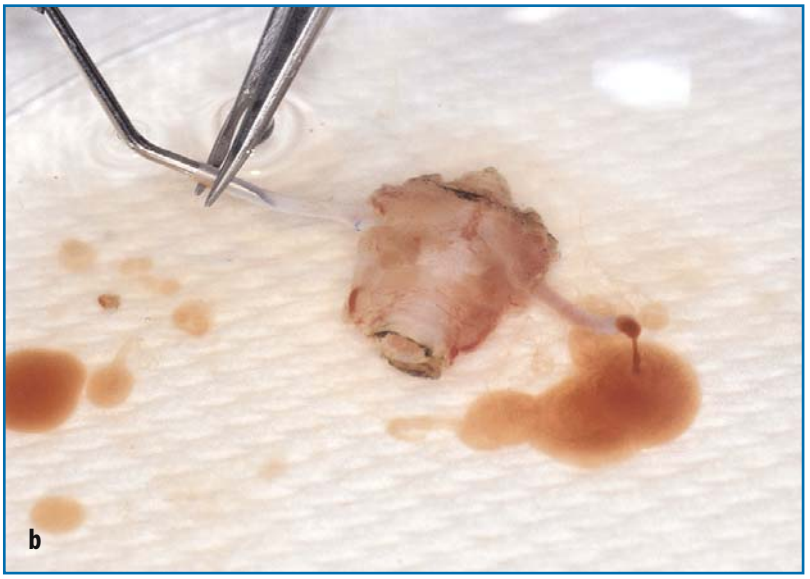
Past findings using the rat laryngeal transplant model include those of Barthel et al. [15], who studied the effect of *in vitro* irradiation on the transplanted laryngotracheal complex. By administering 7.34 Gy to the organ immediately prior to transplantation, doses of CSA could be reduced from 5.0 mg/kg to 2.5 mg/kg, with 10 out of 10 rats displaying no significant rejection at 30 days posttransplantation. These results were compared to those without radiation, which demonstrated that with CSA doses of 5.0 mg/kg, at 30 days, 33% of transplants displayed moderate rejection while an additional 33% displayed severe rejection. The authors concluded that in vitro radiation has some lasting immunosuppressive effects, perhaps reducing the number of viable "passenger" lymphocytes that accompany the transplanted organ.

Using the same model, Lorenz et al. demonstrated that by adding prednisone to CSA, doses of CSA could be further reduced [16]. In a multiarm study containing 220 transplantations, multiple doses of both CSA and prednisone were administered, and the transplanted organs were evaluated at both 15 and 30 days posttransplantation. With the addition of 1.0 mg/kg per day of prednisone, CSA doses could be reduced to 2.0 mg/kg and still demonstrate no significant rejection at 30 days posttransplantation. While this combination of low-dose CSA and prednisone significantly improved graft survival when compared with CSA alone at the equivalent dose, prednisone monotherapy demonstrated rates of rejection similar to no immunosuppression at all.

Haug et al. correlated laryngeal rejection grade, CSA concentration and CSA intramuscular dosing [17]. Despite high variability in their CSA blood levels within groups of rats receiving CSA dosed at either 1.0, 2.5, 5.0, 7.5 or 10 mg/kg/day, significantly different average CSA concentrations were achieved among each group of 5 transplanted rats. While rejection grading within the top three doses of CSA were not significantly different (5.0, 7.5 and 10 mg/kg/day), doses 2.5 or less were shown to have higher rejection grading in this blinded study. Significant pathological allograft rejection correlated with CSA concentrations below 250 ng/cc. This careful evaluation of drug dosing within the model established the correlation between CSA dosing, CSA levels, and graft rejection, as well as established the minimum level of CSA required to obtain optimum graft survival when used as the sole agent of immunosuppression.

When combining newer immunosuppressives within this transplantation model, Nelson et al. demonstrated that decreased levels may be used while maintaining optimum graft viability [18].





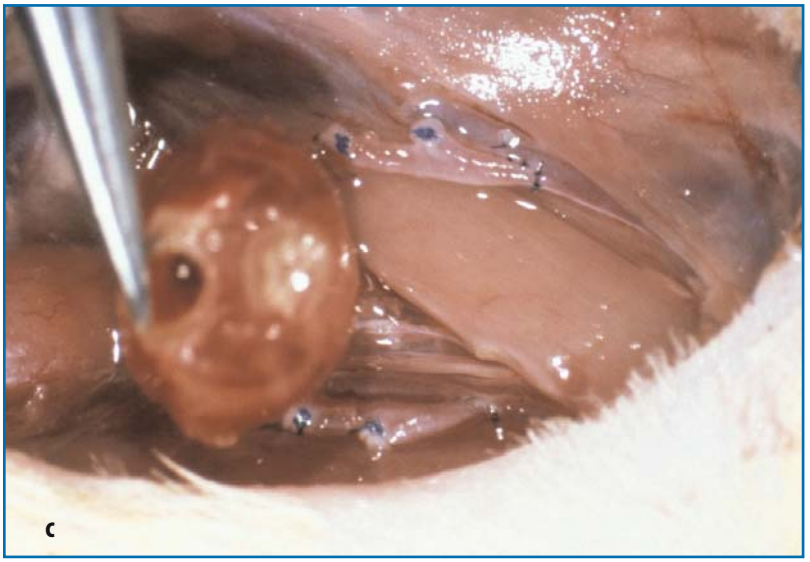


Fig. 4a-c. a The Authors' rat model for laryngeal transplantation. The donor organ is placed in tandem to the recipient's airway, and an arteriovenous shunt is used for venous outflow through the left superior thyroid artery. **b** The donor organ being flushed with University of Wisconsin's (UW) solution prior to revascularization. **c** The donor organ being rotated superiorly in tandem in the left neck of the recipient rat. Arterial and venous anastomoses are shown as blood flow reestablished

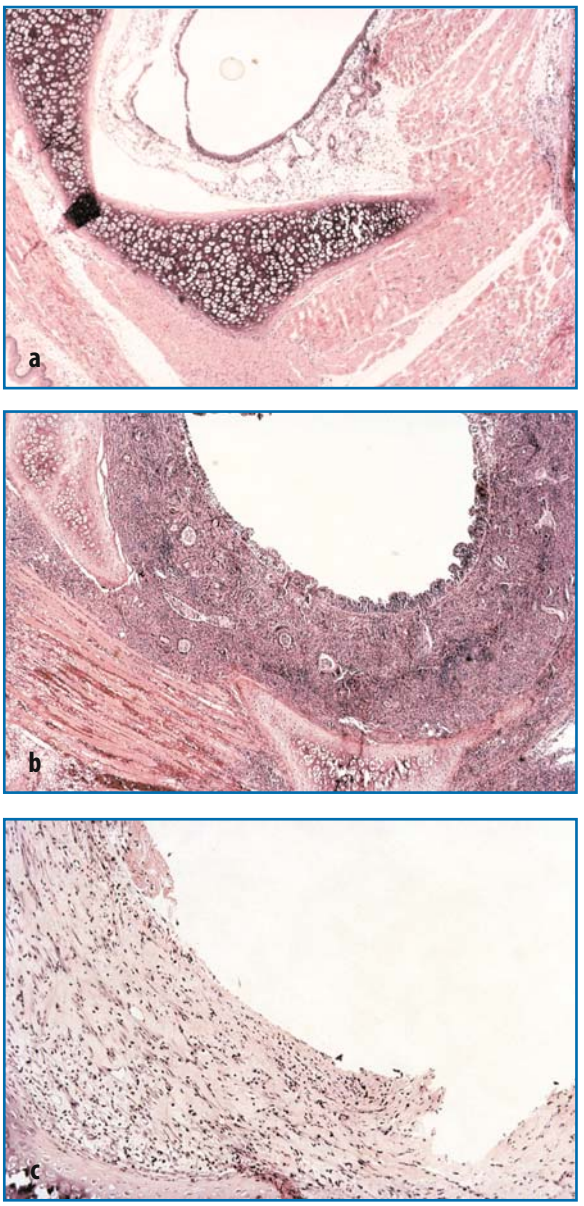


Fig. 5a-c. a Histologic specimen of rat laryngeal transplant in Lewis-to-Lewis rat negative control after 15 days following transplantation (10). Laryngeal architecture, including epithelium, minor salivary glands, muscle, and cartilage, is preserved. **b** Histologic specimen of rat laryngeal transplant in Lewis x Brown Norway (LBN-f)-to-Lewis rat after 7 days following transplantation without immunosuppression (10). Tremendous infiltration of lymphocytes occurs in the subepithelial layer. **c** Histologic specimen of rat laryngeal transplant in LBN-f-to-Lewis rat after 15 days following transplantation without immunosuppression (20). Laryngeal architecture is lost, with destruction of the epithelium and subepithelial minor salivary glands

Ten experimental arms were conducted between FK506 alone at varying levels and (FK506) combined with MMF at varying levels. Groups of 8 to 10 animals were examined at either 15 or 30 days posttransplantation. While increasing levels of (FK506) demonstrated increasing efficacy of immunosuppression, low-dose (FK506) in combination with MMF, achieved comparable results. Immunosuppressive investigation took an exciting step forward in 2003 with the demonstration of the ability to produce tolerance in the rat laryngeal model. Akst et al. treated transplanted animals with FK506 and mouse antirat alpha beta T-cell-receptor monoclonal antibodies for only 7 days following transplant [19]. At 100 days, all grafts demonstrated viability. Skin grafting, mixed lymphocyte reaction, and flow cytometry revealed that tolerance was neither donor-specific nor related to prolonged depletion of T-cell populations. Subsequent studies utilized parathormone production and pulsing of immunosuppressives around this 100-day time period to continue graft survival while avoiding continuous immunosuppression. More recent studies have focused on inducing long-term tolerance, donor-derived bone marrow transplantation, dendritic cell transplantation, as well as pulsing of immunosuppressive therapy

In other laryngeal transplantation laboratories, Genden et al. demonstrated that a single injection of ultraviolet (UV)-B-irradiated donor splenocytes was sufficient to prevent rejection in a rat tracheal graft model [20]. Birchall's group, using multiple color immunofluorescence, described a dense, organized network of immunologically active cells in the laryngeal mucosa in both pig and humans, the morphology of which suggest dendritic cells [21, 22]. Govindaraj et al. studied the role of tracheal epithelium in the rejection process. Those studies of mouse tracheal grafts showed that replacement of the epithelium by host epithelial cells prevents rejection after withdrawal of immunosuppression. The reepithelialization process was significantly quickened with application of vascular endothelial growth factor (VEGF) carried by a fibrin matrix to the mucosa [23].

Reinnervation Research in Laryngeal Transplantation

While the voice quality of our human laryngeal transplant recipient has remained exceptional,

volitional abduction of the vocal fold is not possible given the mass reinnervation of both the abductor and adductor muscle groups by the RLN. Therefore, he remains dependent upon a tracheostoma for his airway and has been reticent to sacrifice voice quality with a laser cordotomy that would allow for transoral breathing and closure of his stoma. Clearly, reinnervation and volitional movement of the vocal folds is critical to the success and wide acceptance of laryngeal transplantation. Stavroulaki and Birchall [24] reviewed the anatomy of the laryngeal nerves in humans and in the four animal models used to study transplantation: dog, cat, rat, and pig. In reviewing innervation of the human larynx, the authors remind us that nerve specification is more complicated than the dogma, which states that the anterior RLN is responsible for adductor function and the posterior RLN controls abductor function. In reviewing the different correlates between human and animal nerve anatomy, the authors even suggest that xenografting a porcine organ may become possible for restoring human phonation.

Sensory reinnervation of the transplanted dog larynx was studied by Blumin et al. [25]. In a randomized controlled study, 10 dogs had their superior laryngeal nerves transected. Half of the animals had the nerves reanastomosed, and all dogs were tested for laryngospasm in response to hydrochloric acid stimulation both preoperatively and 6 months postoperatively. Although none of the dogs regained normal laryngospastic responses, the reanastomosed animals exhibited protective EMG activity and coughing while the control group exhibited no response. In our human laryngeal transplant patient, at 3 months postoperatively, the supraglottis and vocal folds were sensitive to touch, initiating a severe cough. Stimulation through the stoma of the right side of the upper trachea elicited a sensation of touch without cough while stimulation of the left side was not sensed.

The question arises: what happens to nerve function if transplantation is performed several years after laryngectomy? Is there a way to "bank" the recurrent nerve in the neck during tumor resection to better preserve its function later? Again, using the dog model, Peterson et al. [26] attempted to answer this question. While one dog had its transected anterior and posterior branches of one recurrent nerve anastomosed to the distal ends of the ansa cervicalis, a second dog had the transected anterior branch inserted into the strap muscles while the posterior branch was transferred as a nerve-muscle pedicle to the sternothyroid muscle. Six months later, reanastomosis to the original nerve or placing the cut end into the original muscle was performed. Two weeks postoperatively, tensionometry, video, and EMG testing demonstrated that both methods of banking successfully restored vocal fold function and electrical activity specific to abduction and adduction. The Authors recommend that current patients who undergo total laryngectomy should, when it is oncologically feasible, undergo banking of the anterior and posterior RLN branches on at least one side.

Even hemilaryngeal transplantation has been achieved in the canine model. Andrews et al. [27] resected one dog's hemilarynx, including the thyroarytenoid muscle, arytenoid cartilage, and half the thyroid cartilage. Identical structures from a litter mate were then transplanted with reanastomosis of the recipient anterior RLN branch to the donor thyroarytenoid branch, along with an arytenoid adduction. Posterior cricoarytenoid and interarytenoid muscles were reapproximated with their counterparts. Postoperative immunosuppression included CSA, azathioprine, and prednisone. Two months postoperatively, spontaneous EMG recordings were made detecting reinnervation potentials in the thyroarytenoid muscle corresponding to the respiratory cycle. Endoscopic exam revealed that the transplanted hemilarynx was similar in appearance to the native side although the transplanted vocal fold remained fixed in the midline. Histologic sectioning revealed no evidence of graft rejection. The authors concluded that once immunosuppression has become more refined, hemilaryngeal transplantation may become a "theoretically ideal method of hemilaryngeal reconstruction".

Current Investigations

While the risks to transplant recipients are considered acceptable when transplantation is necessary to avoid death, in the case of nonvital or-

gan transplantation, the institution of long-term immunosuppression and its inherent perils is more controversial. One of the largest risks is the potentiation of recurrent malignancy or a cancer *de novo*. While our transplantation in 1998 was performed on an ideal recipient, a relatively fit, young trauma victim, there are only a few hundred such candidates in most countries. Other suitable, though rare, potential recipients would be those with large benign or low-grade malignant tumors of the larynx or those developing laryngeal malignancy who are already on a post-transplant immunosuppression regimen. Patients who have already undergone laryngectomy for cancer might be candidates if their superior laryngeal nerves could be located and there is no sign of recurrent cancer at 5 years or more. However, ultimately, the largest pool of patients who stand to benefit are those presenting with locally advanced laryngeal cancer, approximately 7,000 patients annually in the United States [28]. When the nonrevascularized partial laryngeal transplant was performed in 1969 [4] and again when a tongue transplant was performed in 2003, both in patients with advanced squamous cancer, both patients rapidly succumbed to recurrent disease. Therefore, an important step toward the goal of routine nonvital organ transplantation is development of immunosuppression that does not increase the risk of malignancy. Patients on chronic immunosuppression are known to have a 3- to 4-fold increase in risk for development of de novo malignancies [29].

Everolimus, a derivative of rapamycin, has been shown to have potent immunosuppressive as well as antiproliferative effects [30]. Belonging to the mammalian target of rapamycin (mTOR) class of immunosuppressants, everolimus blocks the translation of mRNA of critical cell-cycle regulatory proteins. In addition, everolimus has been shown to inhibit development of posttransplant lymphoproliferative disorders as well as a variety of other tumors *in vitro* and *in vivo* [31]. Recent data in our rat laryngeal transplant model provided support for use of everolimus as an effective immunosuppressive in laryngeal transplantation. Everolimus' effect upon the growth of a mouse squamous cell carcinoma antigen (SCCA) cell line in both intradermal tumors and pulmonary metastases was more recently studied [32]. Mice received either everolimus 1 mg/kg twice daily, everolimus 0.5 mg/kg twice daily, CSA 7.5 mg/kg per day, or no treatment. Tumor cells were injected either intradermally or pulmonary metastases were established through tail-vein injections. Everolimus showed statistically significant tumor inhibition at 1.0 mg/kg twice daily and 0.5 mg/kg per day when compared with animals treated with CSA and untreated animals ($P<0.0001$), with tumor inhibition evident in both models studied (intradermal tumors and pulmonary metastasis generation). The Authors concluded that everolimus provides potent tumor inhibition in animals inoculated with SCC cells by decreasing local spread of disease as well as distant metastases.

Conclusions

Now, more than 8 years after its attempt, successful total laryngeal transplantation has become a reality. The patient reports a vastly improved quality of life, including smell, taste, daily communication, and emotional expression through a voice that is uniquely his own. As healthy individuals, we take for granted the simple ability to clear our own nose or to express a cry with which others can empathize. But in a 2001, review of the human larynx transplant, the reviewer, a transplant physician himself who underwent a laryngectomy 7 years earlier, stated that given the despair associated with losing his own larynx, "if I were 40 years old I would probably consider undergoing the operation myself." [33]. Clearly, advances in the areas of immunosuppression and reinnervation must continue. But given present-day research in all transplanted organ systems, the day is not far off when immunosuppression without comorbidities will be a reality, and the lessons we learn today will benefit the many potential candidates for laryngeal transplantation in the future.

Acknowledgment
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Section 11-c

Uterus Transplantation

Wafa Fageeh, Giovanna Lucchini

Introduction

Uterine transplant is at a crossroads. It is possibly the best solution for approximately 4% of infertile women who undergo the frustration of being unable to conceive due to the absence of a uterus. In some cases, this absence is due to congenital abnormality (e.g. Rokitansky's syndrome). In other cases, it is due to surgical removal of the uterus at a relatively young age due to clinical emergencies, such as obstetric uterine rupture.

Until recently, only life and death situations warranted organ transplantation. Nonvital transplantation simply to fulfill a patient's wishes or goals was not considered justified. It can be argued, however, that this distinction is not morally significant. Patients with kidney failure, for example, can be kept alive by dialysis, but their quality of life can be greatly enhanced by kidney transplant, which is thus considered a justified procedure. So a spectrum of rationales may justify transplantation. Therefore, the only chance for women without a uterus to have babies was by resorting to surrogate gestation. In many countries, this procedure is prohibited by the law. In some other countries, surrogate mothers are registered as legal mothers of babies born through surrogacy. Another problem that parents may face if they decide to try surrogacy is that they have no control over the course, care or outcome of such pregnancy. These and other ethical, moral and religious issues surrounding surrogacy have left these women to hope for the possibility of uterine transplant. Transplantation of the uterus would relieve the anguish of women who greatly desire to conceive a child and allow them hope for an opportunity to become pregnant.

A clinical milestone was made by Dr. Wafa Fageeh and her team when they successfully transplanted a human uterus in Saudi Arabia in the year 2000 although the transplant lasted only 99 days [1]. The furor that followed this partial success reveals the range of views of the scientific community and the public at large. Of course, the concerned women were enthusiastic and delighted at the potential for progress in this technique. Sceptical scientists raised the question as to whether this "non-life-saving transplant" was necessary at all? Only further progress in transplant science can shed more light on the answer to this critical question. Antirejection drugs are nonetheless becoming safer, and patients with cardiac and renal transplants have had successful pregnancies. Thus, in the progress of the development of nonessential human transplants, the uterus seems to be leading the way because of the successful, if only temporary, transplant in 2000. The three menstrual cycles that followed indicated functionality of the uterine endometrium but the unfortunate incident of thrombus was a drawback.

While other organ transplant donations most often come from cadavers and less often from living donors (kidney or partial liver), the donor

source for a uterus may be an otherwise healthy living patient who requires uterus removal as a care procedure. Furthermore, it should be mandatory to remove the transplanted uterus from the recipient after successful pregnancies so the patient need not be subjected to lifelong antirejection medications.

Since animal uterus transplantation has been done successfully, human uterine transplantation could be considered for select cases.

The interest in uterine transplantation has been increasing in the scientific community since the mid-twentieth century, with the aim of overcoming infertility problems linked to uterine absence or uncorrectable anomalies. It has been stated that 5–10% of cases of infertility are caused by either congenital or acquired uterine disorders, among which are Müllerian agenesis, Mayer-Rokitansky-Kuster-Hauser syndrome, leiomyomas, Asherman's syndrome and hysterectomy are the most common [2]. New reproductive procedures are of no help in these situations. The only chance for women affected by these problems to give birth is to rely on gestational surrogacy, which consists of using gametes of a genetic couple to produce embryos that are then transferred to the womb of a woman who agrees to act as a host for the pregnancy [3]. The technique is legally approved in Canada, UK, Brazil, South Africa, Israel, Hungary, the Netherlands, Australia and some states in the USA. Other countries have no specific legislation on the practice of surrogacy (Greece, Argentina, Belgium, Finland and India). In most Muslim countries and some Asian territories, it is strictly forbidden on the basis of religious or ethical grounds. Even in nations that have accepted this procedure by law, scattered incidences of resistance based on ethical, psychological, religious and economical issues have been reported [4, 5].

Around 15% of all couples are infertile. Most resistant cases have been helped by assisted reproductive technologies, such as in vitro fertilisation (IVF) and intracellular sperm injection (ICSI). However, for women who have healthy ovaries but have had a hysterectomy or serious uterine problems due to injury or congenital conditions, a transplant could provide their only hope for experiencing a pregnancy of their own. At present, they can choose IVF, in which their own egg and their partner's sperm can be used for gestational surrogacy. Technically, this is straightforward, but it may not always be suitable for many couples. Uterine transplants could help up to 47% of infertile women. The surgery would be comparable to a kidney transplant and would offer advantages over surrogacy, especially in countries where it is not allowed.

With a uterine transplant, any health risk of pregnancy, such as high blood pressure, are taken by the genetic mother, which makes it acceptable from an ethical point of view. There is also no financial consideration, a situation often criticised with surrogacy. It also clears up complications regarding who is the legal mother. In some countries, the legal mother is the woman who gives birth regardless of who is the biological mother. With transplantation, the biological mother would be the legal, social and gestational mother.

Since a uterine problem is a factor in 3–4% of infertile women, there would probably be no shortage of women willing to try the technique because according to Dr. Mats Brännström of the Sahlgrenska Academy at Göteborg University in Sweden, he has received hundreds of enquiries from women who have read about his pregnancy success with uterine transplants in mice. Several volunteers contacted Fageeh's team to be donors, and several others as would be recipients, indicating that there is definitely a demand for this procedure even though the numbers may be small.

Landmarks in Organ and Uterine Transplantation

The advent of organ transplants began with that of the kidney in the early 1960s. Inspired by the success, transplantations of other vital organs, such as liver, heart, lung and pancreas, followed. In the beginning, azathioprine (Imuran) and prednisone were the only available immunosup-

pressive agents. In 1980, with the introduction of cyclospine therapy, the prognosis for transplanted organs became better. Progress in safer and more easily tolerated immunosuppressive therapy has opened the doors for the transplant of nonvital organs, such as the uterus.

The first study of ovarian transplantation was published as early as 1896 by Knauer. However, attempts at uterine autotransplantation did not begin until 1918 [6]. Autotransplant of a uterus in a dog by Eraslan, Hamernik and Hardy in 1964 and 1966 was the first to end in a successful delivery [7]. Confino, Vermesh and Gleicher introduced the use of cyclosporine therapy for uterine allotransplantation in rabbits in 1986 [8].

In 2000, a human uterine transplant was performed in Saudi Arabia by Fageeh et al. [1]. Postoperatively, the patient had three spontaneous menstrual cycles followed by amenorrhea. Exploratory laparotomy confirmed uterine necrosis due to vascular thrombosis. There was no evidence of rejection. The attempt, however, raised discussions on many moral and ethical issues. The scientific community, although deeply divided, consider this as the only reference to a human success. Recently, interest has fallen on further exploring the feasibility of human uterine transplantation as a replacement for surrogate gestation.

Work on Animal Models

The story of experimental animal models used for the purpose of uterine transplantation begins in the early 1960s [7–14]. Sheep, dogs, macaques, rabbits and rats were used in both autologous and homologous transplantations. The intention was to understand two main areas of this technique: (1) recreation and stable vascularisation of the uterus, with anatomical network of small vessels, and (2) modulation of immunosuppressive treatment in order to avoid rejection, prevent toxicity for the mother and eliminate teratogenicity for the foetus. Vascular support of the pelvic region was crucial to graft survival and was therefore the most studied element in the first proposed animal models. This led to the development of different techniques to obtain good viability of the transplanted organ. Among these, omentopexy has been commonly used to obtain a milieu that supports spontaneous revascularisation, fixation of the uterus to the broad ligament has been tested with good results and the more classical vascular anastomosis has been improved to the point of becoming the most efficient surgical option. All these studies proved the surgical feasibility of the transplantation and even attempted to recreate the function of the normal uterus by producing some examples of pregnancies and deliveries in the grafted animals. From an immunological point of view, experimental models using azathioprine and prednisolone were tested but never achieved outstanding results in avoiding rejection and because close monitoring of serum drug levels was not easily carried out.

More recent epidemiologic and experimental studies have underlined the effect of some immunosuppressive drugs on the foetus. Azathioprine has proven to be mildly teratogenic on rats whereas corticosteroids seem to be linked to a general augmented risk of cleft palate development [15, 16]. Cyclosporine has shown to restrict foetal growth, resulting in low birth weight. However, these data were collected in a population of patients suffering from autoimmune diseases, and therefore the role of these diseases on gestation is still to be clarified [17].

Fageeh performed 16 autologous orthotopic uterine replantation on baboons and 2 on goats. After a midline abdominal incision, hysterectomy was done so as to preserve tissue and vascular integrity. The uterus was then flushed with Euro-Collins solution and replanted in the same animal with cervico-vaginal anastomosis. The first 8 animals had end to end uterine vascular anastomosis but occlusion and vascular thrombosis was observed in 12 out of the 16 vascular connections. It was therefore decided to change to an end to side anastomosis between uterine vessels and internal iliac vessels, which offered better results (18 out of 20 vessels remained viable). After 6 to 12 weeks the animals underwent abdominal exploration that showed survival of the uterine graft and good vessels patency [1].

