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ORIGINAL ARTICLE

Outcome of allogeneic vascularized knee transplants

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Summary

Transplantation of vascularized knee joints is a novel approach in Composite Tissue Allotransplantation (CTA). In 1996 our group started a clinical knee transplantation project and six transplantations have been performed since. Key problems identified early were the monitoring of acute rejection and choice of an immunosuppressive regime. One graft was lost due to postoperative infection and one due to of noncompliance where the patient discontinued the immunosuppressant regime. In three cases late rejection lead to necrosis and graft dysfunction after 15, 16 and 24 months, respectively. Exit-strategies were arthrodesis in one patient and Above Knee Amputation in two cases. With retrospective analysis after initial five cases the treatment protocol was improved. The immunosuppressive drug regime was altered, femoral diaphysis and knee joint grafting was combined and a vascularized block of donor skin and subcutaneous tissue was harvested with the graft (sentinel skin graft). The sentinel skin graft enabled us to monitor acute rejection by clinical and histological examination and avoid late rejection by rapid treatment with high dose steroids. In summary, over a four-year period, one of six allogeneic vascularized knee transplants has survived, one was lost from a surgical site infection, one by noncompliance and three by late rejection. Analysis of our data leads us to suggest that knee transplantation should be limited to a combined injury consisting of extensive loss of cartilage and bone, deficient extensor mechanism and soft tissue and skin defects without any signs of infection. Transplantation should only be taken into consideration as last option before Above Knee Amputation in an otherwise healthy patient under 35 years of age.

Introduction

Extensive destruction of the knee joint after high-velocity trauma and/or infection is always difficult to treat. The worst-case scenario is the combination of extended loss of bone and cartilage, destruction of the extensor mechanism with skin and soft tissue defects.

The extensor mechanism consists of the quadriceps tendon, patella and patella ligament. If the extensor mechanism is insufficient, active extension of the knee is impossible.

Treatment options are arthrodesis, amputation or reconstruction of the joint.

Arthrodesis [1] results in a stable extremity, but motion is lost and bone loss leads to shortening of the leg.

Above Knee Amputation with rehabilitation is effective, but is associated with an increased risk of limp, dependence of walking aids, anxiety, and loss of independence such as the inability to drive [2].

Reconstruction of the destructed knee joint as described above requires a combination of total knee arthroplasty [3], reconstruction of the extensor mechanism (by tendon

– muscle flaps [4], allograft [5] or synthetic material [6]) and a local [7] or free flap [8] for skin and soft tissue management.

Each of these approaches is technically feasible, but each has inherent complications and limitations. In addition, the outcome of a combination of all three techniques is unpredictable.

As an alternative the concept of allogeneic vascularized knee transplantation was conceived, a combination of orthopaedic and transplant surgery.

After encouraging results in animal models [9,10], increasing experience in composite tissue allotransplantation (CTA) and intense ethical and legal discussions at the Louisville symposium on CTA [11], we launched our clinical transplantation project regarding allogeneic vascularized knee joints in 1996 [12,13]. Six transplantations have been performed since.

In 2000 the first human hand transplantation was done by Dubernard *et al.* [14]. Table 1 summarizes the world experience in allogeneic vascularized joint transplantation.

Patients and methods

Five male and one female patient received an allogeneic vascularized knee joint transplantation.

Table 1. World experience in allogeneic vascularized joint transplantation.

Joint replacement	Time	Number	Graft survival	Reference
Single hand	1998–2005	11	9	[32]
Double hand	2001–2005	6	6	[32]
Knee	1996–2004	6	1	

All patients had extensive loss of bone and cartilage combined with deficiencies in the extensor mechanism of the knee joint. One case had a significant soft tissue defect.

All patients underwent routine pretransplantation investigations with specific morphological (radiography, arteriography) and microbiological testing.

The sterile osseous defect of the knee joint was temporarily stabilised with a hinge arthroplasty or an intramedullary nail in the case of a long femoral defect (Fig. 1a,b).

Assisted passive motion and isometric exercises were possible and necessary to avoid contractions and muscular atrophy. Informed consent was obtained and the patients were placed on a waiting list for the procedure.