To complete the history of experimental surgery in uterus transplantation we need to quote the most recent animal model of this kind. The researcher leading the work on mice, Dr. Mats Brännström of the Sahlgrenska Academy at Göteborg University in Sweden and his team proposed a mouse model for homologous uterus transplantation. They had seen pregnancies in mice with donor uteruses which resulted in healthy babies. The mice used were syngenic (inbred strain) and the vascular technique used was that of end to side anastomosis between donor uterine vessels and recipient inferior cava vein. The viability of the uterus was sequentially examined and proved to be good for 8 weeks post operatively. The function of the transplanted uterus was evaluated inducing pregnancies with good results. This model is still under development [18].

In their original work, the team took a uterus from a donor mouse and transplanted it alongside the recipient mouse's own uterus. This meant they could compare how both worked.

The team led by Mats Brännström grafted one arm of the V-shaped mouse uterus from a donor mouse into another's abdomen, alongside its existing uterus. The implanted partial uterus was connected to the mouse's blood supply. Several days later, tests demonstrated that blood flow in both organs was similar, the team says. Three fertilized embryos were then transplanted into each of the uterus.

Their report, published in the Journal of Endocrinology (V 174, Pg 157), reveals that one of the three in the donor organ, and all three in the mouse's native uterus, developed into healthy fetuses. The experiment was terminated after 13 days, two-thirds of the way through the pregnancies, due to ethical restrictions placed on the research.

The reason their experiment on mice worked is because they connected the vascular system of the implanted uterus directly to the existing blood supplies, rather than using stents which have caused other transplants to fail.

As predicted by the Swedish researchers, the procedure conducted in mice would be easier to repeat in humans. In a woman, the procedure would involve removing the existing organ completely and replacing it by the donor uterus.

The First Human Uterine Transplant

The first human uterine transplant is reproduced in its entirety here to shed clarity on this very important landmark in uterine transplantation.

Introduction

During the past three decades, scientists have made tremendous efforts to solve infertility problems; indeed, the achievements and developments that have occurred in this field have had a considerable clinical impact [18]. Infertility due to the absence of a uterus or to a congenitally malformed uterus with normally functioning ovaries, has remained an obstacle to pregnancy, however, especially in communities where surrogate gestational carriers are approved by neither religious nor ethical authorities.

Uterine transplantation could provide a solution to this problem, but its feasibility, safety and reproducibility remain to be proven. To evaluate the potential for safe, successful, uterine transplantation in humans, we reviewed earlier animal experiments and clinical trials. The main difficulty was vascular anastomosis between uterine vessels of donor and recipient [20]. Unlike other organs where large vessels are the source of blood supply, in the uterus, the blood supply and drainage occur through a net of tiny vessels. Most earlier animal experiments were performed with avascular techniques that led to failure and the formation of pelvic abscesses [8]. Human trials were limited to transplantation of endometrial tissue [21], and no documentation of successful uterine transplantation was available in the English literature.

The Islamic religious position on uterine transplantation was clarified in March 1990, before initiation of this project, when the Islamic Jurisprudence Council approved the transplan-

tation of reproductive organs that do not transfer genetic coding.

Experimental Animal Studies

The project conformed with the Guiding Principles in the Care and Use of Laboratory Animals approved by the authorities of the King Fahd Medical Research Center. Previous experiments had proven the feasibility of uterine transplantation in animals, with successful pregnancy [7]. As the main difficulties lay in uterine vascular connections, some researchers performed avascular uterine transplantation in the animals, which resulted in failure and in the formation of pelvic abscesses. We, therefore, decided to concentrate our animal studies on uterine reimplantation rather than transplantation. We focused on the vascular surgical anatomy and its variations [22], the physiology of the uterine blood flow and mastery of microvascular techniques of uterine arterial and venous anastomosis.

Autologous orthotopic uterine reimplantation was performed on 18 virgin female animals (16 baboons and two goats). The baboons' average age, weight and height were 2–4 years, 15.6 kg and 37 cm, respectively; the goats' average age, weight and height were 2–3 years, 20–30 kg and 60–71 cm, respectively. Surgery was performed with the animals under general anaesthesia without muscle relaxation. Prophylactic antibiotics (tetracycline, 20 mg/kg body weight) were given for 5 days. In each animal, a midline abdominal incision was made. Hysterectomy was modified to preserve tissue and vascular integrity. The extirpated uterus was flushed in both the antegrade and retrograde manner with 60 cm^3 of cold Euro-Collins solution then reimplanted orthotopically in the same animal by doing cervicovaginal anastomosis. The first eight animals underwent end-to-end uterine vascular anastomosis, but anastomotic occlusion and pelvic abscesses occurred due to graft failure and vascular thrombosis in 12 of the 16 (75%) vascular connections. Therefore, the technique was modified so that the anastomosis was performed between the uterine vessel and the internal iliac vessels in an end-to-side fashion using monofilament, nonabsorbable polypropylene sutures. This modification was technically easier to accomplish. It also provided wider anastomotic stoma and a higher success rate in the remaining ten animals, with proven vascular patency in 18 of 20 (90%) vascular connections. All animals underwent abdominal exploration after 6–12 weeks to evaluate survival of the reimplanted uterus, and the following steps were taken:

- assessment of vascular patency by visualising emptying and refilling of veins and pulsatility of arteries, and by palpation of the arteries for presence or absence of thrill

assessment of uterine and fallopian tube viability by evaluation of their color and texture

- observation of bright red fresh bleeding from the tissue on abrasion or puncture
- determination of pelvic infection.

Our animal studies demonstrated survival of the uterine graft and indicated that good mid- and long-term vessel patency could be achieved using skillful microvascular techniques for uterine arterial and venous anastomosis in an end-to-side fashion.

After reviewing the earlier reported experimental work by other researchers [6] and our satisfactory results, we decided to prepare for a human trial. Protocols for human uterine transplantation were designed detailing indications, contraindications, selection criteria, surgical techniques, immunosuppression regimen and clinical follow-up. Detailed informed consent forms were prepared for the donor and recipient according to the guidelines and regulations of the Food and Drug Administration (FDA).

Materials and Methods

The potential recipient was a 26-year-old woman who had undergone a hysterectomy in 1994 because of massive bleeding following a cesarean section. She had consulted us concerning the possibility of uterine transplantation and after thorough evaluation was found to be eligible. The donor was a 46-year-old woman who presented with bilateral multiloculated ovarian

cysts measuring 8×6 cm on the right side and 3×2 cm on the left side. Hysterectomy with bilateral salpingo-oophorectomy was planned, as this patient agreed to donate her uterus. ABO compatibility, human leukocyte antigen (HLA) tissue matching and negative cytotoxic antibodies in the recipient were confirmed.

The Procedure

The Donor

On 6 April, 2000, uterine extirpation was carried out with the patient under general anesthesia. The donor's abdomen was opened through a midline incision; bilateral en-block oophorectomy was performed, and the ovaries were sent for frozen section, which confirmed the benign nature of the cysts. Uterine removal was accomplished using a technique modified so as to maintain the vascular pedicle of the uterus as long as possible and thus maintain tissue integrity. The long vascular pedicle was maintained by transecting the round ligaments as far laterally as possible. Ureters were identified and protected. Infundibulopelvic ligaments were clamped, divided and sutured. Pararectal and paravesical spaces were developed with care to avoid traumatising the numerous small veins in the broad ligaments and paravesical space. Uterine arteries were then encircled with vessel loops. Uterosacral ligaments were serially divided and sutured. The uterovesical peritoneum was incised, and the bladder was separated from the cervix and vagina. At that stage, methyl prednisolone (500 mg) and heparin (20,000 IU) were given IV. Uterine arteries were clamped 1 in. away from the uterine body (Fig. 1). The vagina was entered by circumferential incision and the extirpated uterus immersed in cold saline for topical hypothermia. The graft was flushed with modified, cold (4°C), Euro-Collins solution, antegrade through uterine arteries and retrograde through uterine veins, to ensure removal of all white blood cells and fibrin and to induce central core cooling for tissue preservation during the ischaemia period. The uterosalpingeal graft was additionally trimmed to ensure removal of any remnants of unwanted tissue (Fig. 2). A 6-cm-long segment of the great saphenous vein and an 8-cm-long reversed segment were anastomosed to each uterine vein and artery, respectively, with 6×0 nonabsorbable polypropylene suture (Prolene Ethicon) on a sterile side bench to extend the length of vascular pedicles (Fig. 3). The eight vascular grafts were flushed again with Euro-Collins solution to check for any anastomotic leaks. A small laceration of the anterior wall of the donor's left ureter was found and was splinted with a double J tube and sutured by the urologist.

The Recipient

A preoperative oral dose of cyclosporine (4 mg/kg body weight) was administered 6 h prior to surgery, and methyl prednisolone (500 mg i.v.) was administered to the patient at induction of anaesthesia. The recipient's laparotomy was started when donor uterine extirpation was imminent. A midline subumbilical incision was selected, and intra- and retroperitoneal adhesions were lysed. Internal and external iliac vessels were dissected bilaterally. The bladder and rectum were dissected from the cervical stump, and the latter was excised. The donor uterus was placed in orthotopic position, and the cervix was then sutured to the recipient vaginal vault by single, interrupted, nonabsorbable 2×0 Ti-Cron (Ethicon) sutures. Uterosacral shortening was accomplished using two nonabsorbable 2×0 Ti-Cron sutures. The extended uterine veins and arteries were then anastomosed to the external iliac veins and arteries, respectively, with 6×0 Prolene. No ovarian arterial or venous anastomosis was performed. Five hundred milligrams of methyl prednisolone was given IV on releasing the iliac clamps and reestablishing uterine perfusion. The abdomen was closed in layers after complete homeostasis. The recipient made an uneventful recovery with good wound healing. White blood count, cyclosporine level and creatinine phosphokinase enzyme levels were checked twice a week. Immunosuppression consisted of oral cyclosporine (4 mg/kg body weight) divided into two doses to assure a serum trough level of 200 ng%, azathioprine (Imuran) (1 mg/kg body weight) and prednisolone, with a maintenance dose of 0.2 mg/kg body weight. The adequacy of immunosuppression was monitored by measuring the lymphocyte subpopula-

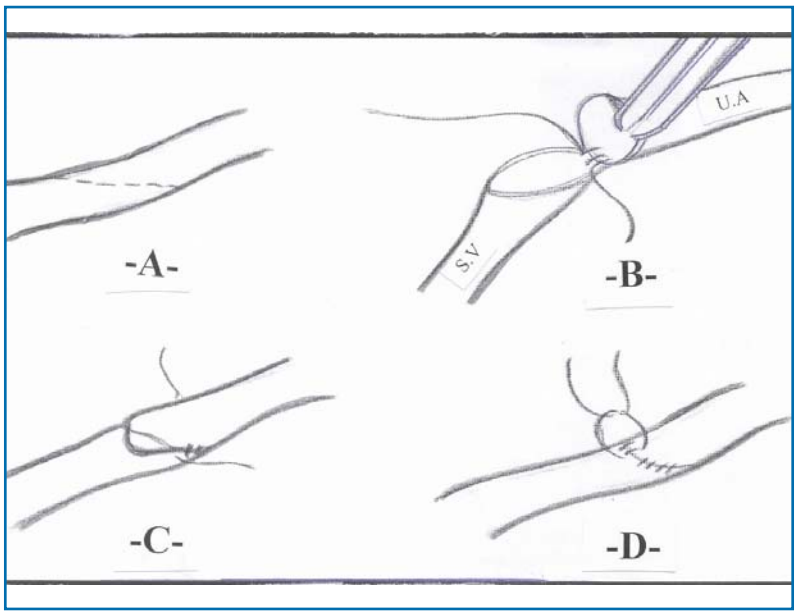

Fig. 1. Preparation of vascular pedicles

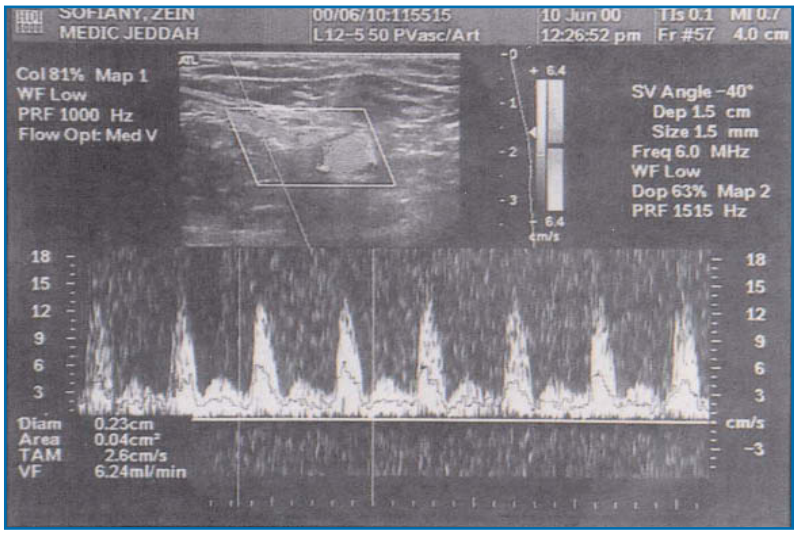

Fig. 2. Doppler ultrasound with excellent uterine artery filling

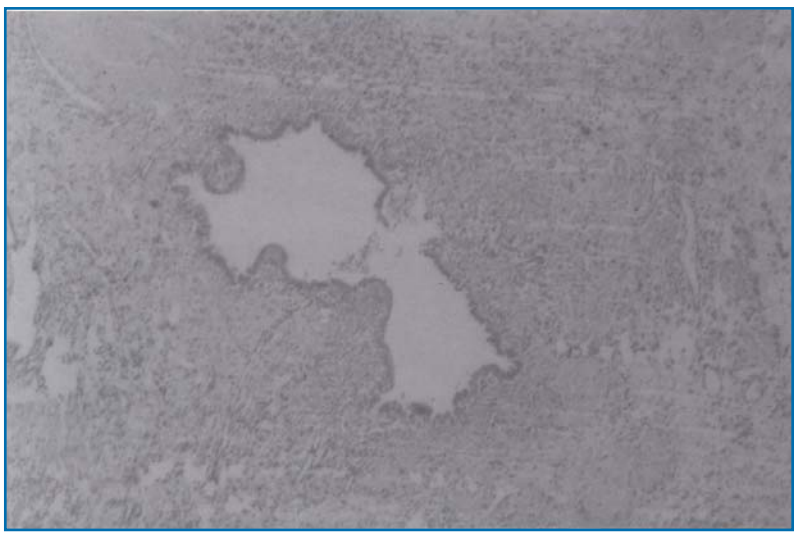

Fig. 3. Tubes with patency and no rejection

tion (CD4/CD8 = helper/suppressor) cell ratio by cytoimmunological cytometer (FACS Scan) and Doppler ultrasound to study flow volume, pulsatility and resistance index [23]. On the ninth postoperative day, the patient complained of low abdominal and back pain, general fatigue, malaise and body aches. She had minimal serosanguineous vaginal discharge, low-grade fever and tachycardia, indicating acute rejection. The CD4/CD8 ratio was found to be reversed to 3.4. Abdominal Doppler ultrasound showed increased brightness due to myometrial oedema. The patient was treated by increasing the oral doses of cyclosporine and azathioprine and administering an intravenous pulse of methyl prednisolone. The rejection did not resolve, however. Antithymocytic globulin (ATG) (2.5 mg/kg body weight) was given, controlling and resolving the rejection phenomenon. Cervical inspection on the 12th day revealed good healing of the cervicovaginal anastomosis, with some venostasis of the lower one third of the ectocervix. Biopsy was not attempted so as to avoid anastomotic disruption. The symptoms of rejection disappeared after 2 days, and the CD4/CD8 ratio was 1.3. Doppler ultrasound revealed excellent bilateral uterine arterial perfusion, with low resistance indices (Fig. 4). Hormonal therapy with oestrogen and progesterone (Progyluton) was given for the first 3 months to build up the atrophic endometrium. Two withdrawal bleedings occurred promptly after cessation of hormonal therapy. These were considered to reflect good blood perfusion and viability of the transplanted uterus.

Removal of the Transplanted Uterus

On the 99th day, the patient experienced a sudden feeling of heaviness, with a foul-smelling vaginal discharge on straining. Speculum examination revealed a dusky-coloured cervix prolapsing into the vagina. Immediate Doppler ultrasound confirmed cessation of uterine blood flow. A diagnosis of mechanical occlusion of the uterine vessels with resulting uterine infarction was made, and the need to perform a hysterectomy became obvious. At surgery, the uterus was found to be infarcted, and the uterine arteries, veins and their supplying grafts were thrombosed. Both fallopian tubes remained pink and viable, however. Histopathologic microscopic examination confirmed the above findings as well as the viability of both tubes and absence of any rejection (Fig. 5).

Discussion

Advances in immunology make organ transplantation for end-stage organ failure a clinical reality [24]. Advances in microvascular surgery and tissue preservation as practiced in ovarian transplantation [25] provide support for major steps in the new era of the surgical management of infertility [26]. Such advances can be applied

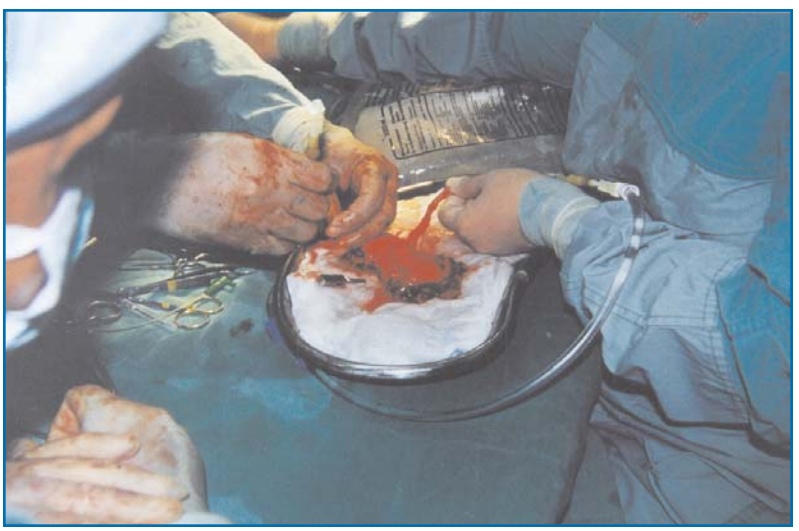

Fig. 4. Irrigation of donor vessels

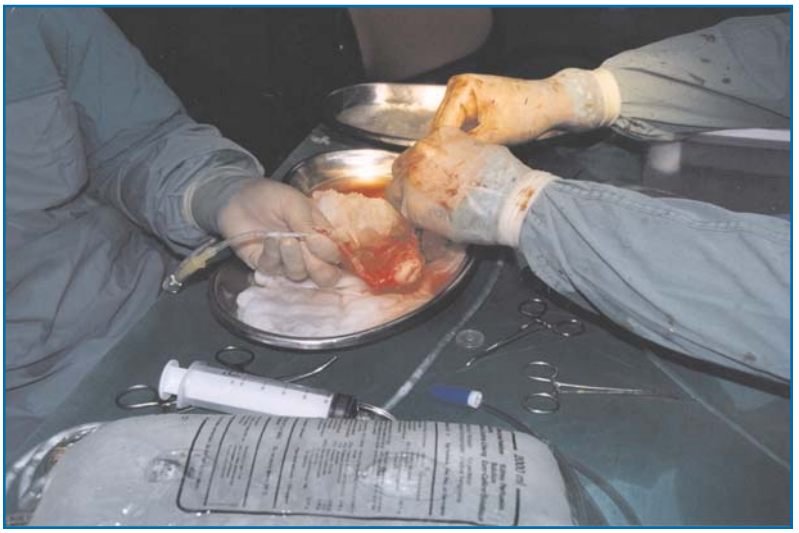

Fig. 5. Preparation of donor uterus

successfully in uterine transplantation, and indeed, our experimental work with microvascular uterine vessel anastomosis provides ample clinical evidence of good mid- and long-term vascular patency and graft survival.

Simple noninvasive techniques, such as Doppler ultrasound, to monitor and detect early rejection are essential. Cytoimmunological monitoring for activated lymphocyte subpopulation (CD3/CD4) cell ratio using monoclonal antibodies is a simple, noninvasive technique to monitor rejection, with sensitivity and specificity approaching 96% and 88%, respectively [27]. Punch biopsy from the endocervix to detect and histopathologically grade rejection seen as myocyte necrosis and perivascular infiltration of lymphocytes is an invasive procedure that could be associated with certain risks. It was, therefore, not applied in our patient.

Modification of the hysterectomy technique in the donor is essential to promote preservation of a longer vascular pedicle and application of a gentle, atraumatic technique to preserve the uterus and differs from conventional hysterectomy. Extension of the vascular pedicle for a required length using a conduit such as the great saphenous vein or the radial artery may be advantageous in selected patients, and application of microvascular techniques by an experienced vascular surgeon is mandatory. The use of fine polypropylene monofilament, nonabsorbable sutures is required. Suspension of the uterus to the anterior abdominal wall (ventrouteropexy) and by uterosacral shortening is essential to avoid displacement of the uterus with consequent tension, torsion or kinking on the vascular pedicle and anastomosis, with obstruction of blood flow and vascular thrombosis.

Conclusion

Our clinical results with the first human uterine transplantation confirm the surgical technical feasibility and safety of this procedure in gynaecologic, surgical and vascular terms. Acceptable short- and midterm outcomes were documented by good endometrial proliferation on hormonal therapy and the occurrence of two withdrawal bleedings in the transplanted menopausal donor uterus.

An understanding of the surgical vascular anatomy and physiology of uterine blood flow and the application of microvascular techniques in uterine vessel anastomosis solved the earlier reported difficulties encountered in that aspect. Cytochemical and cytoimmunological noninvasive techniques for monitoring graft rejection are useful and reliable. Preservation of tissue and vascular integrity during uterine extirpation

is essential. A vascular pedicle of good length with the possible use of an extension conduit, such as the radial artery or the great saphenous vein, could be required. Strong fixation of the transplanted uterus to the anterior abdominal wall and the sacral promontory is required, as the uterus lacks the support of the uterosacral ligaments and could develop slow progressive or acute prolapse with consecutive thrombosis, infarction and loss of the uterus.

Further clinical experience and additional development of the surgical techniques could make uterine transplantation useful in the treatment of infertility, especially in communities where the surrogate mother concept is unacceptable from a religious or ethical point of view.

Controversies

Unlike other organs, which are supplied by large blood vessels, the uterus receives its blood supply from a network of tiny vessels. This means that establishing a blood supply for the transplanted organ is extremely complex and prone to problems. In addition, blood vessels supplying the uterus must be able to expand to three times their normal size during pregnancy if they are to support a developing foetus.

The Future

Uterine transplantation is still supported by gynaecologists who believe that advancement in microsurgery and immunology may allow the achievement of good results without major side-effects or risks for the transplanted mother and her foetus. Two frontiers clearly lie in the path of progress of further development in uterine transplant. One is improving and optimising immunosuppression techniques. The second is to develop an ideal vascular model for uterine transplant, its survival and functionality and subsequent pregnancy.

According to Brännström: “Suitable donors could be either a sister after she has had her own children or a mother since the chance for a good immune and blood type match would be high. It would be possible to carry your own child in the same womb [donated by mother] as you developed during your growth as a foetus” [18]. Commenting on the work by Brännström and his team, Dr. John Mills, chairman of the British Fertility Society and a consultant obstetrician and gynaecologist at Ninewells Hospital, Dundee, UK, said: “This paper has described successful pregnancies in the mouse, at least to the early pregnancy stage, and will obviously give hope to those surgeons who are interested in carrying out a similar operation in humans. More evidence of success in other animals will be required before it is justified to make such an attempt.” He said there was a huge difference between mice and humans, which meant much more work was needed. He also said the Swedish work and successful pregnancies in women who had taken immunosuppressant drugs after kidney or heart transplants showed that progress is being made on the issue of reducing the rejection of transplants.

US experts Dr. Louis Keith and Dr. Guiseppe Del Priore described transplantation of the reproductive organs as the “last frontier” in the field of organ transplantation. To some individuals, childbearing is the greatest event of a lifetime. To such persons, transplantation of organs of reproduction would not be considered frivolous or unnecessary even though these organs do not sustain life [31]. Further clinical experience and additional development of the surgical techniques could make uterine transplantation useful in the treatment of infertility, especially in communities where the surrogate mother concept is unacceptable from a religious or ethical point of view.

Dr. Richard Smith from the Chelsea and Westminster Hospital in London, who has been carrying out laboratory experiments to test the feasibility of a uterus transplant, says that a similar operation should be possible in the UK in 2 years. According to him ,there is a small group of women who are very keen to have children and who would be prepared to undergo that sort of surgery to achieve that end [31]. Peter Bowen-Simpkins, from the Royal College of Obstetricians and Gynaecologists, said he believed the develop-

ment would eventually lead to women without a uterus being able to give birth. "This shows it is technically possible. The womb survived for more than two menstrual cycles, so the first crucial hurdles have been passed" [31].

The Web to Assist the Progress of this Procedure

Web and Internet-based activities have shown an ability to bring together persons keen on further development of techniques as well as clientele looking for venues to discuss their experiences and other cooperative efforts. In relation to uterine transplantation, these sites include:

- www.uterinetransplant.com
- www.uterinetransplant.net
- www.uterinetransplant.org

The latter two were under development at the time of publication of this material. Monitoring contemporary views and progress will continue on www.uterinetransplant.net, which will also provide a platform for publications. Researchers and clients will use the forum available at www.uterinetransplant.org to continue exchanging views and cooperate with each other.

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Section 11-d

Abdominal Wall Transplantation: A Review of the Literature

Giovanna Lucchini, Marco Lanzetta

Introduction

Patients suffering from intestinal failure and total-parenteral-nutrition-derived complications can be treated with either intestinal or multivisceral abdominal transplantation. These operations have greatly increased in number in the last decade (more than 1,000 reported cases) because of the improved survival rate due to advances in surgical technique, better immunosuppressive regime, donor selection and recipient care [1]. However, there are cases where achieving primary closure of the abdominal wall at the end of the surgical procedure is very difficult. Patients requiring intestinal transplantation have often previously undergone intestinal resection, with loss of the small-bowel domain. Their abdominal wall structure may be severely altered because of laparotomies, enterocutaneous fistulae, infections and tumours. Moreover, there is always a donor/recipient size mismatch and severe postoperative graft oedema, and it is important to avoid compression of intra-abdominal viscera so as not to impair vascular supply or alter respiratory dynamics. A number of options to close the abdominal wall and avoid severe postoperative complications include graft-size reduction, skin grafts or myocutaneous flaps to close the defect, abdominoplasty techniques [2] or the use of prosthetic materials [3–5].

Abdominal wall transplantation was first introduced by the group led by Levi in 2003 in an attempt to solve the problems associated with difficult closure of the abdomen [6]. The allograft is a full-thickness, vascularised, myocutaneous free flap taken from a brain-dead donor and includes one or both rectus abdominis muscles, fascia, subcutaneous tissues and skin. Few experimental studies have been reported, but none of them to this extent [7].

Surgical Technique of Abdominal Wall Transplantation

As reported by Levi's [6] technique, graft procurement is part of the cadaver, heart-beating donor harvesting procedure. A median sternotomy and bilateral subcostal incisions are carried out. Longitudinal incisions are made following both lateral edges of the rectus abdominis muscles sheath. Skin incisions are then prolonged to the groins bilaterally, a final suprapubic incision is performed and the common femoral vessels are identified. The wall graft is flushed with cold University of Wisconsin (UW) solution through the aorta. The graft is then removed, together with the femoral and iliac vessels and with a short segment of distal aorta and inferior vena cava. Closure of the donor abdomen is achieved by mobilising skin and subcutaneous tissue flaps from the lateral abdomen and flanks.

The abdominal wall is transplanted to the recipient following reperfusion of the other transplanted visceral organs and adds more or

less 2 h to the surgical procedure. Vessels of the abdominal wall graft are usually connected to the recipient's common iliac artery and vein (but the infrarenal aorta and inferior vena cava or the distal aorta and infrahepatic vena cava may also be used). The graft is rotated and positioned according to the location of the abdominal wall defect then sutured in layers.

Immunosuppressive Treatment

All grafts are carried out without human leukocyte antigen (HLA) matching, assuming that this is of little clinical significance in intestinal transplantation. However, ABO matching is much more relevant. The standard induction treatment consists of anti-CD52 monoclonal antibody (alemtuzumab 0.3 mg/kg) given i.v. immediately preoperatively, immediately postoperatively and on days 3 and 7 postoperatively and tacrolimus (FK506). Maintenance therapy is based on tacrolimus (target serum concentration 10 μ/l) without steroids. A variation of this drug regime was used for the first paediatric case and then abandoned (daclizumab as an induction agent; tacrolimus together with methylprednisolone for maintenance). Corticosteroids are used as rescue therapy in case of rejection.

Patients

Between May 2001 and July 2003, 9 patients (5 males and 4 females; four being paediatric) received a transplantation of the abdominal wall together with either intestinal or multivisceral grafts [8]. Ten abdominal walls were transplanted because one patient received two consecutive grafts. Table 1 shows the details of their clinical history.

The size of the grafted free flap was dependent on the anatomic characteristics of the donor, with an area ranging from 150 cm^2 to 500 cm^2. In two cases, the abdominal wall and internal organs were harvested from two different donors and implanted but not simultaneously (at 2 and 7 days postoperatively, respectively). Clinical reasons for using two donors were the need to find a better size-matched donor in one case and the relatives' refusal to allow abdominal wall harvesting in the second.

Patients were followed up to 33 months. Four were alive and well at the time of this writing; five had died. Causes of death were sepsis (three cases), uncontrollable rejection (one case) and primary nonfunction of the transplanted intestine (one case). In all these patients, the abdomen wall graft was still viable at the time of death. In two cases, the allograft had to be removed – in one because of an impaired venous outflow (at day 6 postoperatively) and in another because of severe hypoperfusion both of the intestinal and of the abdominal wall graft. Removing the abdominal wall did not affect the remaining grafted organs.

Abdominal wall graft did not lead to increased morbidity or mortality and proved to be a far better choice compared with more conventional techniques, such as leaving the

Table 1. Details of the nine patients grafted with abdominal wall

Pretransplantation diagnosis	**Short-gut syndrome (9 cases)**
Etiopathogenesis	Posttraumatic (3 cases) Gastroschisis (2 cases) Gardner syndrome and desmoids (2 cases) Hirschsprung (1 case) Small-bowel resection (1 case)
Type of transplantation	Isolated intestinal (5 cases) Multivisceral (3 cases) Multivisceral without liver (1 case)
Immunosuppression regime	Alemtuzumab + FK506 (8 cases) Daclizumab + FK506 + methylprednisolone (1 case)

abdomen open while waiting for the granulation tissue to provide the bed for skin grafting or plastic procedures leaving disfiguring scars. Moreover, abdominal wall transplantation led to a faster recovery period and discharge from the hospital.

Rejection Episodes

Three patients showed abdominal wall skin rejection consisting of erythema and maculopapular cutaneous rash about 1 month postoperatively, which was confirmed histologically. Rejection was reversed completely in about 10 days by using a salvage therapy of steroids. Interestingly, there was no concomitant internal organ rejection. Similarly, no skin rejection was noted at the time of acute rejection affecting the transplanted visceral organs.

From all nine patients, a total 22 histological specimens were evaluated at a mean follow-up of 23.5 weeks and graded for rejection, which allowed Levi et al. to work out a specific pathological scoring system (Table 2) [9]. Four of these biopsies, coming from three different patients, showed thrombosis of vessels feeding the graft even in absence of clinical or pathological patterns of acute rejection. No evidence of graft-versus-host disease was found.

Conclusions

Current clinical experience demonstrates that abdominal wall transplantation is surgically feasible and immunologically justifiable. This free vascularised allograft allows primary coverage of the abdomen's defect, reducing both postsurgical morbidity and patient discomfort when breathing, moving, and eating. In case of rejection, this seems not to involve internal organ allografts, which are also not damaged in case of removal of the transplanted flap.

One advantage is that the immunosuppression regime is steroid-free, and showed to be quite tolerable. No donor bone marrow cells are included in the allograft, as is the case in hand transplantation, and this difference might be important in avoiding any immune modulatory effect requiring control by either steroids or mycophenolate mofetil (MMF).

Transplanting the abdominal wall at the same time as the internal organs adds extra time to an already lengthy procedure and requires a sound surgical technique in order to avoid thrombosis of the feeding vascular pedicle or insufficient perfusion of the graft. Further experience and longer follow-up will be necessary to perfect this technique, which at this stage seems very promising in solving abdominal closure problems during intestinal or multivisceral allograft.

Table 2. Grading system for abdominal wall acute rejection

Grading score	Pathological description	No. specimens
No rejection (grade 0)	No perivascular infiltrates	9
Indeterminate for rejection (grade 1)	Up to 10% of vessels have infiltrates of small lymphocytes	2
Mild rejection (grade 2)	11–50% of vessels show small lymphocytes infiltration. Mild spongiosis and eosinophils may be found	5
Moderate rejection (grade 3)	More than 50% of vessels show lymphocytic infiltration, and epidermal as well as stromal phlogosis may be present. Spongiosis is absent or mild; endothelial plumping, eosinophils and large lymphocytes may be present	4
Severe rejection (grade 4)	More than 50% of vessels show lymphocytic infiltration, there is dyskeratosis and the epidermis shows heavier lymphocytic infiltration and moderate to severe spongiosis. The stroma shows infiltrates reaching the base of the epidermis. Endothelial plumping, eosinophils and large lymphocytes are present	2

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Section 11-e

First Human Face Allograft: Report at 4 months

Jean-Michel Dubernard, Bernard Devauchelle

Introduction

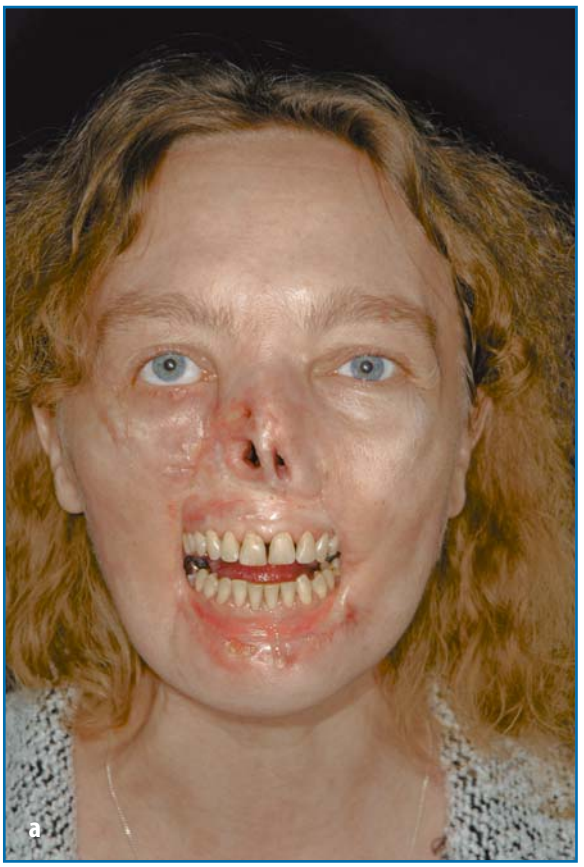
On 27 November 2005, the first face allotransplantation was performed on a patient with an extended soft tissue effect, including the nose, both lips and chin. This type of lesion is very difficult, if not impossible, to reconstruct with satisfactory cosmetic and functional results [1, 2]. Encouraged by the excellent long-term survival and good functional results of human hand allograft [3, 4], we came to the conclusion that composite tissue transplantation was a valuable option in functional facial reconstructive surgery

Patient and Preoperative Management

On 2 June 2005 a 38-year-old woman was transferred to the maxillofacial surgery department of the University Hospital in Amiens, France, 3 days after a severe dog bite that amputated completely her distal nose, both upper and lower lips, the entire chin and adjacent parts of the right and left cheeks. The defect involved all soft tissue of the face down to the skeleton and teeth and was somewhat larger on the right buccal and zygomatic areas (Fig. 1). The soft tissue defects also involved the distal nose, which completely lacked the columella, both nostrils and anterior part of the nasal septum. Physical examination of the patient before operation showed that she retained full integrity of the proximal stumps of her zygomatic and levator anguli oris muscles on both sides of the defect. Clinically, all these muscles remained functional, indicating that they retained their intrinsic motor nerve supply. No depressor muscle remnants were found in the lower part of the face. The maxillary and mandibular bones were intact, and the patient was left with a complete, perfectly healthy denture surrounded by undamaged gingival mucosa.

Preoperative magnetic resonance imaging (MRI) was performed to corroborate the clinical findings, and functional MRI (fMRI) tests were also registered from the time of trauma, aiming to study and compare before and after transplantation the cortical brain behaviour in the face representation frontoparietal areas. Routine pretransplant investigations showed no medical or surgical contraindication. The patient underwent a thorough psychological assessment by three different psychiatrists – one in Amiens and two in Lyon – who agreed with a fourth independent expert that the patient was fully able to cope with the procedure.

In coordination with a lawyer, we drew up a detailed informed consent form and a legal contract. This contract mentioned all possible complications related to this potentially life-threatening and non-life-saving procedure, especially well-known or foreseeable drug-related complications. We ensured that the patient was totally informed and understood this information.



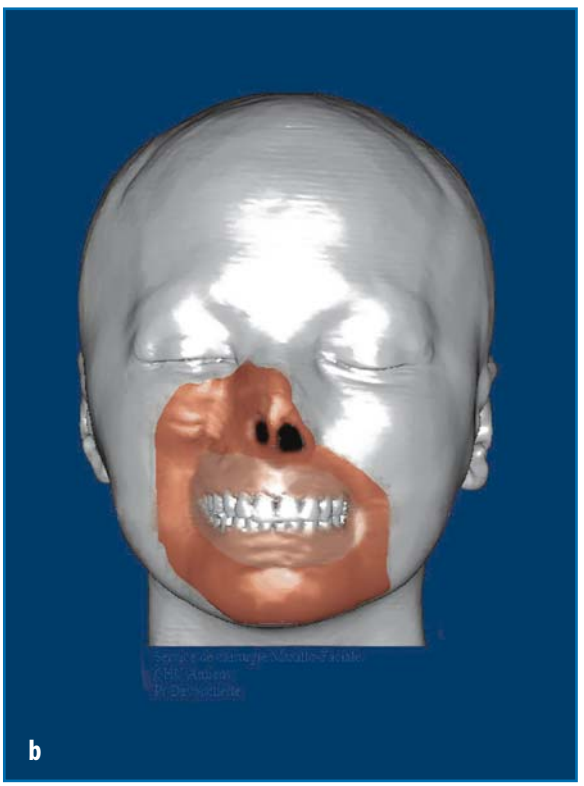


Fig. 1. Preoperative condition. **a** Patient picture 4 months after the dog bite showing wound retraction. **b** Anatomical figure of the lesions (imaging work by Dr. F. Taha, Amiens)

Authorisations were requested according to the guidelines laid down by the French National Ethics Committee. Final approvals certifying that the protocol fulfilled all ethical, medical and scientific rules were obtained from the French agency for health safety (AFFSAPS), the French Biomedical Agency (ABM) in charge of organ procurement in France and the local Consultative Committee in Biomedical Research (CPP, Amiens).

While the patient was waiting for the graft, intensive physiotherapy was performed in order to reduce scar contraction of the surrounding skin and prevent atrophy of the remaining muscles responsible of facial expressions. Despite intensive physiotherapy, scar contracture progressed and finally involved masseter muscles, reducing mouth opening to 19 mm. Elocution was severely affected, mastication was impossible and the patient was fed by a gastric tube.

Donor Operation

The donor was a brain-dead woman aged 46 years. Her skin complexion was close to that of the patient. Her family gave authorization to harvest and transplant part of her face as well as the thoracoabdominal organs. Donor and recipient had the same blood group (O+) and shared 5 human leukocyte antigens (HLA-DR). Prior to face procurement, bone marrow was harvested from the donor iliac crests and cryopreserved in liquid nitrogen. A plaster cast was moulded on her face, which was used to prepare a coloured silicon mask to give normal aspect to the donor after harvest in order to respect her dignity. Finally, a tracheostomy was performed.

Surgical preparation of the graft (Fig. 2) consisted of:

- Exposure of both right and left facial vessels first exposed on the basilar border of the mandible
- Ink skin design of the contour of the skin flap
- Deep dissection first on the surface of the masseteric fascia and cheek fat pad laterally
- Deep dissection in a subperiosteal plane medially in order to include in the graft, skin,

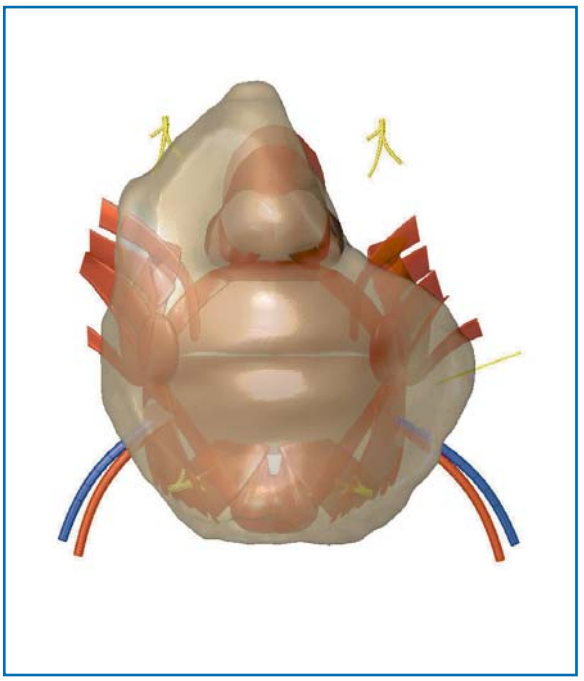


Fig. 2. Preparation of the graft. Anatomical figure of the partial allograft with muscles, facial vessels, motor (left mandibular branch) and sensitives nerves (V2–V3), which were repaired microsurgically during surgery (imaging work by Dr. F. Taha, Amiens)

subcutaneous tissue, all perioral muscles with their intact nerve supply arising from the zygomatic, buccal and mandibular branches of the facial nerve and mucosa of oral and nasal vestibules.

The graft also contained alar and triangular cartilages of the nose in continuity with the anterior part of the septum and both right and left infraorbital and mental sensitive nerves. Simultaneously, a conventional radial forearm flap (Chinese flap) was harvested on the donor's left upper limb to be transferred as a vascularised sentinel graft on the recipient's left thoracodorsal vessels.

After harvesting, both facial graft and sentinel flap were irrigated with 500 cc of IGL-1 organ preservation solution at 4°C then placed in double plastic bags and a standard ice box. After the kidneys, liver and heart were removed, the donor's nose-lips-chin triangle was reconstructed with the coloured silicone mask custom-made inside the plaster cast moulded at the beginning of the procedure.

Recipient Operation

Tracheostomy

Preparation of the graft site consisted of:
- Extended facial dissection in order to remove all scar tissue and isolate each anatomical structure to be joined to those dissected and individually tagged on the graft
- Skin incision along a regular curved line following the borders of the original defect
- Superficial muscle dissection to expose individual stumps of the elevator bellies, with their intact motor nerve supply entering their deep surface
- Exposure of the terminal sensitive branches of the maxillary and mandibular nerves at the point they left the infraorbital or mental foramina
- Exposure of the right and left facial veins and right and left facial arteries, which had a quite small diameter leading us to expose the proximal part of the artery on the right side by a complementary submandibular approach.

Preparation of the graft consisted of:
- Concomitant bench surgery to further dissect and prepare vascular and nervous structures of the graft
- Anastomoses of the right facial artery, sutured end-to-end with 10/0 Prolene; the clamp was released, and the entire composite transplant rapidly achieved normal colour and volume
- Anastomosis of the right facial vein, sutured end-to-end with 9/0 Prolene; total ischaemic time was less than 4 h (Fig. 3)
- Circumferential closure of the oral vestibule with separate 4/0 Vicryl sutures
- Terminoterminal repair of right and left mental and infraorbital sensitive nerves using 9/0 Prolene
- Anastomoses of left facial artery and vein, sutured end-to-end with 10/0 Prolene
- Suture of facial mimic muscles in layers, with attempt to join them individually whenever possible. On each side of the midface, repaired muscles included the buccinator, zygomaticus major and minor, levator angulae oris and levator labii superioris, risorius and

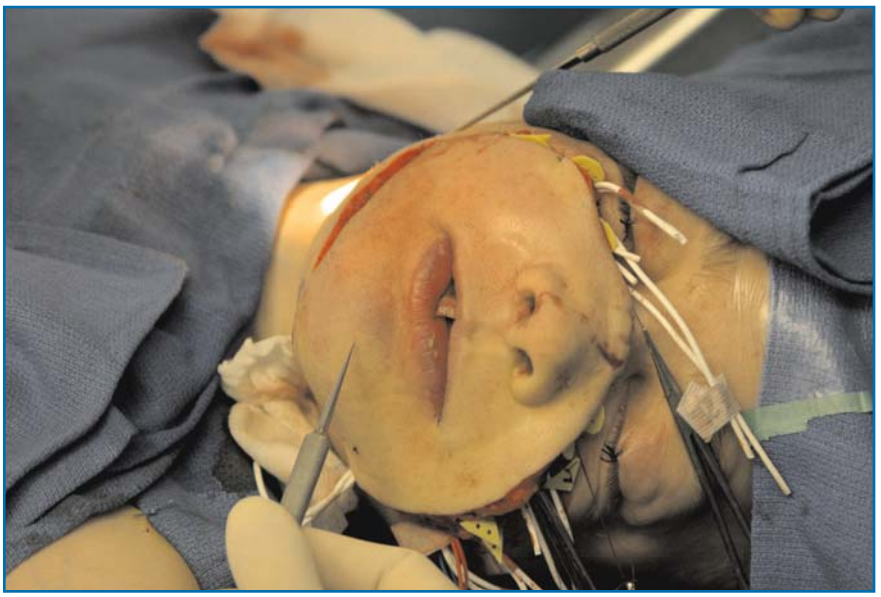


Fig. 3. Surgical aspects of the graft immediately after revascularisation (lips regain normal colour)

platysma. On the lower face, depressor muscles of the lower lip were reinserted on the periosteum of the mandibular border. Since all proximal stumps of the midfacial muscles had kept their original motor nerve supply, the decision was made not to sacrifice zygomatic and buccal rami of the facial nerves and to suture them on the homologous branches dissected on the transplant

- Terminoterminal coaptation of the left mandibular branch of the facial nerve to reanimate the lower face on the right side. This thin nerve was not found in the graft.

Final inset of the transplant included the ascending repair of both nasal vestibules, closure of the nasal superficial musculoaponeurotic system (SMAS) layer and finally subcutaneous and skin suture. The latter was performed with 6/0 after a moderate adaptation of the upper cutaneous edges of the recipient's defect. Silkworm guts were used to drain the subcutaneous space, and wounds were lightly dressed with short Steri-Strips only. The whole graft was left uncovered for postoperative monitoring.

While performing the face transplant reimplantation, the sentinel radial forearm flap harvested from the donor's left upper limb was transferred to the recipient's left submammary fold and suture end-to-end to the thoracodorsal vessels with 9/0 Prolene. This vascularised composite tissue flap, hidden under the hanging breast, was used to monitor indirectly the immunological behaviour of the graft, aiming to avoid damage to the reconstructed face by repeated skin biopsies.

Postoperative Care

The induction immunosuppressive protocol (Fig. 4) consisted of:

- Intravenous antithymocyte globulins (Thymoglobulin, Genzyme, 1.25 mg/kg per day for 10 days)
- Oral tacrolimus adjusted to maintain blood concentration between 10 and 15 ng/ml during the first month
- Mycophenolate mofetil (MMF) (2 g/day)
- Prednisone (250 mg on day 1, 100 mg on day 2, followed by 60 mg/day for 10 days, then progressively tapered to 5 mg/day).

Prophylaxis for cytomegalovirus (CMV) infection consisted of IV Ganciclovir (5 mg/kg BID) for 5 days, followed by Valganciclovir (900 mg/day for 5 months). For prevention of *Pneumocystis jiroveci* pneumonia, the patient received Trimethoprim sulfamethoxazole (400 mg/day) for 6 months following transplantation. Amoxicillin-clavulanate prophylaxis (3 g/day) was given for 10 days to prevent postoperative infection. Finally, antithrombotic prophylaxis

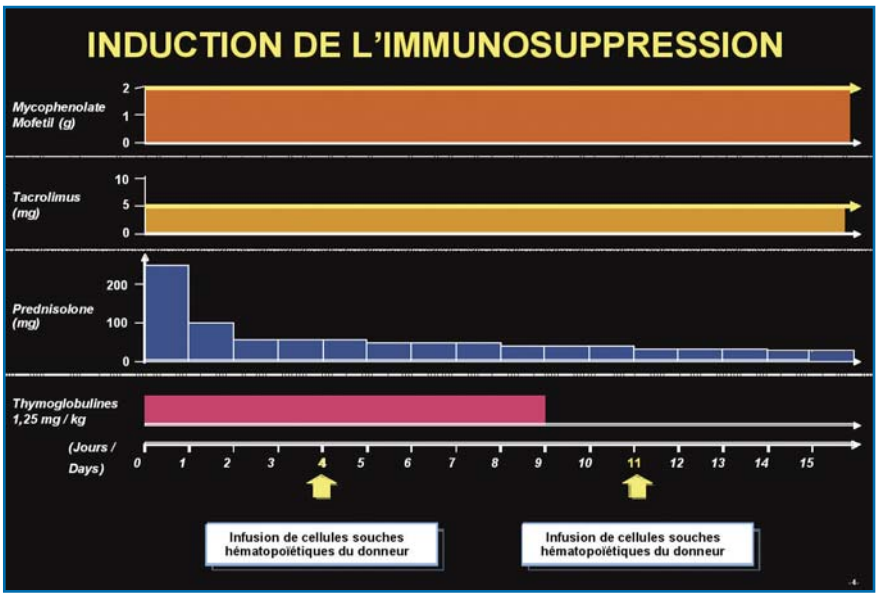


Fig. 4. Induction of immunosuppression

combined subcutaneous heparin and aspirin.

Frozen bone marrow was thawed immediately before infusions, which were performed respectively on days 4 and 11 posttransplant. The total nucleated haematopoietic cells infused were 1.6×10^8/kg on day 4 and 1.8×10^8/kg on day 11. The graft contained 2×10^4/kg and 4×10^4/kg colony-forming units granulocyte macrophage (CFU-GM) cells, 0.12×10^6/kg and 0.12×10^6/kg CD34+ cells and 2.7×10^6/kg and 4.1×10^6/kg CD3+ cells on days 4 and 11, respectively. The decision of adding donor blood marrow cell (DBMC) infusions to the immunosuppressive protocol was based on experimental and clinical data demonstrating its long-term efficacy. DBMC infusions are efficient for tolerance induction in experimental animals [5]. Since the pioneer works by Monaco et al. [6], DBMC infusions have been used in kidney, kidney and pancreas, liver and heart transplantations [7]. Results of DBMC infusions in combination with immunosuppressive drugs including thymoglobulins as induction therapy has been intensively studied in cadaveric kidney transplantation [8]. Over the long term, decreased chronic rejection rates and higher graft survival were demonstrated when compared with noninfused controls, even in the absence of proven microchimerism.

Protocol mucosa and skin biopsies were scheduled every week in the sentinel skin graft and cheek mucosa for 1 month then monthly for 4 months. Physiotherapy was started 48 h after surgery and was offered twice daily for the entire follow-up period. The rehabilitation programme consisted of supervised controlled-motion passive and active exercises as well as early sensory reeducation and cortical reintegration protocol. Psychological support was offered once daily during the first 4 weeks then twice weekly.