The knee joints were harvested in accordance with standard organ procurement guidelines used in multiorgan donation (MOD). Authorization for knee donation was obtained from the donor's family. MODs older than 45 years or those who had an accident involving the same leg were excluded. For additional safety reasons, MODs who had received blood substitutes or fresh-frozen plasma were excluded as well.

Harvesting of the knee joint included perfusion of the External Iliac Artery with 4 l University of Wisconsin (UW) solution at 4 °C, dissection of the femoral artery and vein distally to the proximal level of the adductor canal, transsection of the muscles and osteotomy of the femur, tibia and fibula. Fig. 2 shows a diagram of the graft.

To restore normal appearance of the donor leg a polyethylene spacer was inserted into the bone defect and the skin closed.

The graft was stored in sterile conditions in three layers of plastic bags at 4 °C in UW-solution. Cold Ischemia Time ranged from 18–25 h.

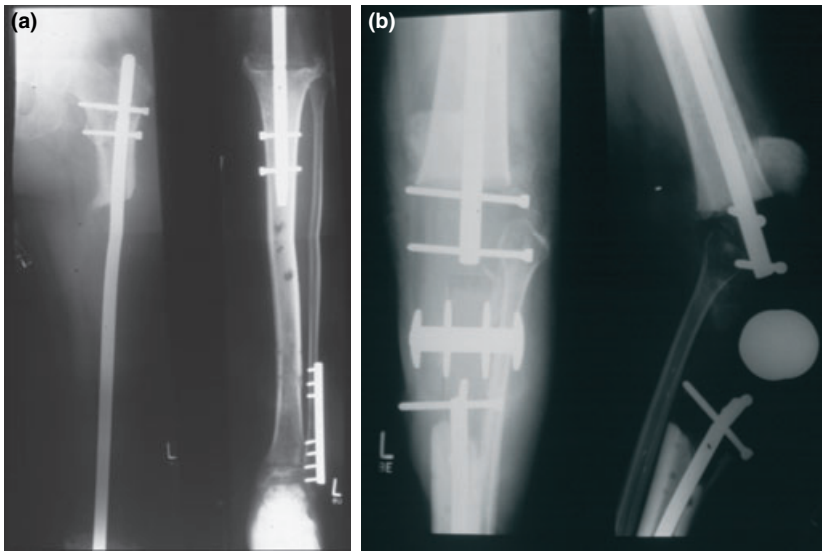

Figure 1 (a) Radiography of left femur with hip and knee joint: Osseous defect including femur, knee joint and prox. tibia (length 44 cm), temporally stabilised with an intramedullary arthrodesis nail (b) Radiography of right knee joint: Osseous defect stabilized with a hinge arthroplasty.