Postoperative Course

The initial postoperative course was uneventful. No microsurgical complications occurred, and no ischaemic or congestive areas were observed on the graft or sentinel flap. Wound healing occurred normally. Minor oedema of both grafts was observed in the early postoperative period but quickly disappeared and did not delay the immediate implementation of the rehabilitation programme. The patient's general condition remained excellent.

On day 18, diffuse erythematic features and oedema were observed on the grafted mucosa (Fig. 5). They were considered and treated as candida stomatitis because of the demonstrated presence of *Candida albicans* on the patient's oral mucosa. From day 20, mild and diffuse erythema and oedema progressively developed on

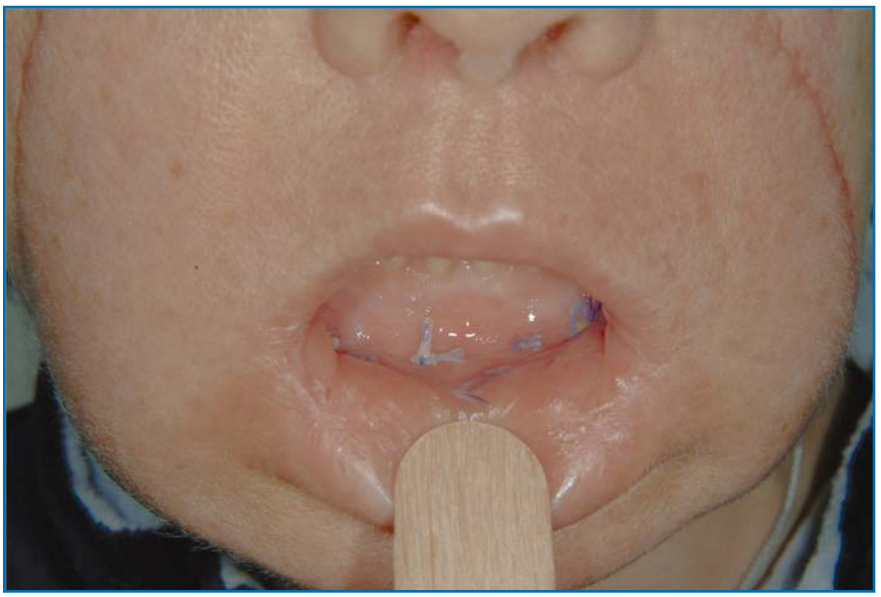


Fig. 5. Graft mucosa on day 18: erythema and oedema

the facial skin (Fig. 6) and sentinel skin flap (Fig. 7) On day 20, mucosa biopsies showed dense mononuclear cell infiltrate, some basal cell vacuolisation and occasional apoptotic keratinocytes (Fig. 8). At the same time, skin biopsies emphasised moderate perivascular mononuclear cell infiltrate in the grafted derma (Fig. 9). The lesions could be graded between I and II according to the classification established for composite tissue acute rejection [9]. Based on the treatment of rejection used in hand-allografted patients, prednisone doses were increased from 25 to 60 mg/kg per day. Tacrolimus and clobetasol ointments and steroid mouth rinses were alternatively applied twice daily. As clinical and pathological improvement was very slow, three pulses of 1 g prednisone were given on day 34, 36 and 38. Tacrolimus doses were increased from 10 to 15 mg/day in order to maintain blood concentration between 10 and 15 ng/ml. MMF doses were increased from 2 to 3 g/day from day 39. Under this regimen, the mucosa aspect rapidly returned to normal. Simultaneously, redness of the graft and sentinel flap rapidly faded. Subsequent mucosa and skin biopsies showed a substantial decrease

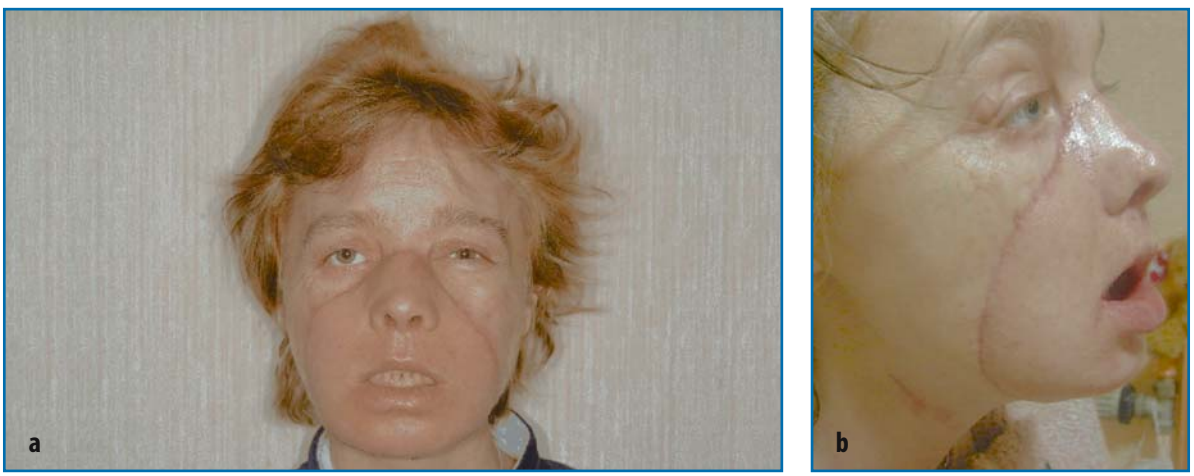


Fig. 6. Graft rejection on day 20. **a** Face **b** Profile

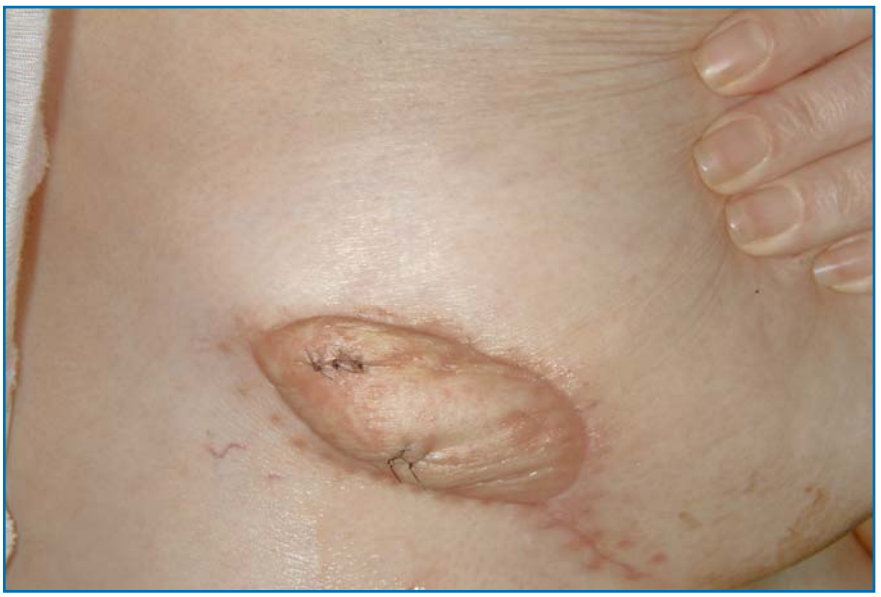


Fig. 7. Rejection of the sentinel flap on day 20

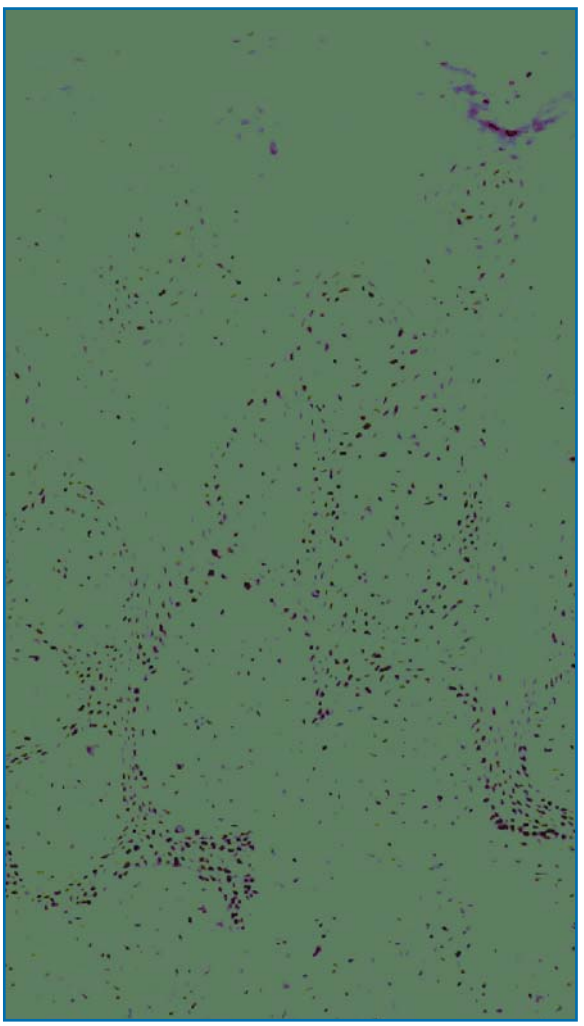


Fig. 8. Pathology of mucosa at time of rejection on day 20 (Kanitakis grade II–III)

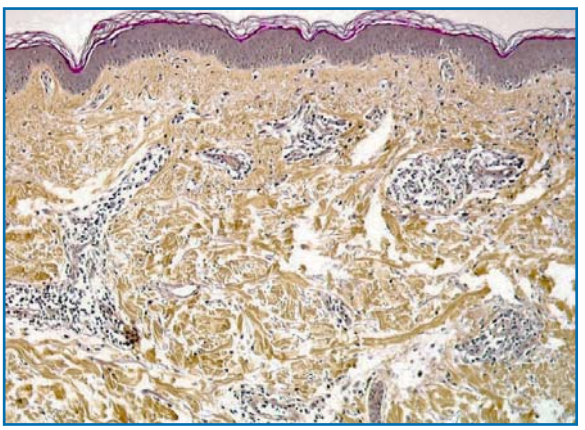


Fig. 9. Pathology of skin at time of rejection on day 20 (Kanitakis grade I–II)

of the cellular infiltrate, with return to normal at day 45. Although forearm and facial skin do not have exactly the same thickness, clinical and pathological changes appeared simultaneously on both during the rejection episode and were grossly parallel in the forearm fasciocutaneous flap and face allograft. Furthermore, our observations showed that clinical and pathological patterns of rejection might appear first on the transplanted oral mucosa and are easier than skin to biopsy. Intraoral mucosal biopsies thus offer another way of monitoring rejection by using a similar grading classification as that previously described in hand transplantation.

Chimerism documentation using microsatellites and quantitative polymerase chain reaction

(PCR) was performed once weekly on total blood and CD3, CD15 and CD56 cells and monthly on bone marrow. All results until day 90 showed a complete recipient profile in blood and marrow.

Functionally, physiotherapy started at day 1, the tracheostomy was removed at day 3 and the patient became able to eat and drink nearly normally at the end of the first postoperative week. Rehabilitation training was performed twice a day and included facial static and dynamic exercises mainly focused on restoration of lip suspension and mouth occlusion. Sensitivity recovered quite quickly. Assessed by the progression of repeated Semmes-Weinstein tests, it reached the lateral part of the upper lip and the lateral mental area on both sides after 10 weeks and thereafter involved the whole skin surface of the transplant, including the tip of the nose, at the 14th postoperative week. Oral mucosa of the graft also became sensate in the same interval so that since the end of the second postoperative month, routine mucosal biopsies needed to be performed under local anaesthesia.

Motor recovery was slower and less effective. Dynamic motions of the upper lip, due to contraction transmission from the repaired levator and zygomatic muscles, were obvious from the beginning of the 12th postoperative week. Smile, however, remains at the present incomplete and still imperfect. Lower-lip motion is for the moment not present, causing a slight sagging of the central inferior part of the graft. Consequently, complete lip closure is not already achieved, and although highly improved compared with the preoperative state, phonation still lacks labial occlusive phonemes.

Psychologically, the transplant was easily tolerated in the immediate postoperative period, and its quick integration in the patient's new body image was highly favoured by the fast sensitive recovery of its skin surface. At the end of the 12th postoperative week, the patient became able to show her new face to the outside world and returned to a normal social life (Fig. 10).

Fig. 10. Result at 4 months

Conclusion

The early outcome of the first human face transplantation confirms what we already learnt from animal studies [10] and retrospective multicentric clinical experience with human hand transplantation Technical feasibility of the procedure is here clearly demonstrated, with no surgical complication. When compared with conventional techniques using serial autologous tissue transfers, face allografting thus brings the advantageous possibility of reconstructing severely disfigured patients in a one-stage procedure, providing simultaneously an entire *ad integrum* restitution of each missing anatomical unit of the damaged face, complete sensitivity recovery of the transplant and promising results in terms of aesthetics and motor function. Face composite tissues, however, are able to trigger an alloimmune response that needs to be prevented and can be controlled by a standard immunosuppressive regimen.

Acknowledgments

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Section 11-f

Lower-Extremity Hindquarter Transplantation in Conjoined Twins

Ronald M. Zuker

Introduction

Limb transplantation is now a clinical reality. The success of upper-limb transplantation prompted us at the Hospital for Sick Children in Toronto, Canada, to apply the concept to the lower extremity. The hindquarter of a failing conjoined twin was transplanted to her healthy sister at the time of separation. Appropriate bony, muscular, vascular and neural repairs were carried out, as will be described in this chapter. Functional return was better than expected and became appropriate and spontaneous in the surviving twin.

Case Report

A healthy 38-year-old woman had a routine ultrasound at 24 weeks estimated gestational age revealing conjoined twins. Further investigations revealed a complex cardiac abnormality in Twin A, including an aortic valve stenosis (ASD), ventricular septal defect (VSD), double outlet ventricle and right ventricle (RV) outflow obstruction. Although the anomaly was concerning, the babies seemed to develop fairly well *in utero*.

At 36 weeks, they were delivered by caesarean section and transferred to the Hospital for Sick Children in Toronto, Canada, for further investigation and discussion regarding separation. They both appeared healthy, were breathing spontaneously, and had excellent colour. The cardiac anomaly in Twin A seemed to be stable, at least initially. On examination, they were united from the lower part of the chest through the entire abdomen and into the pelvis. There was a single pelvic ring, thus classifying them as ischiopagus twins. Each baby had two normal upper extremities, thus adding to the classification quadrabrachius. However, the lower extremities issuing forth from the single pelvis were quite abnormal. There were two relatively normal lower extremities with moderate club feet deformity and one very abnormal extremity. Thus, the babies were classified as tripus. Each baby controlled one relatively normal extremity with the club foot. However, the abnormal extremity was small, significantly deformed, barely mobile only at the hip and had significant deformity of the toes with nails on both sides. This extremity had neural input from both babies but in fact was virtually useless from a functional standpoint. The perineum revealed one anus, a single vaginal opening and a small urinary sinus. Because of the single pelvic ring, the four upper extremities and the three lower extremities, this set of conjoined twins would be classified as ischiopagus, quadrabrachius tripus (Fig. 1).

The babies were quite healthy initially and were started on enteral feeds. A number of investigations were carried out over the course of the next month, and they both gained weight and seemed to do quite well. Investigations included

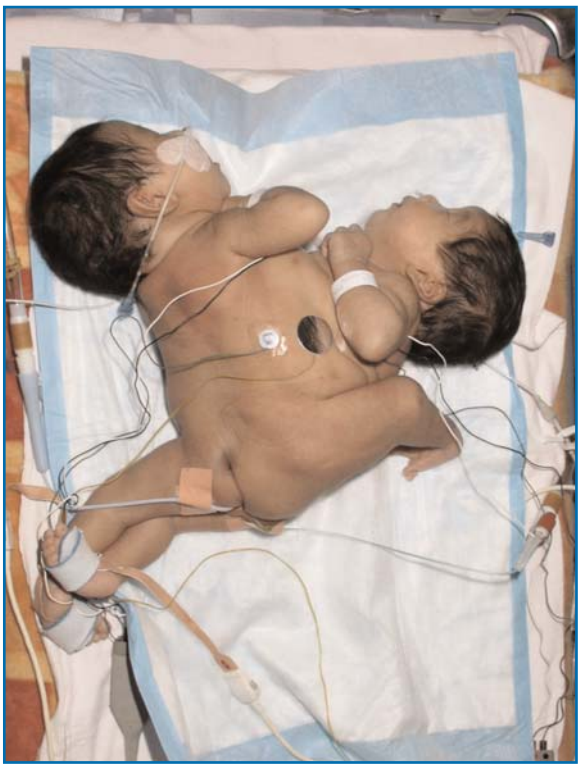

Fig. 1. Ischiopagus quadrabrachius tripus conjoined twin

a computed tomography (CT) scan, which demonstrated clearly the single pelvic ring. Magnetic resonance imaging (MRI) demonstrated a large shared liver, which importantly had separate biliary drainage systems for each baby. The upper gastrointestinal (GI) tracts were separate but became united at the distal ileum. The large bowel was single and shared, with mesentery on both sides. MR angiography (MRA) revealed two iliac arteries for each baby. They shared an iliac and femoral system, which came together to vascularise the third, nonfunctional, shared lower extremity. Urological investigations included a cystourethrogram that demonstrated a large urogenital sinus with only one functional kidney in Twin A and two functional kidneys in Twin B. However, one of the kidneys in Twin B had a ureteropelvic junction obstruction with a dilated renal pelvis. Interestingly, the functioning kidney from Twin A drained into the bladder of Twin B.

The single pelvis revealed three relatively normal hip joints and one symphysis pubis. A variety of cardiac investigations confirmed a double-outlet RV with midcavity outflow obstruction. Twin A also had a very restrictive VSD and ASD with pulmonary hypertension. However, both twins remained relatively stable, and we felt that the cardiac status would allow for separation with survival of both twins. Thus, our initial plan was to separate the children and provide sufficient tissue for reconstruction utilising tissue expansion. We would utilise not only subcutaneous tissue expansion for skin but also intraperitoneal tissue expansion for abdominal wall and pelvic support. Each baby would have only one functioning lower extremity and a hindquarter disarticulation on the other side. Our hope was for the children to become a few months older and stronger and then undergo tissue expansion insertion and 3 months after that, definitive separation.

Unfortunately, at about 3 months of age, Twin A developed acute cardiac decompensation. We had hoped that medical management might improve the situation, but it did not. After various consultations with cardiologists and cardiac surgeons, it was deemed that Twin A had a lethal and inoperable cardiac anomaly and was about to succumb. If Twin A died, then Twin B would also not be salvageable. Twin A's failing cardiac condition necessitated urgent separation. The concept of transplanting the dying twin's limb to her surviving sister was discussed in detail with the family, the surgical team and the psychosocial support network. We felt that this would be technically feasible and superior to a unilateral hindquarter deficit. We did not know whether Twin B would integrate function of the transplanted limb, as there had been no cortical-site developed. All parties agreed that the transplant would be worthwhile for the surviving twin, and plans to proceed accordingly were put in place. We knew that Twin A might not survive the surgical procedure itself, or if she did, she would die shortly afterwards. We were encouraged by the recent success of upper limb transplantation [1–3] and felt confident that this would be a worthwhile procedure for the surviving twin.

Surgical planning was done in concert with general, vascular and orthopaedic surgeons, urologists and our team of plastic and reconstructive surgeons. We would require consider-

able soft tissue from Twin A in order to accomplish a tension-free closure of the chest, abdomen and pelvis of Twin B. We would transplant the entire hindquarter of the dying twin and remove the hip joint of the third, useless limb (Fig. 2). However, our plan was to maintain the musculocutaneous component of the third limb to provide for hip flexion. The quadriceps musculature of this third, shared limb would be implanted into the transplanted extremity to provide for this. In this way, we would preserve as much function as possible, with innervation of this muscle component from Twin B.

Surgical Separation

Anaesthesiologists play a major role in major limb transplantation. This was further compounded by the conjoined nature of these babies and their young age. The operation itself was carried out on 26 January 2003 when the babies were just 3.5 months of age. The operation took 22 h. The surgical procedure began with the incisions in the lower chest and abdomen continuing down to the pelvis. Diaphragm, liver and gastrointestinal tracts were divided by the general surgical team, and then the urologists divided and reconstructed the urinary system. Twin B would be left with her upper GI system as well as the large bowel and two functioning kidneys draining into a single bladder. After the internal organs had been separated, the limb to be transplanted was isolated. It had a normal sciatic nerve coming from Twin A, and this was completely divided. As indicated earlier, we planned to save the anterior thigh musculature of the third, useless limb and implant this into the transplanted limb. Thus, we did not need to divide and reinnervate the femoral nerve. Through pelvis osteotomies and excision of the third, useless hip joint, the limb was transplanted from Twin A to Twin B. Here it was secured with appropriate bony fixation and then revascularised. Revascularisation was through the aorta and inferior vena cava of Twin A. These were removed from Twin A, along with the lower extremity and through end-to-side anastomoses were connected to the aorta and inferior vena cava of Twin B. Excellent revascularisation occurred, with healthy pulses palpable in the transplanted limb. Then the sciatic nerve of Twin B, which originally went to the third lower extremity, was coapted to the sciatic nerve of the transplanted limb. This would be the right

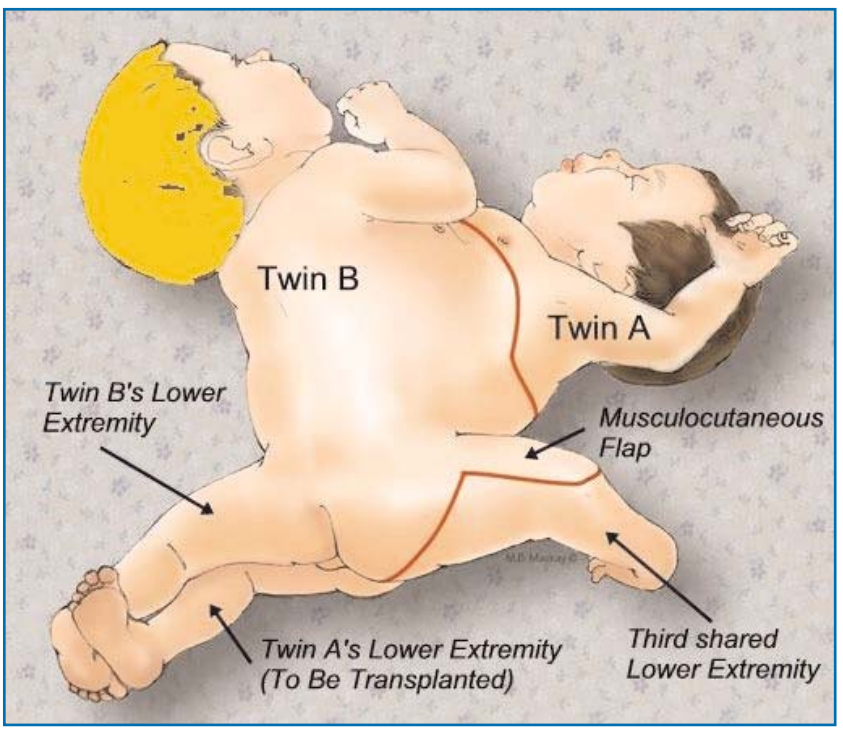

Fig. 2. Schematic of planned surgical separation. Courtesy of Dr. Margot McKay

sciatic nerve of Twin B, which previously had gone to the third, useless limb. The third, useless limb did receive contributions from both babies, specifically, the right sciatic nerve from Twin B and the left sciatic nerve from Twin A. Thus, the right sciatic nerve was coapted to the healthy normal sciatic nerve of the transplanted limb. It is to be noted that this right sciatic nerve of Twin B had no functioning musculature to innervate in the third, useless limb. Thus, one could surmise that the cortical representation of this right sciatic nerve in Twin B was minimal and undeveloped.

Under high power magnification, the sciatic nerve coaptation was carried out, which would hopefully provide for both sensory and motor innervation to the transplanted limb. Thus, the transplanted limb had independent innervation and independent perfusion. The quadriceps musculature was inserted into the quadriceps tendon of the transplanted limb to provide for active hip flexion.

Skin flaps were appropriately positioned and sutured to provide for abdominal and pelvic support in Twin B (Fig. 3). Twin A had her abdomen and pelvic wounds closed. She survived the surgical procedure but died in her mother's arms in the recovery room.

Twin B did extremely well during the postoperative period. She required intensive care unit observation for about 5 days and then was transferred to the ward. Wound healing progressed uneventfully, and she was started on a rehabilitation programme to maintain passive mobility of her hip, knee, ankle and toes.

We then waited for reinnervation of the transplanted limb. The quadriceps musculocutaneous flap regained function and was able to provide for hip flexion. The transplanted limb was in a somewhat abducted position and required a femoral osteotomy to place it in a more functional location. This was carried out 2 years posttransplant, and the tendinous insertion of the musculocutaneous flap was also shortened and adjusted. An abdominal scar revision was also carried out (Fig. 4). Detailed evaluation at almost 3 years of age and over 2.5 years posttransplant demonstrated excellent recovery in the distribution of the sciatic nerve. We were delighted to note that sensation had recovered throughout the entire extremity. This went to the tips of her toes, as demonstrated by withdrawal from tickling. In a 3 year old, it is difficult to know exactly the extent of sensation, but it was clear that the entire extremity was sensate. From a functional standpoint, we were also delighted

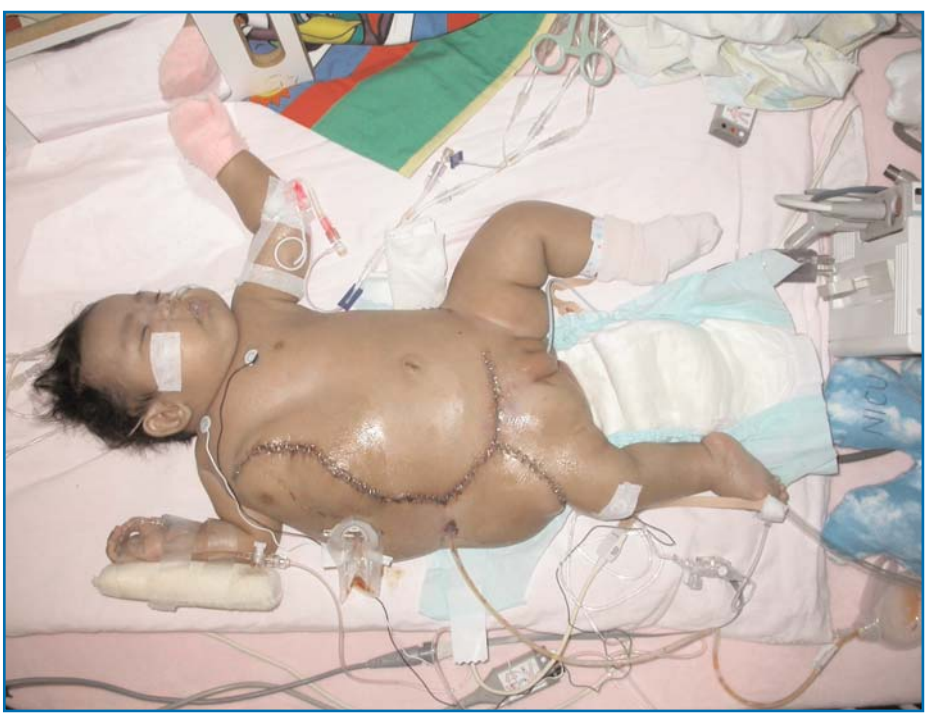

Fig. 3. Immediate postoperative result following hindquarter transplantation

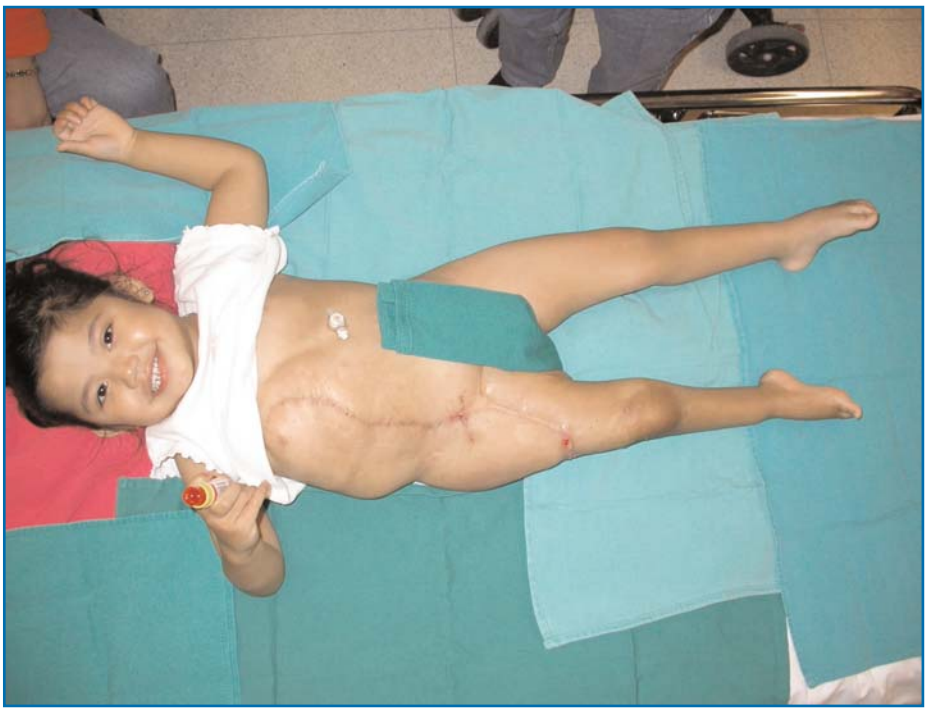

Fig. 4. Postoperative appearance following osteotomy, quadriceps tendon repositioning and scar revision

to see that the sciatic nerve innervated musculature had recovered. There was excellent active knee extension and ankle plantar flexion (Fig. 5) and toe mobility. Regrettably, there is no demonstrable active ankle dorsi flexion as yet. We are, however, optimistic that this may recover, but if it does not then a tenodesis or a tendon transfer will be necessary.

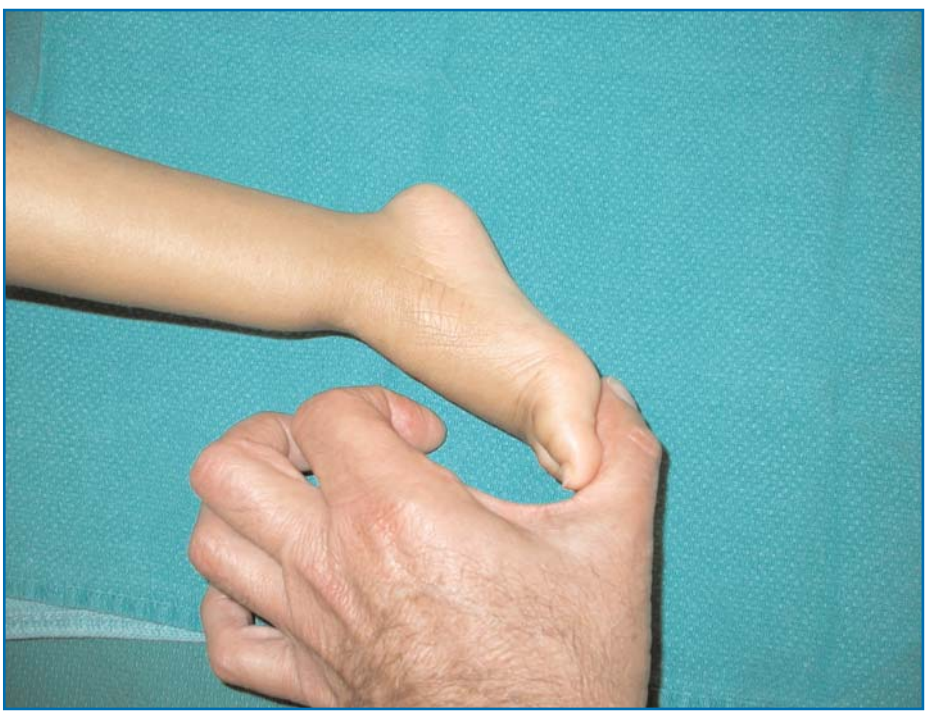

Fig. 5. Active plantar flexion at ankle

Of particular importance is the spontaneity of function of the transplanted limb. When asked to extend her knee, move her ankle or wiggle her toes, these activities are done bilaterally and spontaneously. Thus, cerebral integration has taken place relative to the transplanted limb. The right sciatic nerve, which went to the malformed, useless, third limb, has now innervated both sensory and motor function in the transplanted right lower extremity. The nerve also innervates this in a spontaneous and fully integrated fashion. This would suggest that additional cortical representation has developed in Twin B, representing the transplanted right lower extremity. Thus, the healthy right lower extremity that was appropriately controlled by her sister (Twin A) is now controlled fully in a spontaneous and integrated fashion by herself (Fig. 6a, b).

Discussion

To our knowledge, this is the first successful lower-extremity transplantation carried out in humans. The surgical procedure was carried out on 26 January 2003 at the age of 3.5 months. At 3-year follow-up, the surviving twin is doing exceptionally well and has integrated function of the transplanted extremity. As was the case of

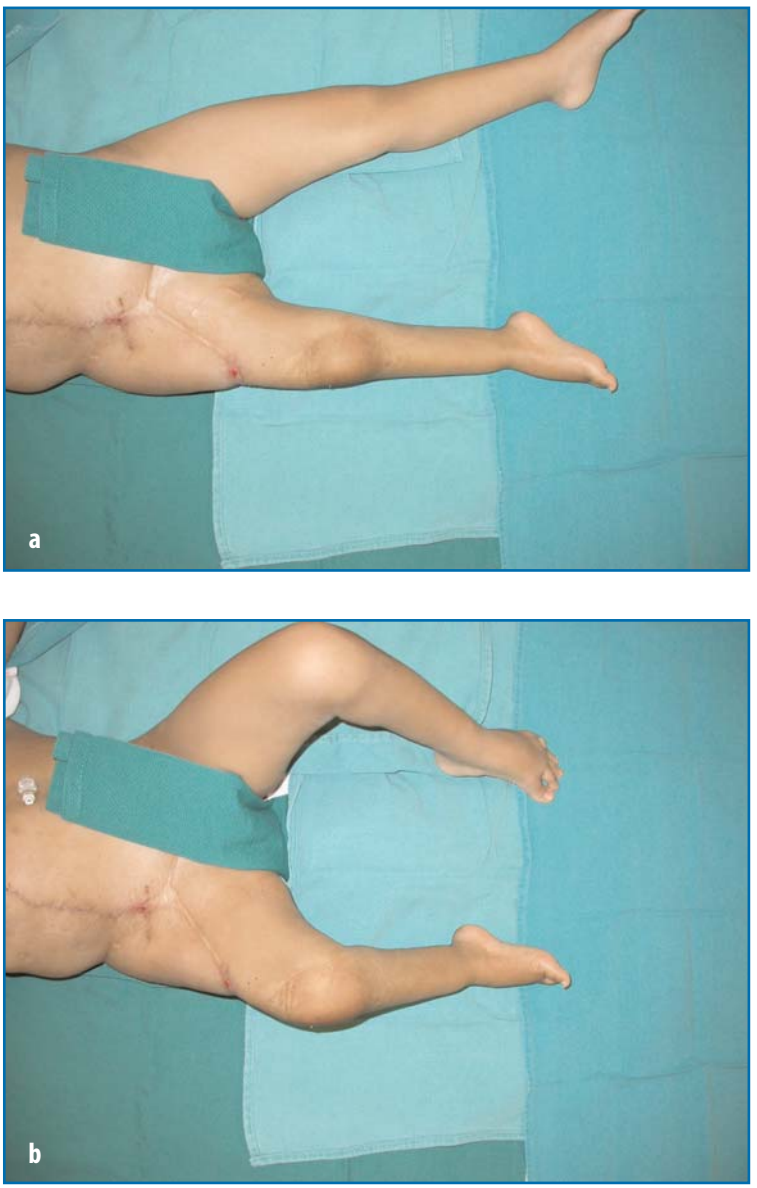

Fig. 6. Functional result at 2.5 years post-transplant. **a** With lower extremities extended. **b** With spontaneous hip and knee flexion

the first kidney transplant, this transplant was carried out in conjoined twins. Therefore, there was no need for immunosuppression, as the genetic structure is identical. One may surmise that because of the lack of immunosuppression needed, the functional recovery was considerably better than it might have been if immunosuppression was required. This is probably true. However, it is important to note that cerebral integration has taken place in a cortical area where representation may have been absent or only minimal at birth. This, we believe, has significant implications with respect to transplantation of parts in congenitally absent locations. Cerebral integration may well have the capacity to develop even when there was none initially.

From a structural standpoint, the pelvic ring seems to be stable, with excellent hip function bilaterally. Vascularisation has been maintained through end-to-side repairs to the aorta and inferior vena cava. The anterior thigh myocutaneous flap from the shared, virtually useless, third limb is functioning well and in fact provides improved hip flexion now that the tendon has been shortened and repositioned. Most impressive, however, is the function achieved through the sciatic nerve coaptation, as outlined above.

This lower-extremity transplant was carried out at the hindquarter level. Although prostheses are available for this level, they are cumbersome and difficult to use and require an enormous amount of energy with ambulation. Thus, this transplant has provided a substantial advantage over a prosthesis for this child. The lack of need for immunosuppression provides additional justification for the procedure. Lower-extremity prostheses are, in general, quite effective, particularly the below-knee prosthesis. At the present time, limb transplantation for the lower extremity would only be considered in such cases as conjoined twins where one twin would not survive, and possibly in hindquarter amputations. The future direction of lower-extremity transplantation thus relates directly to the future of effective, minimal-risk immunosuppression.

Ackknowlegement

The Author wishes to thank Dr. Margot McKay of the Department of Anatomy, University of Toronto for the schematic drawings which were invaluable in the planning for the complex separation of these children.

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12. Future Directions

Section 12-a

Limb Transplantation in Congenital Deformities

Aram Gazarian, Davit O. Abrahamyan

"Science sans conscience n'est que ruine de l'âme".
François Rabelais, *Pantagruel*, 1532

Introduction

This chapter presents current issues for possible limb transplantation in newborns and indicates directions, which will be likely to produce some answers on different feasibility aspects. Even if total hand transplantation (HT) will never be used in congenital individuals, current research advancements may allow composite tissue allotransplantation (CTA) to be an adequate tool for managing many currently "incurable" malformations presenting with different missing anatomical parts. There are numerous cases of congenital limb deformities (CLD) in which failure of formation or development has no surgical answer because we are unable to restore or properly replace the missing parts. "Confusion arises also from the circumstance that these anomalies are never exactly alike" [1].

Is not it a situation where composite tissue allotransplantation may be a solution?

The given topic evokes many questions. This chapter does not pretend to provide answers to these issues but merely demonstrates a retrieval of possible solutions.

The field of CLD experimental treatment is currently occupied with competitive innovations in prosthetics, tissue engineering, gene therapy, intrauterine surgery and up to the idea of an artificial hand [2–6].

The current protocol for CTA conducted in France under the direction of Prof. J.M. Dubernard, has opened a novel pathway in limb reconstruction [7–11]. Amongst the wide varieties of CLD, only transverse failures of formation (TFF) at the forearm level are chosen here in order to narrow the limits of the topic, keeping in mind, however, that the future of CTA may not necessarily involve the whole hand but parts of it. TFF refers to the absence or hypoplasia of limb distal structures, producing an amputation-like stump [12, 13].

As TFF in adults are mainly unilateral, and as these adults almost never request HT [14], they are not envisaged as candidates for this surgery, which is mainly accepted for bilateral amputees [15–19]. Peculiarities of the neonatal immune system, however, mean that one can consider HT in newborns, even those with unilateral TFF. With our current state of knowledge, one cannot propose this procedure unless it can be done using newer tolerating regimens without recourse to immunosuppression [20]. Moreover, the immunological issue is not the only one to be solved (Fig. 1).

Ethical and Psychological Aspects

"This idea of hand transplantation makes me afraid" [21].
Even if hand transplantation were feasible, would it be desirable and acceptable?

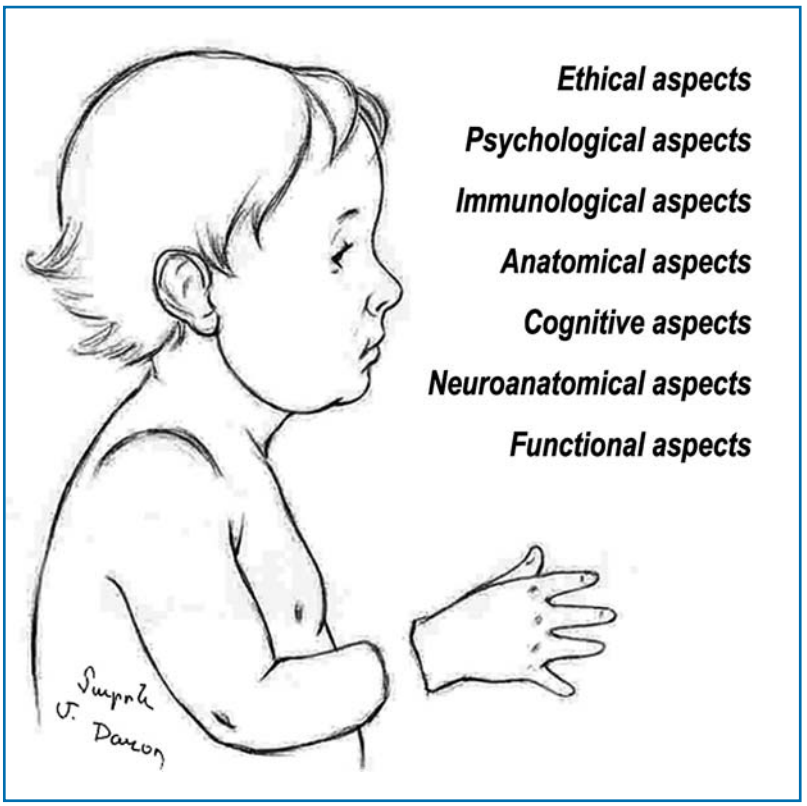

Fig. 1. Feasibility aspects of hand transplantation in a newborn with TFF). Courtesy of Daron Mouradian, painter, Yerevan, Armenia

Patients and Parents

The patient's perspective is missing because the newborn is unable to pose questions and give responses. Future acceptance of the allograft and reaction to the fact of having "another's hand" should be considered. How will a growing child accept the decision made for him or her, "ignoring" his or her own opinion and wishes? "Babies never ask to enter this world. It is their parents, who 'ask' them to come", says Père Peillon [22]. "This charisma comes with the duty that parents should make everything possible to them for the child's welfare".

Epidemiology

The prevalence rate of TFF is 1 in 20,000 live births; the most frequent is at the forearm level (Fig. 2) [23–26]. Congenital limb deficiencies in the paediatric population are much more common than acquired ones [25, 26]. Risk factors are nonspecific – parents' age; maternal use of alcohol, tobacco or cocaine [27–29].

Genetics

The majority of TFF are nongenetic, occur sporadically and unilaterally with normal unaffected feet and are not usually associated with systemic conditions [24, 30–33]. An extensive workup for them is unnecessary [1, 34–36]. However, inheritance of unilateral hypoplasia has been described [37]. TFF may occur if several mutated genes are present, or it may be caused by single gene mutations with low penetrance [37].

Embryology

The apical ectodermal ridge, a zone of tissue at the most distal aspect of the developing limb, governs the proximal to distal axis. Under the control of certain genes, it releases fibroblast growth factors that influence the limb's proximal to distal development (intrauterine life weeks 4–8) [38–40]. Various harmful agents, gene mutations or their specific co-occurrences can generate TFF [41]. Parents rarely pose similar requests. In most cases, they quickly "bear with" the situation.

Fig. 2. A 2.5-year-old child with transantebrachial transverse failure of formation (TFF)

However, there are instances of abortion in such cases. In central-eastern France from 1999 to 2004, transantebrachial TFF occurred in 37 foetuses (1/16,500), which was established antenatally in 20 cases (Fig. 3). From these 20 diagnosed cases, eight were aborted, of which three had significant associated anomalies. Thus, of 17 pregnancies with antenatally diagnosed isolated TFF, five (almost one third) were interrupted. What can be concluded about "acceptance" of such a malformation by future parents? What would these couples have decided had there been an opportunity for HT for their offspring?

Current Care

"I have learnt to use what I have, as another child learns to serve himself with his 10 fingers" [21].

Care of TFF patients comprises psychological support and functional and aesthetic prosthetics [15, 26, 30, 31, 42]. However, unilateral TFF up to the upper quarter of the forearm is not invalidating for children. They have a level of independence comparable with that of other children of the same age [26, 30, 31, 42]. The adaptation potential of patients presenting unilateral TFF allows astonishing autonomy in daily life activi-

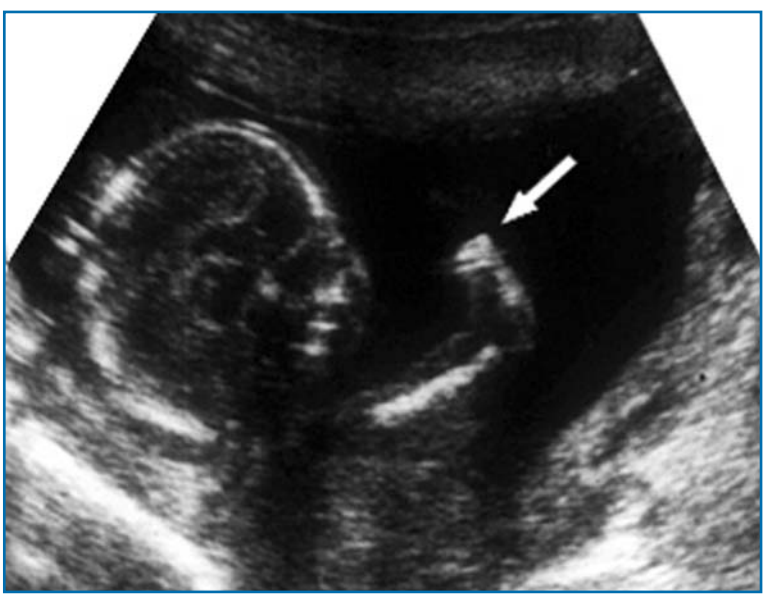

Fig. 3. Foetus with transantebrachial transverse failure of formation (TFF). Limb stump (*arrow*). Courtesy of Dr. Bisch

ties, which is considerably higher than that of adult traumatic amputees. Indeed, "agenesic patients are functionally complete but differently constituted individuals" [31].

The trouble of relational origin is often important, burdened by the attention given by others [21, 26, 42]. In many cases, merely this issue to resemble others is the reason for prosthesis [31]. The use of prosthesis is "team dependent" [26, 30]. It should be mentioned that prosthesis brings relief not only for the baby but also for the parents. Adults who wear prostheses systematically began wearing them early in childhood [31]. The myoelectric prosthesis has some disadvantages: it takes away "stump" sensation, [30]; and it is heavy, noisy and expensive [43].

To investigate the opinion of persons with TFF, a survey is to be performed by the Lyon team, including utilisation of the French version of Disabilities of the Arm Shoulder and Hand (DASH) [44] and a specially developed questionnaire assessing daily life activities, prosthesis experience, subjective estimate of the affected limb, the impact of others' reactions and opinion on possible hand allograft.

Doctor, our baby was (or is going to be) born with only one hand! Would it be possible to graft him one?

A sizable responsibility also lies with the parents of a potential donor neonate born with life-incompatible pathology and normal limbs. Will a "Nicholas Effect" be possible in this case (Nicholas Green was a child whose family donated his organs after his murder) [45].

Doctors: An agenetic patient can lead an autonomous, independent and happy life with a single hand . . . Nevertheless, would not it be better with two?

A preliminary survey has been recently been performed in Europe during two different meetings in 115 doctors (70 paediatricians and 45 microsurgeons) while presenting the topic: "Consideration on the feasibility of hand transplantation in congenital deformities". A simple questionnaire assessed participants' opinion regarding HT in a newborn in the absence of prolonged immunosuppression and with 50% expected functional outcome. Eighty-four (53+31) doctors responded. A slight majority, i.e. 48 (57%) (25+23) were favourable to HT, 19 (13+6) had no opinion and 13 (11+2) were unfavourable. Therefore, the advisability of this surgery at least needs to be confirmed in the medical population as well.

Is here a new manifestation of the deleterious propensity for surgeon to feel oneself as God?

"God created us not equal to him, but in his image endowing us with opportunity to create" [22].

In fact, transplanting a hand does not mean creating a "superman". You just try to change a form created by nature into a form more expected in nature. The team, which considers grafting a hand to a neonate, should be *convinced* and *convincing* that it intends to perform an intervention destined for the patient's welfare (functional, aesthetic and psychological), with careful estimation of the benefit–risk equilibrium [46, 47].

Immunological Aspects

The key component of allotransplantation is immunological [47, 48]. In the context of nonvital surgery, one may consider HT in newborns only in the absence of deleterious immunosuppression. Tolerance, the "holy grail" of transplantologists [49], could enable avoidance of the main drawback of HT in the newborn. Two situations can be considered on an immunological plane:

1. Isogenic graft: One monozygotic twin is missing a hand while the other one, presenting a life-incompatible anomaly, may serve as a hand donor. Immunosuppression is not necessary in such cases. However, this clinical picture is unlikely to present itself.
2. Allogeneic graft after immunotolerance induction: The peculiarities of the neonate "immature" immune system represent a chance that immunotolerance can be obtained more easily than in adults, avoiding immunosuppression [50–52]. There are

promising investigations concerning the exposition of alloantigen (bone marrow cells) to foetal or early neonatal rats, inducing lifelong tolerance uniquely to that alloantigen and permitting further CTA from the same donor [53–56]. Optimistic data were obtained from the recent clinical trial of paediatric heart transplantation by intrathymic inoculation of unmodified donor bone marrow prior to sternal closure [57]. This manoeuvre significantly diminished late acute cellular rejection. Experiments on larger animal neonates (e.g. piglets) using identical and novel tolerating models need to be conducted to better understand the issue of "donor-specific tolerance" in CTA [58–60].

Anatomical and Functional Aspects

How can a recipient's hypoplastic stump adjust to a donor's eutrophic limb?

We found no anatomical study dedicated to TFF, as it concerns longitudinal failures of formation (e.g. radial club hand) [1]. Forearm pronosupination is often restricted because of proximal radioulnar abnormalities [13]. Transantebrachial TFF can be associated with congenital radial head dislocation, radioulnar synostosis and other anomalies of bone shape and position [61]. The residual limb in TFF is usually well cushioned, and rudimentary nubbins or dimpling can be found on the end [36]. Abnormal development or absence of various anatomical structures are systematic findings in TFF. For example, in cases of transmetacarpal TFF, while planning toe-to-hand transfer, we inform parents that the procedure can be cancelled during the surgery because we may "fail" to find adequate vessels and other structures for planting the normal toe.

Certain technical problems may occur during transplantation of a hand allograft harvested from a "normal" donor. Potential issues are incongruence of donor and recipient vessels and nerve diameters; immature, nonfunctional muscle bellies and tendons; and underdeveloped radius or ulna. Indeed, the functional elements of the donor limb, harvested with some excess, can replace the recipient's corresponding aplastic structures. For example, one can excise the aplastic nerve, vessel or bone up to the normally developed "healthy" levels and replace them with those of the donor. This manoeuvre will probably supply the hand allograft with sufficient neurovascularisation and skeletal framework, providing optimal conditions for functional recovery. However, one must always keep in mind the "main rule" of HT: no or minimal residual limb shortening or iatrogenic impairment after possible allograft failure.

Cognitive and Neuroanatomical Aspects

Is an individual born with only one hand capable of using two?