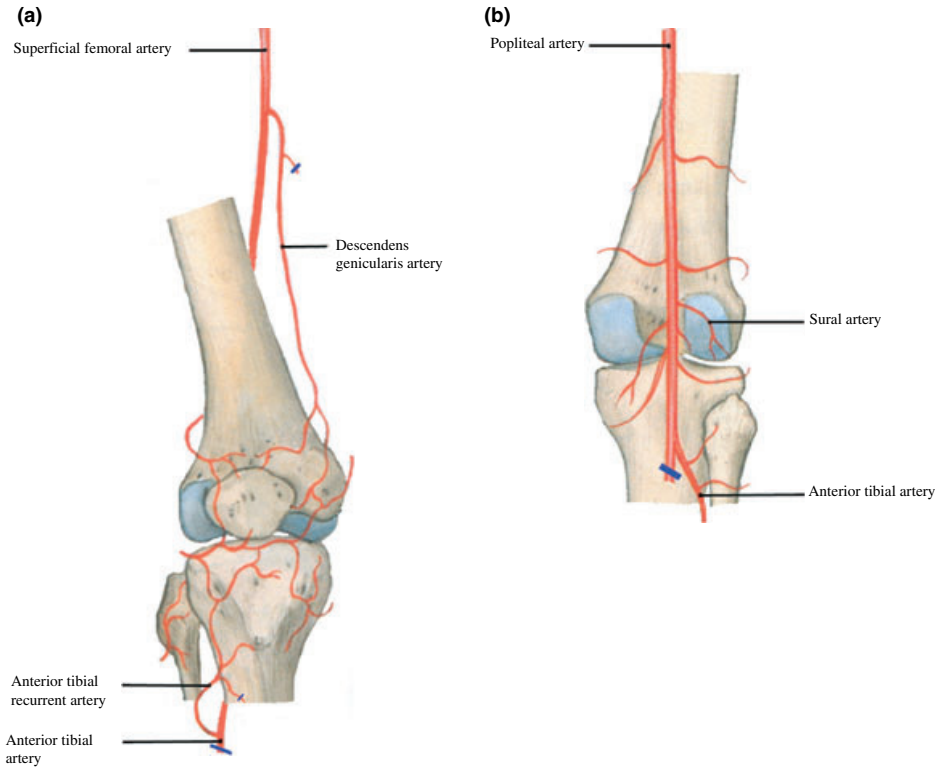

Figure 2 Diagram of the graft [view from ventral (a) and dorsal (b)]. [Additional information added after online publication 8th February 2007: Modified from Sobotta: Atlas der Anatomie des Menschen, 22. Auflage © 2006 Elsevier GmbH, Urban & Fischer Verlag].

Allograft preparation involved dissection from the surrounding soft tissue with the quadriceps tendon and the articular capsule intact, vessels perfusing the muscles ligated and vessels to the bone preserved.

The graft arterial pedicle was perfused with methylene blue to confirm adequate perfusion for transplantation.

The surgical procedure commenced with removal of the spacer or nail. The graft was inserted and fixed by an anterograde femoral and a retrograde tibial interlocking compression nail.

The grafts vessels were anastomosed to the recipient's superficial femoral artery and vein using the end-to-side technique. Reperfusion commenced immediately while the ligaments and tendons (Quadriceps Tendon, Iliotibial Tract, Gastrocnemius, Hamstrings) were reconstructed.

AB0-compatibility and negative cross-match were confirmed in all transplantations. HLA profiles were determined but not matched due to logistical restrictions in donor acquisition.

Immunosuppression was started immediately after reperfusion of the graft and consisted in the first five cases of Antithymocyte Globulin, 4 mg/kg bw IV as an induction therapy and Cyclosporin A, 1, 5 mg/kg bw IV; Azathioprine, 1.5 mg/kg bw IV; and Methylprednisolone, 250 mg IV for the first 3 days.

During the first two weeks, Methylprednisolone was reduced in a stepwise regime. Immunosuppression was continued with an Oral Double-Drug maintenance therapy with Cyclosporine A (CsA, 6 mg/kg bw; blood level 100–150 ng/ml) and Azathioprine (1.0 mg/kg bw).

In the sixth case Tacrolimus (10 mg p.o.) and Mycophenolate Mofetil (MMF; 2 g p.o.) were used instead of CsA and Azathioprine, following the immunosuppressive regime used in hand-transplantation [15,16]. Oral Double-Drug maintenance therapy was continued with MMF (2 g p.o.; serum level 2–6 μg/ml) and Tacrolimus with a serum level between 8 and 10 μg/ml from the beginning of the third week. A Heparin infusion was administered for the first 3 days and then continued SC to maintain Partial Thromboplastin Times (PTT) within the range of 60–80 s.

Postoperative monitoring included clinical examinations, routine laboratory tests, Duplex-sonography, radiological assessment and single photon emission computerised tomography (SPECT).

Local inflammation of the knee and fever were the only clinical indicators of infection or rejection in the first five cases. In patient six, the sentinel skin graft was used to monitor for possible rejection.

Differential white blood cell count, erythrocyte sedimentation rates and C-reactive protein were measured

daily as well as CsA Tacrolimus blood levels and PTT. Later CsA Tacrolimus levels were monitored twice a week. Radiographs were used to visualize bone healing. Digital subtraction angiograms (DSA) were employed to monitor the macrocirculation of the graft vascular pedicle on the first postoperative day and as required to investigate clinical symptoms and possible complications. Duplex sonography was performed daily during the first week. 99 m-Tc-MDP scintigrams demonstrated perfusion, metabolism and vitality of the graft. Moreover, SPECT was used to exclude tracer uptake from the overlying soft tissue [17]. If clinical signs of rejection were present, arthroscopy was performed and biopsies taken for histological examination.

Postdischarge, patients were seen weekly by a nominated General Practitioner in their locality and every 2 months at our Outpatient Department.

Results

To date, six allogeneic transplantations of vascularized human knee joints have been performed. Preliminary outcomes and follow-ups have been published in detail elsewhere [12,13,18–22]. In the following, each patient is described separately. After the first five cases, the treatment protocol was changed, as mentioned above. Table 2 summarizes the outcomes of all cases.

Patient 1

The first grafting was done on April 27th 1996. There was no intraoperative or early postoperative complication. Sufficient macrocirculation of the graft was shown by duplex sonography and angiography. Undisturbed microcirculation and intact cellular metabolism were demonstrated by scintigraphy and SPECT-technique. Wounds healed by primary intention. The patient was discharged after 2 weeks, mobilising on two crutches and partially weight-bearing. Radiographs showed complete osseous integration of the transplanted knee after 6 months. Full range of motion was achieved on clinical examination at 12 months.

15 months postgrafting, the patient complained of pain and reduced range of motion at the grafted joint. The knee showed clinical signs of inflammation. A diagnosis of rejection crisis was made. SPECT showed an increased accumulation of tracer in the sub cartilaginous zone of the graft. Biopsy of synovial membrane revealed vital and perfused tissue; however, there were signs of perivascular infiltration of lymphocytes. Subsequently, the patient developed occlusion of the allograft vascular pedicle (duplex sonography), hence transplant failure. Immunosuppression was discontinued and the patient received a total knee arthroplasty (TKA) using the graft as bone stock that resulted in good stability as well as mobility.

Unfortunately, the patient moved to a different town, stopped visiting our Outpatient department against our advice and contact was lost. We understand from written correspondence that a periprosthetic infection occurred in 2002 and despite surgical and antibiotic treatment, a chronic infection of the TKA developed. With severe sepsis and impending multi organ failure (MOF), an Above Knee Amputation was performed 2004.

Patient 2

The second transplantation was in November 1996 with no postoperative complications. At 12 months the patient had a stable joint, was able to walk, to do sports and had returned to work. During a personal crisis in 1999, the patient discontinued his immunosuppressants. Acute rejection occurred and most parts of the graft were lost. A TKA was performed, using the remaining graft as bone

Table 2. Outcome of allogeneic vascularized knee transplantation.

Case	Date of surgery	Outcome of graft	Outcome of extremity	Major complications
1	April 1996	Late rejection after 16 months	Above Knee Amputation	Fatigue fracture, implant related infection
2	November 1996	Acute rejection after 3 years	Above Knee Amputation	Noncompliance, patient discontinued immunosuppressants
3	December 1996	removed after 5 weeks	Arthrodesis	Surgical site infection
4	July 1997	Late rejection after 24 month	Above Knee Amputation	Fatigue fracture
5	February 1998	Late rejection after 14 months	Arthrodesis	Fatigue fracture
6	April 2002	Still vital for 4 years	Vital leg	Acute rejection

stock. Later an implant-related infection was diagnosed. On request of the patient, Above Knee Amputation was performed in July 2001.

Patient 3

One week after grafting in December 1996 a surgical site infection of the allograft was detected. Despite antibiotic therapy, reduction of the immunosuppressive regime and aggressive surgical debridement, infection persisted. Immunosuppression was discontinued and allograft removed 5 weeks after transplantation. With infection controlled, a temporary, articulating spacer was implanted and the patient rescheduled. Due to difficulties in the size match (small size of the recipient's knee), a donor could not be found within a year. A number of options were discussed with the patient and family. Arthrodesis of the knee, bridging the bone defect with a callus distraction in Ilizarov's technique was performed in 1997.

Patient 4

The 4th grafting took place in July 1997. Two years following transplantation the patient developed a stress fracture of the tibial plateau of the grafted knee joint whilst running downstairs. On admission SPECT revealed no tracer uptake of the whole graft. A TKA using parts of the remaining allogeneic bone as bone stock was performed. Postoperatively the patient had a stable joint, good mobility, was able to walk and returned to work. In March 2000, 3 years post-transplantation, a periprosthetic infection occurred. The patient declined further surgical treatment for control of the infection and on request the leg was amputated.