The human central nervous system (CNS), especially the neonatal brain, possesses excellent plastic properties. Integration and adequate functioning of transplanted hand allograft in the traumatic amputee depends on multilevel plastic reorganisation potential both in the CNS and peripheral nervous system [62, 63]. This "allointegration" is possible thanks to the reversible "invasion" of hand cortical representation by face representation, which cedes its captured territory following HT [64]. However, some doubts have been cast on similar successful integration following HT in the agenetic newborn. The main issue is whether such a neonate has cortical representation of the absent part of the limb. Hand representation has been shown to be shrunken in syndactyly and dysmelia [65, 66]. Moreover, the deficiency of the target organ can contribute to underdevelopment of not only relevant cortical but also spinal representations [67–70]. Thus, the hypoplastic peripheral nerves of the stump may contain fewer axons, sufficient only for sensory and motor function of the residual limb. On the other hand, accounts of phantom sensations (10–20%) in their missing limbs from individuals born without limbs [71–74], as well as excellent clinical integration of transferred toes after

toe-to-hand transfers in transmetacarpal and transphalangeal TFF (Fig. 4), argue for a priori existence of central representation or of another reorganisation mechanism [22, 75–81]. Due to the absence of univocally accepted classification and nomenclature, one cannot ascertain if those born without limbs had TFF or another type of CLD [74]. Ongoing research in collaboration with Dr. A. Sirigu , research director at the Institut des Sciences Cognitives (CNRS, Bron, France) will help shed light on the cognitive aspects of the discussed subject.

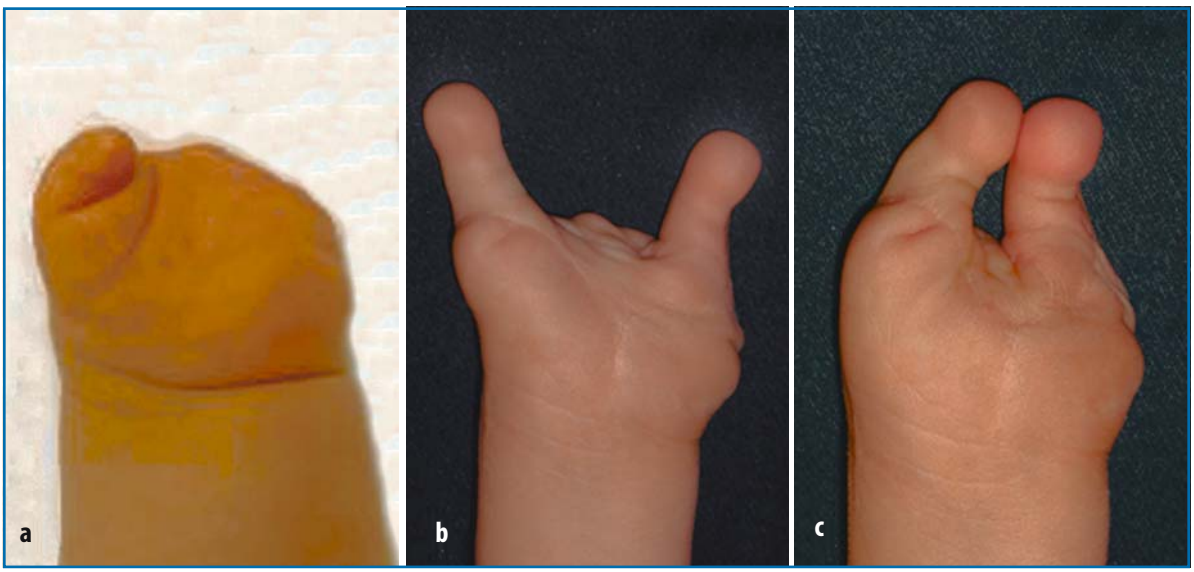

Fig. 4a-c. Transmetacarpal transverse failure of formation (TFF). **a** Before. **b**, **c** After two consecutive second-toe-to-hand transfers; follow-up 2.5 years. Fast cognitive integration of the transferred toes. Courtesy of Prof. Gilles Dautel and Dr. Aram Gazarian

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Section 12-b

Mechanisms Involved in the Induction of Tolerance in Allogeneic Hand Transplantation: A Proposal

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Introduction

The first human unilateral hand allotransplantation was performed in Lyon, in September 1998, followed by other cases all over the world. The technical feasibility of this procedure has thus been demonstrated. Whereas antigenicity of the skin was considered as the major obstacle to human composite tissue allotransplantation, clinical outcomes have demonstrated that hand allotransplantation seems to be well tolerated without drastic immunosuppressive drugs. This chapter briefly describes current advances in the field of tolerance to organ transplantation and elaborates suggestions for the reasons why hand transplantations are likely to be immunologically well tolerated by the host.

Chimerism and Tolerance to an Organ

Mechanisms of Tolerance to Autoantigens

Tolerance induction based on clonal deletion of self-reactive T cells in the thymus upon interaction with dendritic cells (DC) is a well-known mechanism to prevent autoimmune reactions in the periphery and is defined as central tolerance [1]. However, not all self-antigens are represented in the thymus. Thus, autoreactive T cells can reach the periphery. Therefore, additional mechanisms for tolerance induction and maintenance must be present in the periphery [2]. These mechanisms of peripheral tolerance include anergy, which is the functional inactivation of the T-cell response to restimulation by autoantigen (Ag); immunoregulation, which is an active process whereby one population of cells controls or regulates the activity of another population; clonal exhaustion, which can occur as a result of chronic stimulation; or ignorance of Ag, usually due to sequestration [3]. This review article presents data on central and peripheral tolerance to alloantigens following organ transplantation.

The evolution of tolerance to donor alloantigens *in vivo* is a dynamic process involving many mechanisms at different stages. Persistence of alloantigens is thought to be essential for most of the mechanisms that have been reported since, in the absence of alloantigens, tolerance is lost either immediately or gradually [4, 5].

Central Tolerance Through Clonal Deletion of Donor-Alloantigen-Reactive T Cells can be Induced by Mixed Chimerism

Clonal deletion of donor-alloantigen-reactive T cells can be achieved centrally in the thymus through infusion of donor bone marrow into a recipient who has been conditioned by myeloablative irradiation or nonmyeloablative irradiation and immunotherapy [6]. This enables donor antigen-presenting cells (APCs), notably, DCs, to access the thymus and trigger deletion of matur-

ing thymocytes. Indeed, allogeneic haematopoietic stem cells that have engrafted into recipient bone marrow environment will participate to the generation of haematopoietic lineages, including: (1) DCs that mediate negative selection in the thymus, and (2) thymic progenitors. Thus, all newly maturing thymocytes (which are of host and donor origin in that case) recognising either donor or host antigens will be eliminated during the process of central deletion. The newly developing immune system will consider the donor as self, and as long as donor and host haematopoietic stem cells coexist, the thymus will not generate mature T cells with reactivity to the donor or the host. Intrathymic deletion of donor-reactive thymocytes was shown to be the dominant mechanism for the maintenance of tolerance whereas no evidence for peripheral mechanisms was found. However, in these models, a constant source of donor APCs was required to ensure intrathymic deletion of newly developing thymocytes. This was demonstrated by depletion of donor chimeric cells with donor class I major histocompatibility complex (MHC)-specific monoclonal antibody (mAb) in mice and resulted in breaking tolerance with appearance in the periphery of T cells with receptors recognising donor antigens [7].

Macrochimerism

Macrochimerism, defined by the presence of more than 10% cells of donor origin, is efficient in inducing tolerance to organ graft even in humans [8]. Indeed, in humans, kidney allografts have been successfully performed in recipients of bone marrow from the same donor without any long-term immunosuppressive therapy. This tolerance was related to a complete haematologic chimerism since following bone marrow transplantation, T cells are of donor origin. However, induction of macrochimerism through ablation of the host haematopoietic compartment and peripheral T-cell repertoire using lethal total body irradiation (TBI) have little clinical potential since severe complications, such as graft-versus-host disease (GVHD), can occur due to the presence of donor T lymphocytes that can recognise antigen-presenting cells from host origin.

Microchimerism and Persistence of Donor-Derived Passenger Leukocytes

Subsequently, nonmyeloablative protocols have been developed. In this setting, depletion of recipient T cells by antilymphocyte antibodies or costimulatory blockade and subsequent repopulation by donor and recipient haematopoietic cells are prerequisites for tolerance induction. The dose of donor haematopoietic stem cells is a critical factor influencing the efficacy of this tolerance-inducing regimen. Indeed, Taniguchi et al. [9] reported that mice with >30% chimerism could accept skin grafts whereas mice with <10% chimerism showed prolonged but not permanent graft survival. Incomplete depletion of residual host T cells associated with <10% chimerism was likely to be responsible for failure of allograft tolerance induction. Other important parameters include MHC class II expression by donor cells and engraftment of donor T cells. These settings resulted more often in microchimerism due to passenger leukocytes originated from the donor. But microchimerism however can also contribute to induction of tolerance, especially when passenger leukocytes are DCs. Indeed, these professional APCs are able to educate T lymphocytes inside the thymus towards central tolerance or to migrate into secondary lymphoid organs and induce apoptosis of donor reactive T cells [10].

Although microchimerism is often associated with graft acceptance and tolerance, it has been difficult to demonstrate a true causal link between microchimerism and the absence of rejection leading to long-term graft survival. Thus, whereas Kanamoto et al. showed that chimeric donor cells play an active role both in the induction and maintenance phases of allograft tolerance [8], in another study using where skin from mutant mice deficient for leukocyte subsets, it was found that grafted in immunologically mature hosts, chimerism can result in immunity and stronger graft rejection [11] as opposed to in immature hosts. Finally, in humans, the majority of both clinical and experimental studies did not show correlation between microchimerism and long-term tolerance. Thus, as proposed by Wood, an alternative interpretation could be that microchimerism is a consequence of long-term graft acceptance

rather than the cause [10].

In conclusion to this section, the impact of donor-derived passenger leukocytes on immune response after transplantation is not strongly correlated with long-term tolerance. However, the presence of passenger leukocytes early after transplantation may play a role to achieve an immunomodulatory effect. Thus, augmenting the number of donor leukocytes present at the early stage of the response may be beneficial to initiate tolerance but not sufficient to maintain long-term tolerance, which more likely depends on the presence of the graft.

Peripheral Tolerance to Organ

Dendritic Cells: The Link Between Central and Peripheral Tolerance

As mentioned above, DCs play a major role in the induction of central tolerance through clonal deletion of alloreactive donor T cells in the thymus. But there is growing evidence that at the immature stage, DCs can also participate in mechanisms leading to peripheral tolerance through induction of a hyporesponse to allo-Ag. This induction of antigen-specific T-cell unresponsiveness can be related to cytokines secreted by immature DCs such as interleukin (IL)-10, to activation of indoleamine 2,3-dioxygenase (IDO) enzymatic activity, or to secretion of soluble factors such as prostaglandin E2 (PGE2). Cyclosporine, corticosteroids, mycophenolate mofetil (MMF) or other immunosuppressive agents might function at least in part by preventing DC maturation [12–14]. Recently it has been shown that some subsets of DCs can directly activate regulatory T cells [15], notably, immature DCs can induce differentiation of naïve T cells into regulatory T cells rather than into effector cells [16].

Regulatory T Cells

In 1995, Sakaguchi et al. described for the first time a subpopulation of CD4 T-helper cells characterised by a constitutive expression of the IL-2 receptor α-chain (CD25) that is essential to control autoimmune responses in mice [17]. After subsequent *in vitro* studies by several groups, this population is now referred to as CD4 CD25 T regulatory cells (Tregs). Comparable T-cell suppressor populations with identical phenotype and functional activities have been defined more recently in rats and humans. They represent 5–10% of all peripheral CD4 T cells. Freshly isolated CD25 Tregs do not proliferate after allogeneic or polyclonal activation *in vitro* but suppress activation and cytokine release of CD4 and CD8 T cells in a cell contact-dependent manner [18].

In organ transplantation, multiple reports using animal models have established that activation of CD4+CD25+ regulatory T cells constitutes an essential element of the immunoregulatory pathways that create peripheral allograft tolerance. In the absence of this T-cell subset, a variety of potent therapies to induce tolerance lose their ability to do so. Indeed, some of these therapies appear to be acting, at least in part, by directly modulating the function of Treg. Thus, it has been shown that treatment with nondepleting mAbs, such as anti-CD4 and anti-CD8, at the time of organ transplantation without infusion of dendritic cells can induce tolerance [19]. Subsequently, it was shown that the blockade of other cosignaling pathways, namely, CD28/B7, CD40/CD154, or LFA-1/ICAM-1, also resulted in transplantation tolerance [20]. This peripheral tolerance is under the governance of regulatory T cells that inhibit nontolerant naïve T cells (dominant tolerance). The presence of these regulatory T cells facilitates, then, the emergence of new regulatory T cells from the naïve lymphocyte population (infectious tolerance). The mechanism that leads to infectious tolerance might result from direct contact between regulatory T cells and coactivated T cells, converting them into suppressor T cells secreting IL-10 and transforming growth factor (TGF)-β [21, 22]. Moreover, in the context of costimulatory blockade, DCs are likely to be immature and to induce anergy through IL-10 secretion, amplifying thus the presence of suppressive T cells (Fig. 1). Recent work in rats, has demonstrated that tolerant cells can be found inside the tolerated organ. This may indicate that they have a protective role within that tissue. Interestingly, similar results were obtained in mice treated with anti-CD4 and

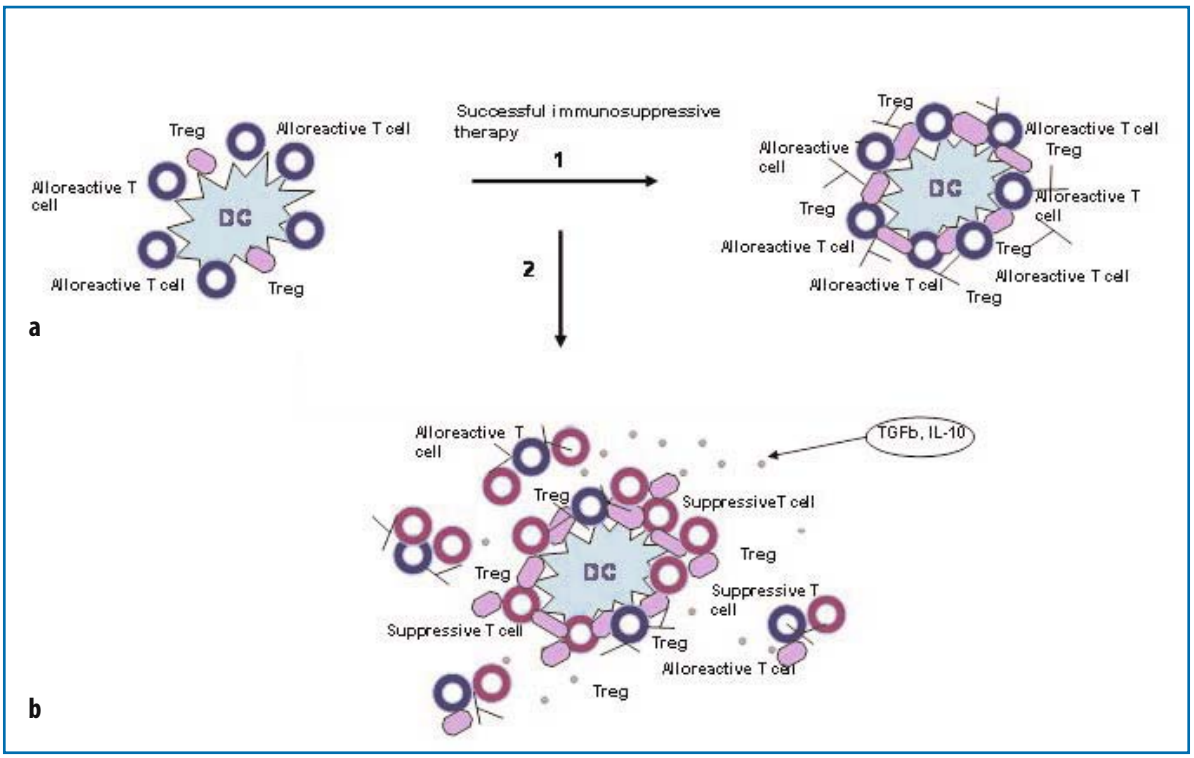

Fig. 1a, b. a Dominant tolerance, local, contact-dependent T cells. **b** Infectious tolerance, systemic, cytokine-dependent T cells

anti-CD8 following skin transplantation. In this study, the Authors showed that tolerance was due to infiltration of the graft by regulatory T cells. It was suggested that regulatory T cells may recirculate through the body and accumulate preferentially at the sites where their target antigens are present [23]. At present, it is unclear whether regulatory T cells are induced at a specific period of development in the thymus and then expand in the periphery in the target site of inflammation or whether they can directly develop in the periphery. However, a recent study may give an answer to this question since it has been demonstrated that conversion of CD4+CD25- cells into CD4+CD25+ regulatory T cells can be induced in thymectomised mice, suggesting that these cells can, indeed, develop in the periphery [24].

The elucidation of Tregs specificity in transplantation has been more difficult to achieve due to the use of lymphopenic adoptive transfer systems in which proliferation of regulatory T cells could not be shown. However, a recent study using an immunocompetent animal model has demonstrated that allo-Ag-specific Tregs, once stimulated through their T-cell receptors (TCR), can proliferate under the influence of the IL-2 secreted by alloreactive T cells. Thus, donor-specific Tregs are likely to be activated together with alloreactive T cells by a common donor: APC.

In conclusion to this section, and based on the suggestions of Cortesini [25], one can imagine that early after transplantation, in the inflammatory milieu created by the operative trauma, recipient CD4 T cells may become activated through direct recognition of alloantigens expressed on the membrane of donor APC within the graft. T cells may proliferate, produce and induce a cascade of cytokines, and elicit the generation of CD8 cytotoxic T lymphocytes, which will damage the graft, causing an acute rejection episode. Successful immunosuppressive therapy will reverse rejection by inhibiting proliferation of alloreactive CD4 T-helper cells and favouring activation of CD4+CD25+ Tregs. Tregs will act directly by inhibiting the action of alloreactive T cells (dominant tolerance) but also by propagating suppression through direct contact with naïve T cells and under the influence of a favourable cytokine environment (infectious tolerance). This anergy would also be amplified by migration of immature donor APC out of the graft and by ingestion of

their apoptotic and necrotic bodies by host DCs, which will process them into peptides and present them to T cells in the regional lymph nodes. Under the coverage of immunosuppression or costimulatory blockade, activated CD4+ T cells that recognise donor allopeptides would be unable to secrete IL2 and proliferate. In contrast, immunosuppressive T cells, which do not require costimulation via the B7 or CD40 molecules, would be activated and secrete IL-10 and TGF-β, which will then in turn maintain DCs in an immature state and favour infectious tolerance following migration of these suppressor T cells into the organ where Tregs are present.

Hand-Graft Transplantation: A Human Model of Tolerance Induction

In humans, no such direct evidence of involvement of Tregs in long-term allograft survival has been shown. Therefore, since in our team bilaterally hand grafted 2 patients [26, 27], we investigated whether alloresponses to donor Ags inside the graft would bring informative data on the status of T cells that infiltrate the graft. Indeed, the opportunity to easily isolate T cells infiltrating the graft was offered by our hand-transplant model. These tissue-composite grafts allowed us to perform skin biopsies, isolate and expand T cells, and monitor their responses against donor Ag at 1, 3, 6, 12 and 18 months posttransplantation independently on the presence of cutaneous lesions in one patient and at 3 and 5 years posttransplantation in another patient. Results obtained by our team strongly suggested the presence of regulatory T cells that modulate the alloresponse in the 3-year old graft whereas alloreactive cytotoxic T cells were preponderant very early after hand transplantation and remained present until 18 months in the other patient.

Indeed, as shown in Figure 2, T cells isolated from skin and expanded *in vitro* were preponderantly CD4+ in the 3-year grafted patient but CD8+ in the recent graft. Moreover, as shown in

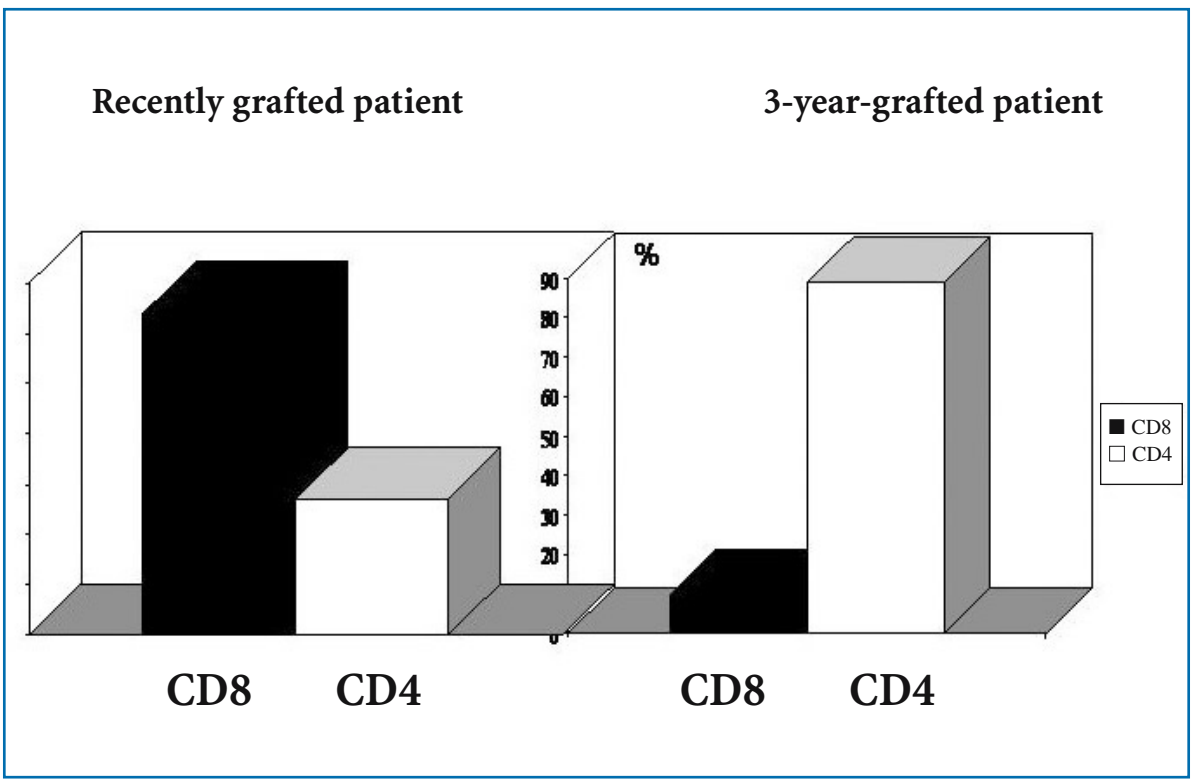

Fig. 2. Phenotypic profile

Figure 3, skin T cell isolated from the 3-year-grafted patient were unresponsive towards donor allo-Ag, albeit they were able to recognise APC sharing human leukocyte antigen (HLA)-class II Ag with the donor, notably, HLA-DR15, DQ6. This unresponsiveness against donor Ag was likely to be related to the presence of approximately 5–10% of regulatory T cells inside the graft, as assessed by immunohistological staining of skin biopsies with FoxP3 mAbs. These skin T cells were indeed capable of inhibiting donor-directed blood T cell responses. In contrast, in the more recently grafted patient, T cells harvested from skin were cytotoxic and were specifically activated by donor allo-Ag (Fig. 3).

These results are highly concordant with those of a very recent study that has shown in a nonlymphopenic mouse model of cardiac transplantation that whereas only 5% of CD4+ T cells were Tregs, this was sufficient to suppress donor-directed specific cell responses and to prevent allograft rejection when cognate Ag was present in the graft, demonstrating that Tregs require TCR stimulation by allo-Ag [28].

Conclusion

Human Mesenchymal Stem Cells, Regulatory T Cells, and Tolerance to Hand Allografts: A Possible Link

The implication of mesenchymal stem cells is not clearly debated at present in human organ transplantation whereas their role in the modulation of allogeneic immune responses is strongly demonstrated in bone marrow transplantation. While CD34+ cells and dendritic cells have been widely implicated in microchimerism-induced tolerance, the question of the possible involvement of mesenchymal stem cells deserves to be asked. Indeed, not only are these cells known to inhibit cytolytic activity and T-cell proliferative responses against alloantigens through direct contact with T cells, they are also able to secrete suppressive cytokines such as TGF-β or soluble factors such as PGE2 [29, 30]. Moreover, numerous studies have shown that these cells are able to inhibit maturation of dendritic cells, which in turn may inhibit allore-

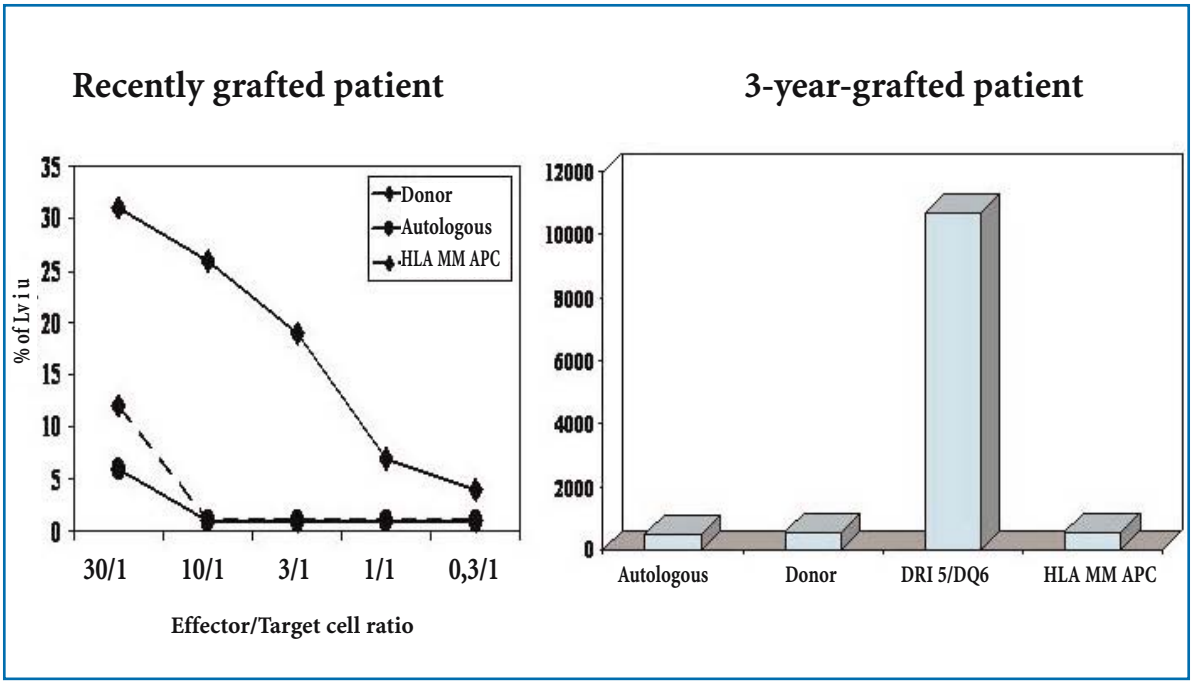

Fig. 3. T-cell response of skin T lymphocytes against allo-Ag

sponses. Thus, in a skin graft model performed in a baboon, it has been shown that infusion of mesenchymal stem cells (MSCs) at the time of transplantation resulted in delayed graft rejection [31]. While one of the most important findings of these recent years was the ability that MSCs could migrate into tissue, another very interesting report has shown that culturing naïve T cells with MSCs leads to expansion of regulatory T cells, demonstrating a direct relation between expansion of regulatory T cells and the presence of MSCs [32].