Patient 5

The first year postgrafting in February 1998 was uneventful. At 14 months a stress fracture of the lateral tibia plateau was detected. A TKA was performed, using the remaining graft as bone stock. At discharge the patient had a full range of motion at the knee joint and no signs of infection. Eighteen months later a periprosthetic fracture following a fall onto the knee was diagnosed. The femoral component of the TKA was removed and a custom designed femoral component implanted. At 5 years the patient reinjured his grafted knee sustaining a patella fracture. Open reduction and internal fixation with screws failed. The patella ligament was reconstructed using autologous fascia lata at the research hospital. Late rejection resulted in necrotic bone stock and infection. The graft was removed and an intramedullary arthrodesis performed, resulting in a 8 cm shortening of the leg.

Patient 6

The final allotransplantation was done in April 2002. A block of donor skin and subcutaneous tissue was harvested at the same time as the graft. This was inserted into the skin of the recipient on the lateral tight to monitor graft rejection or perfusion dysfunction (Sentinel Skin Graft).

Eighteen months post-transplantation the patient was mobile, full weight bearing and had returned to his previous job. Radiography showed the complete osseous integration of the graft (Fig. 3a). Range of motion (ROM) of the left knee was 0–0-90 (Fig. 3b). The sentinel skin graft (SSG) was fully integrated within the recipient skin (Fig. 3b). Arthroscopy showed vital cartilage without degeneration, intact menisci and ligaments.

Two years and 4 months post-transplantation, the patient noticed redness, itching and pain at the sentinel skin graft at his left lateral thigh. Suspecting a rejection crisis skin biopsies of the SSG were taken and histology revealed acute cellular rejection (Fig. 4). Immediate treatment with high-dose steroids (Methylprednisolone 250 mg IV) was commenced for 3 days and reduced stepwise over 2 weeks. Two days later arthroscopy with biopsies of the grafted knee was performed. Histology showed the same signs of acute rejection as seen in the skin biopsy (Fig. 5). Inflammation of the SSG and grafted joint resolved with high dose steroid therapy. It is now, 4 years post-transplantation and the graft remains vital. The patient is mobile, full weight bearing and has a satisfactory range of motion at the grafted knee.

Discussion

As there were no problems or complications during surgery or in the early postoperative period we suppose that the procedure is technically feasible. Side effects of immunosuppression such as transient hyperglycaemia, hypertension, increased serum creatinine or opportunistic infections did not occur in any of our patients. Patient 3 had a cytomegaly virus (CMV) antigenaemia and was treated with a 10-day course of Gancyclovir (3 mg/kg BW per day).

Cold ischemia time (CIT) ranged from 18 to 25 h. This is longer than CIT in other CTAs, e.g. human hand transplantation (from 310 minutes [15] to 11 to 12.5 h [23]). In this study, the same team harvested, prepared, transported and performed the allotransplant. This explains the longer CIT compared with transplantations performed by two teams.

Despite no microbiological evidence of infection in biopsies taken from all recipients prior to grafting, one case (patient 3) had a surgical site infection in the imme-

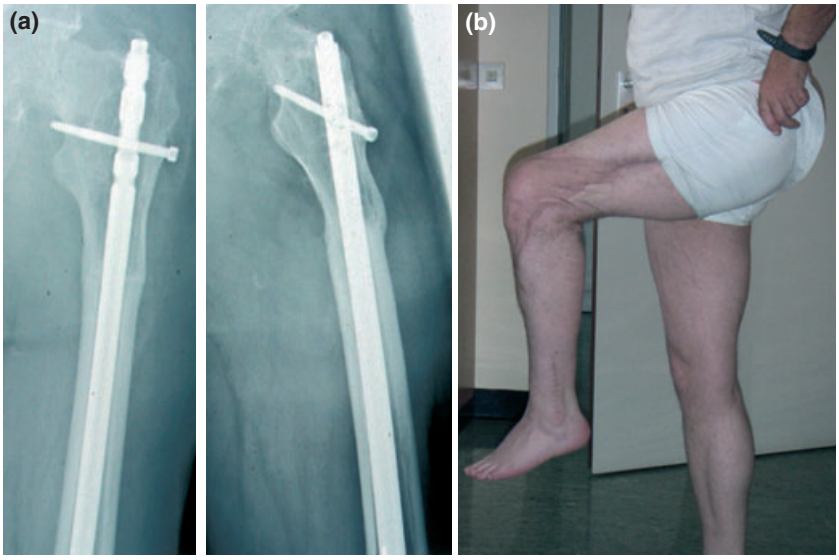

Figure 3 (a) Radiography of left femur with hip joint: control after 1.5 years following transplantation. The graft is completely osseous integrated (b) Range of motion 1.5 years after transplantation.

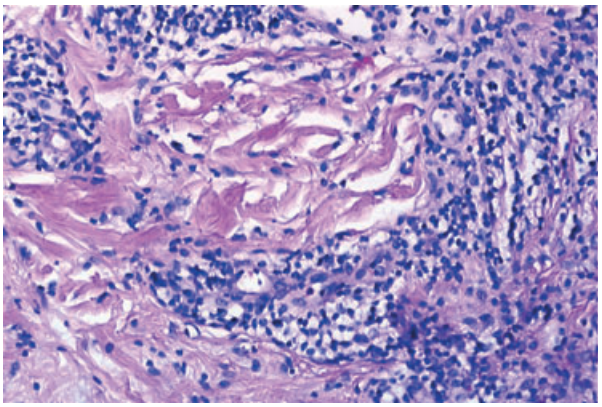

Figure 4 Histology of sentinel skin graft: extensive peri- and intravascularly arranged infiltration of the dermal stoma by activated mononuclear lymphatic cells.

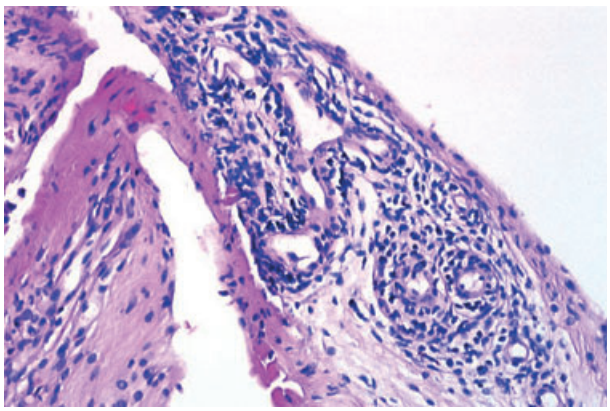

Figure 5 Histology of synovia: infiltration by activated mononuclear lymphatic cells consistent with acute rejection.

diately postoperative period. In this case the indication for grafting was an extensive bone and joint destruction after a post-traumatic osteomyelitis. Presumably postoperative immunosuppression caused reactivation of a latent microbe at the site. To minimize this risk, previous site infections should be an exclusion criteria for transplantation.

One patient (patient 2) discontinued the immunosuppressants 3 years post-transplantation in a personal crisis leading to rejection of the well-functioning graft. A similar case of a noncompliant patient was reported from Kanitakis and co-workers [24].

This emphasises that a comprehensive set of psychological tests are necessary to assess the personality profile of the patients, their ability to understand the potential risks and complications of the procedure and their motivations [25,11]. Confronted with the noncompliance of two patients, the psychiatric assessment has to be carried out with greater care in the future.

Moreover, both cases indicate that lifelong immunosuppression seems to be necessary in CTA of extremities. CTA is considered to improve the quality of life, but is not in itself life-saving as with standard organ transplantations. Therefore, the question arises, do the risks posed by the immunosuppressive drugs, i.e. opportunistic infections, end-organ toxicity and malignancy [26], justify the benefits of CTA procedures? It is very difficult to assess the 'relative risk' of CTA. Brouha and co-workers recently published a study outlining the relative risk that individuals were willing to accept in order to receive the benefits of CTA procedures [27]. They used the Louisville Instrument for Transplantation (LIFT)-Questionnaire, composed of time trade-off questions and the standard gamble method [28]. They concluded, that CTA proce-

dures convey benefits to recipients that are perceived by subjects, despite the risk of immunosuppression, to warrant the risks of these procedures. The question remains is it appropriate to use a risk/benefit ratio to assess CTA. Other authors see the subject more critically, with the incidence of neoplasms among the organ transplant recipient population increasing, estimated to be between 4–18% [29]. Thus the ultimate goal in CTA research is to minimize the risk of immunosuppression. This could be achieved by induction of tolerance without long-term immunosuppression. So far, tolerance in CTA has only been reported in animal models [30].