Therefore, because when a hand is grafted bone marrow from donor origin is present, an important question that deserves investigation is whether MSCs present in the hand-grafted bone marrow could be involved in the expansion of regulatory T cells inside the graft.

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Section 12-c

Induction of Tolerance in Allotransplantation

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Introduction

Since the birth of the field of transplantation, progress in transplantation medicine has been rapid. Chemical immunosuppression with corticosteroids and 6-mercaptopurine was first used to enable transplantation between nonidentical individuals in the early 1960s. The introduction of newer immunosuppressive agents and improvements in surgical techniques and ancillary care have made transplantation a routine and preferred therapy for treatment of end-stage renal, cardiac, hepatic and pulmonary failure; pancreatic transplantation provides similar benefits for diabetic patients. At least in the case of renal failure, studies indicate that patients who undergo transplantation have lower morbidity and mortality rates than appropriately matched nontransplanted control patients [1].

Currently available immunosuppressive medications provide outstanding short-term results in renal, cardiac, liver, lung and pancreatic transplantation with around an 80–95% 1-year graft survival (Fig. 1a). However, improvements in short-term graft survival rates have not been accompanied by improvements in long-term outcomes [2] so that the half-life of the grafts that function after 1 year has changed little over the past 40 years (Fig. 1b, c), with few exceptions [3] (Table 1). Furthermore, the transplant recipient must be treated with immunosuppressive agents for life, a therapy that trades the morbidity and mortality of organ failure for the increased risks for opportunistic infections and malignancy [4]. These drugs also likely contribute to increased mortality from cardiovascular disease, the major cause of premature death in kidney transplant recipients [5]. In addition, there is the problem of chronic rejection, which arises at least in part because immunosuppressive strategies do not completely inhibit alloimmune responses and result in slow, progressive deterioration in graft function [6]. These challenges together with the increasing demand of organs for transplantation create an urgent need for optimising the outcome of transplanted organs by achieving long-term, drug-free, graft acceptance with normal graft function, a condition defined as “transplantation tolerance”.

Since the seminal experiments conducted by Billingham, Brent, and Medawar in 1953 [7], there was unequivocal proof of the concept that specific tolerance to a defined set of donor antigens can be acquired throughout life. They showed a way of preventing graft rejection by challenging the developing immune system in the embryo or neonate with specific donor antigens. They first found that this occurred naturally in nonidentical cattle twins that shared their blood circulation in the placenta. They were then able to repeat this effect in planned experiments, injecting cells from donor mice of one inbred strain into the potential foetal or neonatal recipient of another inbred strain. The injected foetal or neonatal mice were tolerant to the donor of the cells and usually accepted grafts from the donor strain for long periods.

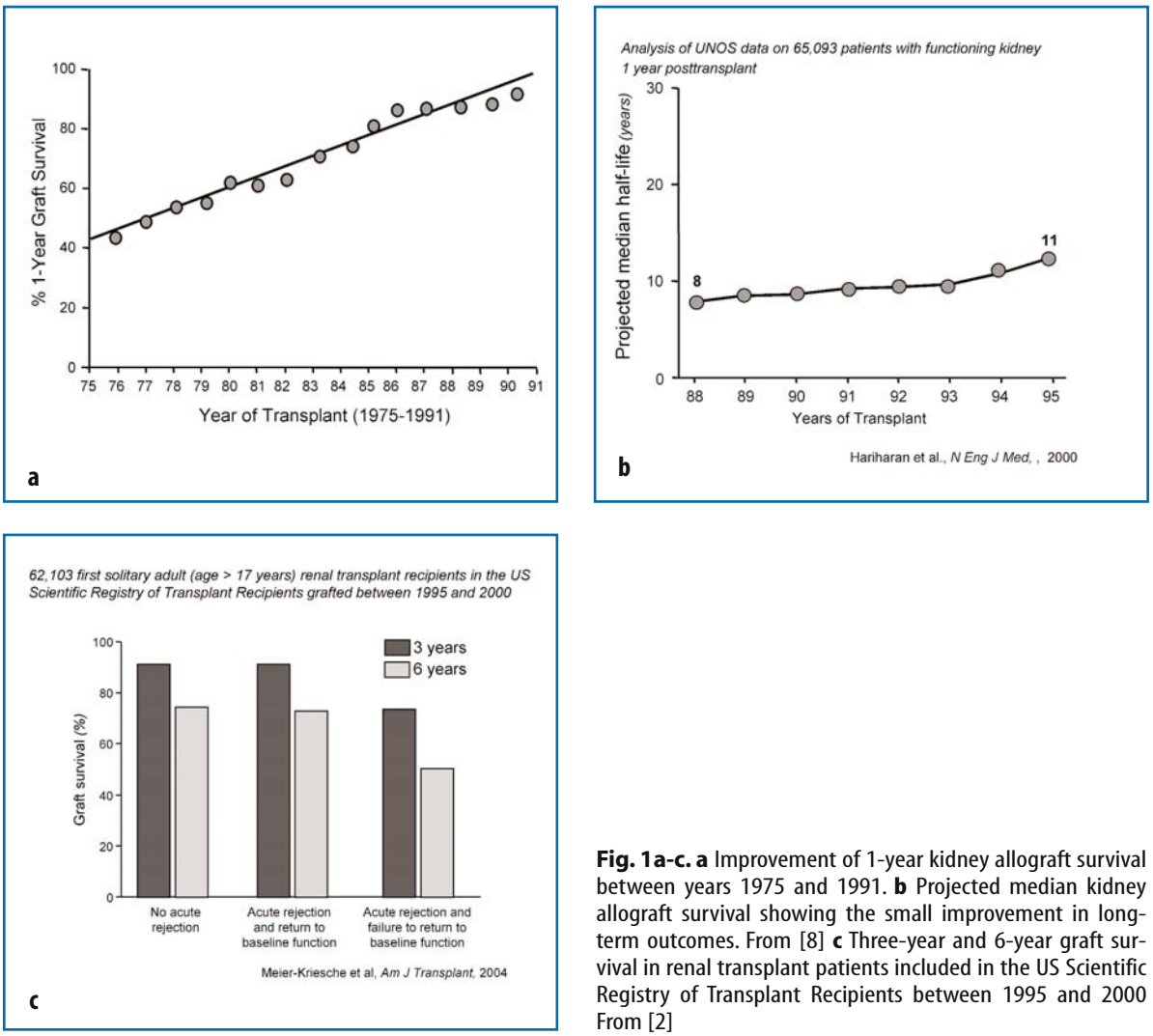

Fig. 1a-c. a Improvement of 1-year kidney allograft survival between years 1975 and 1991. **b** Projected median kidney allograft survival showing the small improvement in long-term outcomes. From [8] **c** Three-year and 6-year graft survival in renal transplant patients included in the US Scientific Registry of Transplant Recipients between 1995 and 2000 From [2]

Table 1. Longest surviving patients who currently have functioning transplants (2004)

Transplant	Years of continued graft function	Centre
Kidney (living)	42	Denver
Kidney (cadaver)	38	Minneapolis
Liver	35	Denver
Heart	26	Standfort

Achieving the specific goal of donor-specific tolerance would not only minimise the risk of the recipient to suffer from serious side-effects resulting from continuous immunosuppressive therapy, but also it would prevent loss of long-term graft function caused by chronic rejection processes [9–11], thus making more organ available for primary (first) transplant recipients.

The Concept of Transplantation Tolerance

By definition, tolerance can be described in general terms as a state of unresponsiveness to self or foreign antigens in the absence of immunosuppressive therapy while the remainder of the immune system is left intact. Thus, lack of response to the alloantigen is specific, and the recipient is capable of responding to potentially pathogenic microorganisms and malignancies.

T cells are the vital elements that orchestrate the alloimmune response and interact with graft alloantigens by direct and indirect pathways, recognising foreign major histocompatibility complex (MHC) molecules directly on the donor antigen-presenting cells (APC) and processed donor antigens as peptides on self APCs, respectively [12]. T cells reacting to their specific antigen can undergo a number of different responses – namely, "activation" followed by proliferation and differentiation into effector and memory cells – and "termination". Physiologic termination of T-cell immune response forms the basis of inducing donor-specific tolerance in clinical transplantation. Several mechanisms, not necessarily mutually exclusive, have been proposed as the basis of transplantation tolerance: deletional mechanisms (actually in the thymus and in the periphery) in which donor-reactive T-cell clones are destroyed, and non-deletional/immunoregulatory mechanisms (including anergy, immune deviation, active suppression/regulation) [13, 14] (Fig. 2). A further possible mechanism of immunologic tolerance unique to the transplant setting is microchimerism, the persistence of a small number of donor-derived bone marrow (BM) cells in recipients [15]. Microchimerism may be strictly related to and be the inciting mechanism for activating both deletional and nondeletional mechanisms of tolerance.

Central and Peripheral T-cell Deletion

Studies in experimental animals have indicated that clonal deletion of maturing T lymphocytes may occur centrally in the thymus following donor haematopoietic cell (HC) infusion. T-cell receptor (TCR)-transgenic mouse models [16] and Vα tracking of T cells responding to superantigens presented by donor MHC class II molecules on APC [17] have been used to document the process of central deletion in mixed chimeras. For instance, mice transgenic for a H-2L^d specific TCR, receiving L^{d+} BM cells underwent intrathymic deletion of H-2L^d CD8+ clono-

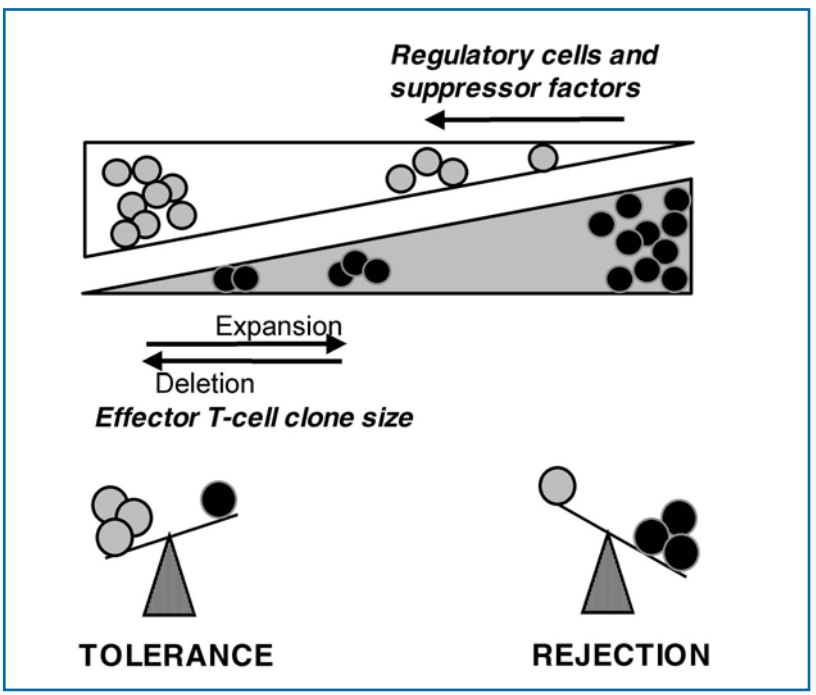

Fig. 2. Balance between rejection and tolerance. Rejection is determined by the number of T cells reacting to their specific antigen that undergo activation followed by proliferation and differentiation into effector and memory cells. Tolerance occurs by termination of the T-cell immune response by deletional mechanisms (in which donor-reactive T-cell clones are destroyed) and by formation and expansion of regulatory cells with suppressive functions

typic cells, as shown by the reduction of CD8 single positive thymocytes expressing the transgenic TCR. The decline in CD8+ cell number correlated with the presence of donor dendritic-like cells in the thymus [16]. These data point to intrathymic clonal deletion of donor-reactive T-cells as one of the major mechanisms maintaining tolerance in allogeneic chimeras. However, thymic deletion cannot account for tolerisation of preexisting mature donor-reactive T cells that is achieved in the presence of an intact recipient T-cell repertoire by the use of BM transplant protocols. This observation led to exploration of peripheral mechanisms through which mature donor-reactive T cells are rendered tolerant to donor alloantigens. Experimental studies of allogeneic BM transplantation with costimulatory blockade in thymectomised recipients have documented clonal deletion of donor-reactive CD4+ T cells, which provides support to the possibility that tolerogenic mechanisms also operate by deletion processes in the periphery [18, 19].

Nondeletional Mechanisms

T-cell anergy and regulation are highly complementary with deletion processes and may well both be necessary for long-term transplant tolerance to be achieved. Anergy is a state of functional inactivation in which antigen-specific T lymphocytes are present but unable to respond. Unresponsiveness can be assessed in vitro by failure of proliferation and cytokine production [20] and in vivo by failure of clonal expansion [21]. Sustained exposure to antigens can also result in generation of anergic T cells with regulatory capacity, even in the absence of tutoring by any preexisting regulatory T cells (Tregs) [22, 23]. These data support the notion of a form of peripheral tolerance, expounded over a decade ago [24], where anergic T cells can compete out emerging naïve responding cells that then default to tolerance themselves.

Several subsets of Tregs with distinct phenotypes and mechanisms of action have now been identified. They contribute a network of heterogeneous CD4+ or CD8+ T-cell subsets (Fig. 3) and other minor T-cell populations, such as nonpolymorphic CD1d-responsive natural killer T cells [25]. Tregs not only contribute to maintain self-tolerance and prevent autoimmune disease but can also be induced by tolerance protocols (*see* later in this Section).

Approaches to Transplantation Tolerance

Over the last 25 years, several strategies have been used successfully to induce transplantation tolerance. Each of these has been validated in at least one rodent model, with varying degrees of success upon extension to large animals, nonhuman primates and humans.

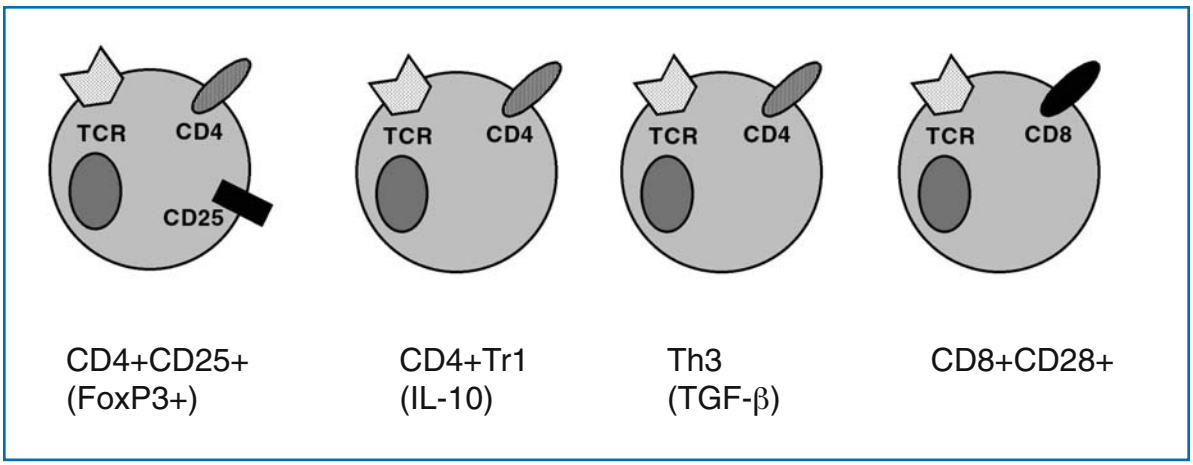

Fig. 3. Subset of regulatory cells that have been associated with tolerance induction in experimental animals

Tolerance Through Donor Haematopoietic Cells

Infusion of donor HC, especially BM cells, associated with different immunological manipulations of the host immune system, represents one of the most promising ways to induce tolerance of solid-organ allografts [26]. Using this approach, mixed chimerism – the coexistence of two genetically different cell components in the host with multilineage chimerism reflecting engraftment of donor pluripotent HCs [27] – is induced and is believed to be the driving factor for tolerance induction.

Patients receiving BM transplantation to cure haematological malignancies are prone to develop tolerance to a subsequent kidney allograft by mixed chimerism. This has been recently documented in two patients with end-stage renal disease secondary to kappa light-chain multiple myeloma who underwent a combined BM and kidney transplantation after a conditioning regimen of cyclophosphamide, thymic irradiation and antithymocyte globulin [28, 29]. After 70 days of posttransplant cyclosporine A (CsA) therapy, the drug was discontinued, and no further immunosuppression was given. Patients have been free from immunosuppression for more than 2 years without evidence of either acute or chronic rejection of the renal allografts. Although effective and appropriate for patients with haematological malignancies, the risk of infection, aplasia and ultimately death associated with the actually available conditioning regimens significantly outweigh the potential benefit of tolerance, particularly when considering the excellent short-term outcomes currently achieved with conventional immunosuppression in organ transplantation. Thus, studies have been undertaken in experimental animals and in humans with the aim of introducing donor hyporesponsiveness by HC infusion with no or minimal host conditioning.

Experimental Studies on Nontoxic Approaches to Reduce Recipient Conditioning

More than 20 years ago, Ildstad and Sachs showed that mice in which the haematopoietic system was ablated by lethal whole-body irradiation and then reconstituted with a mixture of syngeneic and allogeneic BM exhibited donor-specific tolerance to skin allograft [30]. The approach was later rendered much less toxic by using specific nonmyeloablative conditioning involving T-cell-depleting antibody treatment. In this strategy, initial rejection of donor BM is prevented in mice by depletion of peripheral and intrathymic T cells with T-cell-specific antibodies, with or without local thymic irradiation and without myeloablation [31, 32]. Once the immune compartment has begun to reconstitute itself, tolerance is induced and maintained by intrathymic deletion of potential donor-reactive T cells. Subsequent studies in miniature swine and nonhuman primates [33] have clearly established the principle that nonmyeloablative mixed chimerism is a highly effective mean to induce tolerance.

More recent studies in rodents indicated the possibility of achieving high levels of allogeneic chimerism after donor BM infusion in the presence of an intact recipient T-cell repertoire by blocking costimulatory signals of T-cell activation. In addition to specific antigen, naïve T cells require costimulatory signals for optimal activation. The best characterised T-cell costimulatory receptors are CD28, the two ligands of which, CD80 (B7-1) and CD86 (B7-2), are expressed on APC [34]; and CD154 (CD40L), the counter-receptor of which on APC is CD40. Allogeneic mixed chimerism and tolerance in rodents without any cytoreductive host treatment have been recently achieved by combining very high doses of BM and the transient use of costimulatory blockade with CTLA4-Ig (a soluble receptor-immunoglobulin fusion protein that binds CD80 and CD86 with higher affinity than CD28) and anti-CD40L antibody [17]. However, such tolerogenic protocol, although nontoxic, cannot easily be extended to clinics due to the very high amount of donor BM needed to achieve sufficient levels of donor chimerism. On the other hand, noncytoreductive strategies employing lower donor BM doses combined with donor-specific transfusion and costimulatory blockade have been shown to allow permanent mixed chimerism and long-term acceptance of mouse

skin graft in a donor-specific manner [35].

Unfortunately, the translation of tolerance protocols from rodents to large animals has been frustrated by crucial differences either in the level of mixed chimerism and T-cell depletion attainable or in different acquired immune history that results in a more established memory T-cell repertoire [36]. Indeed, a critical distinction between pathogen-free mice and primates or human patients is exposure of the latter to environmental pathogens, which provides a potent barrier to transplantation tolerance. Relevant to this issue are recent data in mice that indicate how viral infection leads to generation of alloreactive memory CD8+ T cells that confer resistance to tolerance induction by costimulatory blockade and donor BM infusion [37]. On the other hand, in another study, tolerance could be achieved in Cynomolgus monkeys conditioned with anti-CD40L antibody, fractionated total body irradiation, local thymic irradiation and splenectomy before transplantation of BM and kidney from the same donor. This regimen has resulted in long-term survival in most animals despite loss of chimerism [38]. At variance with rodent models, these studies in nonhuman-primates failed to demonstrate a significant association of chimerism and graft tolerance, thus leaving open the question of whether chimerism is the cause or the consequence of tolerance.

Donor Haematopoietic Cell Infusion in Organ Transplant Recipients

Some investigators are now extending the above approaches to pilot clinical trials designed to induce donor-specific immune unresponsiveness using donor BM cell infusion without myeloablative conditioning [39–44]. In one study, kidney transplant patients were given donor BM a few days after surgery while on quadruple immunosuppression with antilymphocyte globulin, CsA, azathioprine and prednisone [38]. After 16 months follow-up, lower incidence of acute rejection and higher rate of graft survival were found in the BM group compared with the control group receiving quadruple immunosuppression alone. However, long-term follow-up data showed that allograft survival and frequency of chronic rejection were not significantly affected in the BM group compared with the control group. More encouraging results were reported in 63 cadaveric renal allograft recipients given 1 or 2 donor-specific BM infusions under triple immunosuppressive therapy [40–43]. Compared with 219 noninfused controls treated with identical immunosuppression, the actuarial graft survival at 5 years was superior in the BM-infused patients. Moreover, the incidence of chronic rejection was significantly reduced in BM-treated patients. Others have tested the effects of high doses of donor peripheral blood CD34+ stem cells instead of BM infusion to induce mixed chimerism and tolerance in paediatric renal transplant recipients [44]. Graft survival at 18 months follow-up in the treated group was 100% compared with 80% in controls. Although all these clinical studies are promising, they do not provide evidence of tolerance induction since immunosuppressive drugs were not withdrawn at any time posttransplant. So far, the only attempt to achieve true tolerance in human renal transplantation with the HC approach has been done using donor granulocyte colony-stimulating factor (G-CSF)-mobilised peripheral blood CD34+ cells under a nonmyeloablative conditioning regimen of total lymphoid irradiation and antithymocyte globulin [45]. Three out of four patients achieved multilineage macrochimerism without evidence of graft-versus-host disease (GVHD). Maintenance immunosuppression with CsA and prednisone was withdrawn in a patient by month 12 posttransplant. In another patient, prednisone was discontinued at month 9, and CsA was tapered thereafter. All patients, however, eventually developed some form of rejection and returned to immunosuppressive therapy although maintenance immunosuppression was considerably lower than conventional regimens.

In summary, although these trials represent some progress in the use of donor HC, the goal of stable mixed haematopoietic chimerism resulting in life-long tolerance remains elusive.

Peripheral T-cell Depletion in Combination with "Tolerance-Permissive" Drugs

A variety of strategies have focused on T-cell depletion as a means of eliminating alloreactive T cells and reset the immune system. This approach was first tested in animal models to show that depleting CD4- plus CD8-specific monoclonal antibodies could be used to achieve tolerance to foreign proteins [46]. Transplantation tolerance to MHC-matched skin was induced in mice using nondepleting CD4- plus CD8-specific monoclonal antibodies [47]. In a cardiac allograft model in nonhuman primates, T-cell depletion by irradiation and reconstitution with T-cell-purged autologous BM, rejection was delayed, but tolerance was not observed [48]. In contrast, using the combination of diphtheria toxin conjugated to a CD3-specific monoclonal antibody (to create a T-cell-depleting agent for primates) plus the immunosuppressive drug deoxyspergualin, long-term allograft survival was achieved in rhesus monkeys [11].

Three T-cell-depleting agents are currently available for use in humans: a CD3-specific monoclonal antibody, polyclonal antilymphocyte sera, and the CD52-specific monoclonal antibody Campath-1H. The antibody to CD3 effectively clears T cells from the peripheral blood, but it is not believed to provide effective removal of T cells from lymph nodes and spleen. Polyclonal antilymphocyte sera provide potent depletion of T cells from blood, but their effect in lymphoid tissue has not been clarified yet. A number of clinical trials are underway using these agents, in some instance combined with donor BM, as part of tolerance trials [48, 49].

Campath-1H is a humanised complement-fixing antibody that reacts with the CD52 receptor, the most prevalent cell surface antigen on T and B lymphocytes, and to a lesser degree on natural killer cells and monocytes, inducing complement-mediated cell lysis not only in the peripheral blood but in secondary lymphoid organs and BM [50]. As a consequence, Campath-1H causes a rapid, profound depletion of peripheral T cells that lasts for up to a year following a short-course induction therapy whereas the effect on monocyte depletion is more delayed and transient. Originally approved for treatment of malignancy, it has been used in studies to minimise or avoid immunosuppression. Its use in renal transplantation was first described by Calne et al. in 1998 in a steroid-free and low-dose maintenance CsA regimen with an acceptable rate of acute rejection and good patient and graft survival at 2 years [52]. Puzzling, renal transplant patients treated with Campath-1H alone or in combination with rapamycin have frequently had acute rejection characterised by graft infiltration of monocytes and memory T cells [53, 54]. Notably, a recent report indicates that memory T cells are relatively resistant to depletion by Campath-1H [54], which may be a particular barrier to tolerance induction. More recent studies successfully combined Campath-1H with tacrolimus and mycophenolate mofetil (MMF) in a steroid-free regimen with a very low incidence (around 10%) of acute rejection [55–57], but it is too soon to know whether tolerance has been achieved. This suggests that calcineurin inhibition has a direct effect either on monocytes or on residual memory T cells not eliminated by Campath-1H induction.