In three patients (patient 1, 4, 5) late rejection of the graft was found after 15, 16 and 24 months, respectively. As opposed to the transplantation of parenchymal organs, where graft failure is noticed immediately by reduced organ function and laboratory testing, it seems impossible to detect graft rejection in knee joints early enough to institute appropriate immunosuppression. Our patients presented with reduced function of the grafted joint. Radiographs showed stress fractures of the transplants. In surgical exploration of the sites we found necrotic bone and soft tissue. Histology showed ischemic necrosis with no signs of acute or vascular rejection. We propose that rejection had taken place some weeks prior to the first symptoms. After rejection, only the avital bone matrix remains, which is integrated with recipient bone. This matrix is predisposed for fractures, explaining why stress fractures were the first signs of rejection found in our cases.

The mechanism of rejection (acute cellular or vascular) could not be classified by histological examination.

Compared with hand transplantations [14–16], the perfusion of the grafted knee cannot be observed by visual inspection of the skin. Due to this limitation, nuclear scintigraphy and single photon emission computed tomography (SPECT) was employed to assess the microcirculation of the graft [17]. To monitor rejection, we decided to harvest a vascularized block of skin and subcutaneous tissue with a vascular pedicle together with the graft and integrate the allogeneic skin in the recipient skin [31]. A similar technique had previously been used by Lanzetta and colleagues in their hand transplantation project, where an additional full-thickness skin was transplanted onto the left hip area [23]. The skin served as a source of biopsies and as an additional area to monitor rejection, hence called 'distant sentinel skin graft' [23].

In patient six the immunosuppressive regime was altered and a SSG used.

The first sign of late rejection 3 years post-transplantation was redness of the SSG. Biopsies of the SSG and synovia, taken 2 days later by arthroscopy, showed the same signs of acute cellular rejection. This indicates, that the grafted skin can be used as a monitor of rejection. In this patient, rejection was overcome by treatment with steroids and the transplant has remained vital 4 years now [31].

Moreover, we could show by histology, that the mechanism of late rejection was not chronic vascular but acute cellular in this last case. We surmise, that in the three cases of late rejection discussed above, acute cellular rejection may led to the loss of the graft. The acute rejection crisis itself was not noticed; only the following graft dysfunction some weeks later.

In 12 of 18 patients with hand transplants, acute rejection episodes were seen [32]; all of them were reversible.

After the experience of late rejection in three cases and one surgical site infection, we changed our immunosuppressive regime to Tacrolimus (10 mg p.o.) and Mycophenolate Mofetil (MMF; 2 g p.o.) with omission of steroids after 2 weeks. We speculate, that this immunosuppressive regimen is a major factor along with the SSG for the longest graft survival so far in case 6. In the hand transplants a similar effect was seen with this immunosuppressive regime [23].

In all cases bone healing and osseous integration of the graft was completed after 6 months with immunosuppression. There was no delayed bone healing bone or nonunion. This correlates with the results of bone healing in hand transplants [33], where no negative influence of immunosuppression on bone healing was found.

In summary, one of six allogeneic vascularized knee transplants has survived for over 4 years. One was lost due to a surgical site infection, one by noncompliance and three by late rejection.

Critical analysis of this data presents some issues, which should be addressed. First, the indication for transplantation must be a combination of extensive loss of cartilage and bone, deficient extensor mechanism with soft tissue and skin defects, necessitating a free flap. In this indication, the joint can be replaced and the soft tissue defect covered with an allograft in a one-stage procedure. Transplantation should only be performed as a last option before Above Knee Amputation in an otherwise healthy young patient ideally under 35 years of age.

Patients with postinfectious joint defects should be excluded to minimize the risk of a postoperative surgical site infection.

Psychiatric assessment of candidates for knee transplantation and psychological support after transplantation must be carried out with great care, as recommended by the Louisville group for CTA [25,11]. Monitoring of immunosuppression and the use of the sentinel skin graft needs to be refined, e.g. regular skin biopsies should be carried out.

This can only be achieved with an Interdisciplinary Team consisting of a Clinical Psychologist, an experienced Transplant Immunologist to manage immunosuppression at the practical level, an Anaesthetist, a Physiotherapist, an Occupational Therapist, a General Practitioner, a Transplant Surgeon and an Orthopaedic Surgeon.

Unless these criteria are met, a knee transplantation cannot be recommended for the reconstruction of an extensively damaged knee joint.

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