In Vivo Induction or Ex Vivo Expansion of Regulatory T Cells

Suppression by regulatory Tregs has emerged as an essential tool by which the immune system can actively either silent self-reactive T cells or turn off activated T cells thus controlling immune responses to self-antigens and maintaining immune homeostasis (Figs. 2, 3). Tregs can be distinguished into innate and adaptive. Innate Tregs spontaneously arise during thymic ontogeny. In contrast, adaptive Tregs have been shown to be specific for antigens not present in the thymus and, similar to Th1 and Th2 cells, arise from naïve precursors and can be differentiated *in vitro* and *in vivo*.

Natural Regulatory T Cells

In both humans and rodents, the best characterised populations of Tregs are the CD4+CD25+ T cells, a subset of Tregs constitutively coexpressing CD4 and CD25 [interleukin (IL)-2Rα chain] antigens. CD4+CD25+ Tregs, which constitute 5–10% of peripheral CD4+ T cells, are defined as "naturally occurring" or "innate" regulatory cells since they arise during thymic ontogeny and are selected as a result of relatively high affinity interactions with self-peptide/MHC complexes [58]. These cells play a main role in maintaining self-tolerance and preventing autoimmune diseases [59, 60].

$CD4^+CD25^+$ Tregs are anergic cells that, once activated, are able to inhibit both proliferation and cytokine production by CD4+ and CD8+ T cells in a cell contact-dependent and partially cytokine-independent manner. The contribution of cytokines – in particular, TGF-β1 – to the suppressive activity is a controversial issue [61, 62]. The main mechanism of suppression by CD4+CD25+ Tregs seems to be the inhibition of IL-2 production by responder T cells [63]. Interestingly, both in mice and in humans CD4+CD25+ Tregs have been shown to constitutively express CTLA4 (CD152). Fallarino et al. [63] demonstrated that mouse CD4+CD25+ Tregs block the immunostimulatory function of APCs through CTLA4 engagement of the B7 molecule, which attributes a key role to CTLA4 in Treg function. Thus, CD4+CD25+ Tregs can exert their regulatory activity either by directly suppressing T cells or indirectly through modulation of APC function.

Identification of a specific marker for Tregs remains a controversial issue since activated effector CD4+ T cells also express CD25. Finding that mice carrying the X-linked *scurfy* mutation in the FoxP3 gene display multiorgan autoimmune disease and lack conventional $CD4^+CD25^+$ Tregs [64] has focused the attention on FoxP3 as a specific marker of Tregs in mice. In mice, FoxP3 has been shown to be expressed exclusively by CD4+CD25+ Tregs and is not induced upon activation of CD25- T cells. In addition, transfection with FoxP3 converts naïve CD4+CD25- T cells into Tregs.

Adaptive Regulatory T Cells

In addition to naturally occurring Tregs, it appears to be possible to steer an uncommitted T cell towards regulatory function (induced or adaptive Tregs). Adaptive Tregs can be generated either in vivo from mature CD4+ T-cell populations under particular conditions of antigenic stimulations or ex vivo by culturing naïve $CD4^+$ T cells with antigen or polyclonal activators in the presence of immunosuppressive factors.

CD4+CD25+ Tregs can be induced in vivo by tolerance protocols and play a role in preventing allograft rejection, as demonstrated in many animal models [66]. In a model of rat kidney allograft tolerance induced by preinfusion of donor peripheral blood leukocytes, it has been shown that lymph node cells from long-term surviving rats inhibit naïve T-cell proliferation against donor antigens and that this immunoregulatory activity is confined in the CD4+CD25+ subset [66]. Furthermore, CD4+CD25+ Tregs with the capacity to prevent skin allograft rejection were generated in mice by pretreatment with donor alloantigens under the cover of nondepleting anti-CD4 therapy [68]. Of great interest, the same group has recently shown that such Tregs are generated in the periphery from CD4+CD25- precursors, indicating that their ontogeny is distinct from that of naturally occurring CD4+CD25+ Tregs [69].

Interestingly, Cobbold et al. recently investigated whether CD4+CD25+ Tregs induced by a nondepleting anti-CD4 monoclonal antibody (mAb) tolerance protocol express FoxP3 likewise their naturally occurring counterpart [70]. The Authors used a model of skin graft with female transgenic mice, which have no detectable preexisting CD4+CD25+FoxP3+ Tregs in the thymus or periphery, as recipients. Long-term skin graft tolerance was associated with the presence within the graft of Tregs that expressed CD4, CD25 and high levels of FoxP3 messenger ribonucleic acid (mRNA) and that appear to have arisen de novo in the periphery.

Efforts to study the role and relevance of CD4+CD25+ Tregs in regulation of alloimmune responses in transplant patients have only recently emerged. The effect of Tregs on the

direct pathway was evaluated on peripheral blood leukocytes (PBLs) isolated from 12 stable renal transplant patients by using mixed leukocyte culture, limiting dilution assay, and enzyme-linked immunoabsorbent spot (ELISPOT) for interferon (IFN)-γ. depletion of CD4+CD25+ cells from patients' PBLs did not increase the low frequency of donor-specific alloreactive T-cell clones, thus excluding a role of CD4+CD25+ Tregs in maintaining hyporesponsiveness [71]. On the other hand, other Authors have suggested that CD4+CD25+ Tregs may control T-cell response through the indirect pathway. In stable renal transplant patients chosen for having low reactivity to the mismatched donor-derived human leukocyte antigen (HLA)-DR antigens, Salama et al. detected significant increase in the frequency of IFN-g-producing T-cells in response to donor HLA antigens presented by self APC after depletion of the CD25+ subset [72].

Another CD4+ T-cell subset with suppressive activity has been induced in vitro by antigenic stimulation of naïve CD4+ T cells in the presence of IL-10 [73]. These Tregs, designed Treg type 1 cells (Tr1), are characterised by a unique cytokine profile distinct from that of Th0, Th1, or Th2 cells. They produce IL-10, TGF-β, some IL-5 and IFN-γ, and little or no IL-2 and IL-4 [73]; express very low levels of CD25 in resting conditions [74]; do not proliferate in response to IL-2 unless at extremely high concentrations and strongly suppress activity of both Th1 and Th2 T cells through the release of IL-10 and TGF-b in a completely cell-contact-independent manner.

Tr1 cells have been shown to prevent development of colitis induced by transfer of naïve $CD45RB^{hi}$ cells into SCID mice, a model of Th1-mediated autoimmune disease [73]. In addition, Tr1 cells differentiated with dexamethasone and vitamin D3 suppressed the induction of experimental autoimmune encephalomyelitis (EAE) in mice [75]. Protection from EAE was dependent on the presence of the antigen being recognised by Tr1 cells, indicating that they must be activated *via* TCR in order to exert their regulatory effect. However, once activated, Tr1 cells suppress T-cell response in an antigen-nonspecific manner, as documented by data that Tr1 clones specific for filamentous haemagglutinin from *Bordetella pertussis* inhibited proliferation and cytokine production by a Th1 clone against an unrelated antigen: influenza virus haemagglutinin [76].

Whether Tr1-cell generation could be induced by a dedicated cell population has also been investigated. Recent data suggest that immature dendritic cells (DCs), i.e. under steady-state conditions, play a crucial role in maintaining self-tolerance by inducing the formation of Tr1-like cells. Indeed, Jonuleit et al. [77] showed that repetitive stimulation of naïve cord-blood-derived CD4+ T cells by allogeneic immature dendritic cells generated IL-10-producing T cells displaying most of the typical properties of Tr1 cells. Rat dendritic cells made immature by transfection with an adenoviral vector encoding dominant negative IKK2 to block the NK-kB pathway and incubated in vitro with allogeneic CD4+ T cells generated Tregs that were $CD25^-$ and acted through the release of soluble factors [78]. The regulatory effect was very potent since these cells were capable of inhibiting proliferation of naïve T cells towards donor antigens until a dilution of 1 Treg in 10^5 target cells. More importantly, the above Tregs given in vivo to syngeneic naïve rat recipients prolonged the survival of a completely MHC-mismatched kidney allograft from the same donor strain used for generating the immature dendritic cells.

Immunoregulatory activity is not exclusively confined to CD4+ T cells; indeed, data on the existence of a subset of CD8+ T cells with strong regulatory properties are emerging. In humans, IL-10 producing CD8+ Tregs have been induced either in vitro by interaction of naïve CD8+ T cells with CD40-L activated plasmacytoid dendritic cells [79] or *in vivo* by injection of immature dendritic cells in healthy volunteers [80]. As with Tr1 cells, these CD8+ Tregs exert their suppressive activity in a cell-contact-independent manner. Another subset of CD8+ Tregs has recently been found, which are characterised by the lack of CD28 receptor and are referred to as CD8+CD28- Tregs [81]. Suppressive activity of CD8+CD28- Tregs is cell-contact dependent; they recognise MHC class I peptide complexes

on APCs, rendering them tolerogenic by upregulation of inhibitory receptors such as immunoglobulin-like transcripts 3 and 4 (ILT3 and ILT4) [82]. It has been shown that human CD8+CD28- Tregs arise in the course of repeated in vitro allostimulations, which lead to a hypothesis that they may also develop in vivo in recipients of allogeneic transplants. In this regard, Ciubotariu et al., by performing flow cytometry analysis of blood samples from heart, liver, and kidney transplant recipient, detected donor-specific CD8+CD28- Tregs in all patients with a stable graft function. In contrast, these cells were not detectable in the circulation of patients undergoing acute rejection [83]. These data provided evidence that the presence of CD8+CD28- is relevant to the outcome of transplants and that these cells participate in the induction and maintenance of peripheral tolerance.

Intragraft Gene Therapy

The opportunity to perform ex vivo manipulation of the graft during organ retrieval makes transplantation an ideal condition to achieve local immunosuppression, leaving the systemic immune response intact. In the last decade, gene transfer of immunomodulating molecules into the graft emerged as a new strategy in organ transplantation, showing promising results in experimental animals [84]. Kidney, liver and heart are receptive to gene transfer *ex vivo* by the currently available vectors. However, the efficiency of gene transfer can strongly vary depending on the vector used and on the biologic characteristics of targeted cells as well as on the activation of the host immune system in response to the vector and to the transgene product. Indeed, most vectors, particularly adenoviruses, induce an innate immune response that can impair the efficiency of this approach.

Different bioactive molecules have been delivered to the donor organ with the aim of blocking the antigraft immune response by inhibiting costimulatory signals or inducing apoptosis of immune cells. Direct injection of a recombinant adenovirus encoding CTLA4Ig (AdCTLA4Ig) into the renal artery of the donor kidney before transplantation significantly prolonged graft survival of rat renal allografts without the need for systemic immunosuppression [85]. In another study, survival was indefinite in rat recipients of cold preserved liver transduced with AdCTLA4Ig that developed donor-specific unresponsiveness [86]. Targeted gene therapy with adenovirus encoding CD40Ig fusion protein, which blocks the CD40/CD154 interaction, has been attempted in liver and heart transplantation. Rat liver allografts transduced before transplantation survived more than 100 days [87]. Similarly, the same approach resulted in long-term heart allograft survival in rats and induced donor-specific unresponsiveness. However signs of chronic rejection were detected in the long-term surviving grafts [88].

Activated T cells are usually eliminated by apoptosis triggered by the interaction between their Fas antigen and its counter receptor Fas-ligand (FasL). Theoretically, expression of FasL within the graft should protect it from infiltrating T cells that, by expressing Fas antigen, should undergo apoptosis. Renal allografts adenovirally transduced to express FasL showed prolonged graft survival, an effect correlating with enhanced mRNA expression of immunomodulatory cytokines (IL-4 and IL-10) [89].

Other gene transfer strategies were pursued to directly transduce the graft with immunomodulatory cytokines. Several studies in rodents used viral IL-10 (vIL-10) that shares some biological activities of mammalian IL-10 but lacks the immunostimulatory functions, making it a potentially potent immunosuppressant. Prolonged but not indefinite survival was obtained after retroviral [90], adenoviral [91] or lipid-mediated [92] gene transfer of vIL-10 in cardiac allografts before transplantation. However, high levels of expression of vIL-10 are not always beneficial. Indeed, heart grafts from transgenic mice for vIL-10 failed to exhibit prolonged survival when transplanted in MHC fully mismatched animals [93].

Finally overexpression of antioxidant and antiapoptotic genes have been attempted with the aim of protecting the graft from injury that derives either from ischhaemia/reperfusion injury at the time of transplantation of from toxic molecules released by infiltrating inflammatory cells. Adenovi-

ral transduction of the liver with the gene encoding Cu/Zn superoxide dismutase, an endogenous scavenger of oxygen radicals that are produced during ischaemia/reperfusion, allowed 100% survival of transplanted animals [94]. In another study, overexpression of another cytoprotective antioxidant, HO-1, in the donor rat liver before transplantation increased graft survival and improved liver function, decreased macrophages infiltration and increased intragraft expression of antiapoptotic genes Bcl-2 and Bag-1 [95].

From the experimental data obtained in rodents, it emerges that intragraft gene transfer could represent a promising tool to avoid or at least reduce the need for systemic immunosuppression. However, many hurdles must be overcome. Extensive work has to be done in rendering the vectors safer and less immunogenic. Another fundamental requisite for success in gene therapy is the possibility of modulating and regulating transgene expression for the appropriate length of time.

Conclusions

While many lessons have been learned during the past 50 years of transplant immunology, progress towards tolerance has been slower than expected. Experimental studies have clearly documented that transplant tolerance is achievable in particular animal models with different approaches, and it has been even intentionally induced in a few humans. Identifying the most successful of these strategies and then translating them to larger animals to test their suitability for the patients is the next step. Although we are currently only at a very early stage, there is no doubt that in the near future some of these approaches will have a major impact in transplant medicine, opening a new prospective of indefinite graft survival without the complications of long-term immunosuppressive drugs and contributing to make a reality donor-specific tolerance in human transplantation.

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13. The International Registry on Hand and Composite Tissue Transplantation (IRHCTT)

Section 13

The International Registry on Hand and Composite Tissue Transplantation (IRHCTT)

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Introduction

The first hand transplantation was performed in Lyon, France, on 23 September 1998 by an international team of surgeons [1]. Since then, hand transplantation programmes have been launched in the United States, Austria, China, Italy, Belgium and Poland [2–6], and the teams felt the need to create a worldwide registry to provide a basis for cooperation and to share their experiences (Fig. 1). Since May 2002, all groups [7] performing hand transplantations have supplied detailed information to the International Registry on Hand and Composite Tissue Transplantation (IRHCTT; www.handregistry.com). Follow-up period ranged from 2 to 85 months (Table 1). A good number of composite tissue transplantations other than the hand have been performed around the world in the period 1994–2006, including the femoral diaphysis, the knee, the larynx, the uterus, the abdominal wall, a lower limb in conjoined twins, and most recently, the face in two centres. These allografts are listed in Table 2.

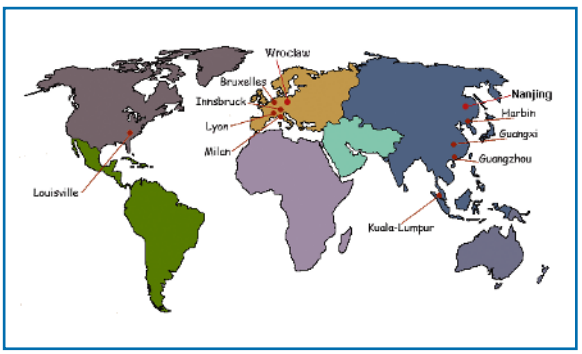

Fig. 1. Hand transplantation centers around the world

Clinical Cases

From September 1998 to February 2006, 18 men underwent 24 hand/forearm/digit transplantations requiring immunosuppression (11 unilateral and four bilateral hand transplantations, two bilateral forearm transplantations, one thumb transplantation). Average recipient age was 32 (19–52 years). The level of amputation was mostly at the distal forearm or wrist. Time since hand loss ranged from 2 months to 22 years. The donors were all male, with an age of 16–50 (average 33). Donor selection was based on negative lymphocytotoxic cross-matching, race, gender, size, age as well and skin-color matching (Table 3).

Transplantation Procedure

In 50% of transplantations, limbs were harvested prior to solid organs while the remaining 50% were procured after extraction of solid organs. University of Wisconsin (UW) solution was used for cold flush and limb preservation in 16 cases; in two cases, heparinized saline solution was used. Cold ischaemia time ranged from 30 m to 13 h (mean 5.3 h), largely depending on local circumstances, including geographical distance between donor and recipient.

Table 1. Hand transplantation cases

Date	Single-hand transplantation (*n*)	Double-hand transplantation	Digital transplantation
September 1998	Lyon, France (1)		
January 1999	Louisville, USA (1)		
September 1999	Guangzhou, China (2)		
January 2000	Guangxi, China (2)	Lyon, France (1)	Guangxi, China (1)
March 2000		Innsbruck, Austria (1)	
May 2000	Kuala-Lumpur, Malaysia (1)[a]		
September 2000		Guangzhou, China (1)	
October 2000	Milan, Italy (1)		
November 2000	Nanning, China (2)[b]		
January 2001		Harbin, China (1)	
February 2001	Louisville, USA (1)		
October 2001	Milan, Italy (1)		
June 2002	Bruxelles, Belgium (1)		
June 2002	Harbin, China (1)[b]		
July 2002	Nanjin, China (1)[b]		
October 2002		Harbin, China (1)	
November 2002	Milan, Italy (1)		
February 2003		Innsbruck, Austria (1)	
May 2003		Lyon, France (1)	
February 2005			Nanjing, China (1)
February 2006	Wroclaw, Poland (1)		
June 2006		Innsbruck, Austria (1)	
Total patients	17	8	2
Total hands/digits	17	16	2

[a]No immunosuppression required – identical twins
[b]Unofficial data

Table 2. Composite tissue allografts other than the hand between 1994 and 2006

Date	Composite tissue transplantation(*n*)	Location
1994–1996	Femoral diaphysis (3)	Murnau, Germany
1996–2000	Knee (6)	Murnau, Germany
1998	Larynx (1)	Cleveland, USA
2000	Uterus (1)	Jeddah, Saudi Arabia
2001–2003	Abdominal wall (9)	Miami, USA
2002–2006	Larynx (15)	Medellin, Colombia
2003	Lower limb (1)[a]	Toronto, Canada
2005	Face (1)	Amiens, France
2006	Face (1)	Xi'an, China
Total number of cases	38	

[a]No immunosuppression required – conjoined twins
n, number of cases

Table 3. Recipient characteristics

Gender	**Male: 100%**		**Female: 0%**
Age (years)	19–52 (average 32)		id.
Smoker (%)	Yes: 19%		No: 81%
Civil status (%)	Married: 65%		Not married: 35%
Working status (%)	Employed: 91%		Not employed: 9%
Time since amputation	2 months–22 years (average 5.5 years)		
Cause of amputation	***Crush: 60%***	***Explosion: 33%***	***Clean cut: 7%***
Level of amputation	Distal forearm: 10 Wrist: 6		Proximal forearm: 5 Digit: 1
Side of hand loss (%)	Dominant: 71%		Nondominant: 29%
Use of prostheses (%)	Yes: 56%		No: 44%
Time on waiting list	2 weeks – 3 years (average 6.5 months)		

The repair sequence of the different tissues varied considerably; however, bone fixation and arterial repair were performed first by all groups. After completion of bone fixation and arterial anastomosis, venous anastomoses and reperfusion followed in most cases. Median and ulnar nerves were always repaired while the radial nerve was reconstructed in only 13 limbs. Innine cases, tendon repair was achieved by suturing individual tendons while in the remaining cases, it was necessary to repair them in groups or by using a mixed individual/group technique.

All patients followed a rehabilitation programme, which included physiotherapy, electrostimulation and occupational therapy.

Immunosuppressive Treatment

Induction Therapy

The most commonly used treatment (n=11) included anti-thymocyte globulins (ATG), tacrolimus, mycophenolate mofetil (MMF) and steroids. The second-most used treatment (n=5) included monoclonal antibodies (Basiliximab), tacrolimus, MMF and steroids. Two patients were treated with tacrolimus, MMF and steroids plus a steroid cream (Table 4).

Maintenance Therapy

A triple combination was widely employed for maintenance of immunosuppression, similar to that currently used in standard treatment of solid-organ transplantation. Fifteen patients followed a protocol consisting of tacrolimus, MMF and steroids while in one case, a regime of tacrolimus and steroids was used. In one patient, rapamycin and MMF were administered; in another, only rapamycin; the last one received only topical applications of steroid and tacrolimus ointments.

Complications and Side-effects

Complications requiring additional surgical intervention included early postoperative necrosis of a small skin area (n=2), arterial thrombosis (n=1) in the first postoperative day, and the occurrence of multiple arteriovenous fistulas (n=1). All these events were successfully treated. The majority of reported side-effects were infections [opportunistic infections, including cytomegalovirus (CMV) reactivation, with two cases presenting clinical signs of infection; *Clostridium difficile* enteritis, herpes simplex blisters, cutaneous mycosis, ulnar osteitis by

Table 4. Immunosuppression: induction therapy

Protocol	Drugs	Dose (range)	Patients (*n*)	Patient (%)
I	ATG	1.25–5 mg/kg per day	11	61
	Tacrolimus*	5–25 ng/ml		
	MMF	750–2,000 mg/day		
	Steroids	500–1,000 mg on POD 1 10–20 mg at 6 months		
II	Basiliximab	20 mg on POD 1 and 4	5	28
	Tacrolimus[a]	10–20 ng/ml		
	MMF	2,000 mg/day		
	Steroids	185–500 mg on POD 1 25–35 mg at 6 months		
III	Tacrolimus[a]	9–12 ng/ml	2	11
	MMF	2,000 mg/day		
	Steroids	80 mg on POD 1 10 mg at 6 months		

ATG, Antithymocyte globulins; *MMF*, mycophenolate mofetil; *POD*, postoperative day
[a]Through-blood level

Staphylococcus aureus] and metabolic complications, such as transient hyperglycaemia, increased creatinine, Cushing's syndrome, and avascular necrosis of the hip. Most of these adverse effects were transient and reversible. At this stage, no malignancies or life-threatening conditions have been reported.

Rejection Episodes

Rejection episodes were first suspected by visual inspection of the transplanted hand and usually confirmed by histological evaluation of a skin biopsy. Acute rejection episodes occurred in 12 patients within the first year. Other episodes occurred when patients were not compliant with the immunosuppressive regimen or the regimen was decreased for different reasons (i.e. side-effects or team decision). It is important to note that all rejection episodes were completely reversible in all compliant patients. Treatment of rejection episodes included high-dose i.v. steroids, increase in oral steroid treatment, ATGs, Basiliximab or Campath-1H. Tacrolimus, corticosteroid or flumix ointment, either administered alone or in combination between them, was applied in all cases displaying signs of rejection (Table 5).

Table 5. Acute rejection episodes in the first year posttransplantation (*n*=12)

Rejection episodes (*n*)	Patients (*n*)	(%)	Onset of rejection (weeks)	
None	3	(25 %)	-	
1	2	(17 %)	2–52	(average 17.5)
2	5	(42 %)	5–27	(average 13.3)
3	1	(8 %)	7	(average 7.0)
4	–		–	
5	1	(8 %)	11	(average 11.0)

Patient and Graft Survival

Patient survival was 100%. Of the 23 hands transplanted, all were viable at 1 year after transplantation; then eight graft failures occurred, caused by progressive rejection in a noncompliant patient [8] and in the Chinese patients who did not take the immunosuppressive treatment. Pei communicated these last failures in Tucson in January 2006.

Functional Results

All viable hands presented normal skin colour and texture, as well as normal hair and nail growth. Arterial blood supply and venous outflow have been satisfactory in all patients. Protective sensation recovery (i.e. the ability to detect pain, thermal stimuli and gross tactile sensation) occurred in all grafted hands. Nerve regeneration allowed a certain degree of discriminative sensation although this was not to the same degree at all parts of the graft. Twenty-one hands were evaluated for static, two-point fingertip sensory discrimination 2 years after transplantation. Five hands showed excellent return of discriminative sensation according to the Highet Scale (grade S4; 2–6 mm). Two hands showed good results (grade S3+; 7–12 mm), and ten hands displayed a satisfactory degree of recovery (grade S3; >15 mm). In four hands, no discriminative sensation was detected (grade S2). Motor recovery began with extrinsic muscle function, allowing all patients to perform grasp and pinch activities. Function of intrinsic muscles was observed only at a later stage, starting at 12 months posttransplantation in the majority of patients. A variable degree of thumb opposition and hand lumbrical/interossei muscle activity was apparent in 14 patients. Activation of intrinsic muscles was confirmed by electromyographic studies in several hands. Motility in some patients continued to improve, even after years. Extrinsic and intrinsic muscle recovery enabled patients to perform most daily activities, including eating, driving, grasping objects, riding a bicycle or a motorbike, shaving, using the telephone and writing. Where performed, functional magnetic resonance imaging showed that sensorimotor activations of the brain cortex progressively regained the classical hand area within 6 months postoperatively [9] (Table 6).

Table 6. Patient daily activities 1 year after transplantation

Activities	Patients (%)
Driving	36
Combing hair	82
Grasping a glass	100
Pouring water	91
Brushing teeth	82
Riding a bicycle	64
Using cutlery	82
Holding hands	100
Shaving	64
Writing	73

Conclusions

In conclusion, hand transplantation became a clinical reality, with immunosuppression comparable to transplantation of solid organs, but it is important to note that this immunosuppressive treatment is indispensable for graft survival.

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14. An Extensive Bibliography on Hand Transplantation

Section 14

An Extensive Bibliography on Hand Transplantation

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