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Allogenic grafting of vascularized bone segments under immunosuppression. Clinical results in the transplantation of femoral diaphyses

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Abstract Trauma surgery lack, substitute, for the reconstruction of large defects of the long bones. Encouraged by the promising results of bone allotransplantation in animal models, we successfully performed vascularized bone transplantation in humans. Vascularized femoral diaphyses were allogenically transplanted into three patients suffering from chondrosarcoma or post-traumatic osteomyelitis with postoperative immunosuppression. The bone segments were harvested from multi-organ donors and perfused with UW solution. After back-table preparation, the grafts were transplanted into the defect zone. Interlocking devices were used in these operations. Vascular anastomoses were performed in end-to-side technique. The early clinical course of the patients was not free of anatomical, technical, or immunological complications. However, all patients are currently free of malignancy and infection. They are also free of pain and full weight bearing. We conclude that allogenic grafting of vascularized bone segments has the potential to become an alternative for the replacement of large bone defects.

Key words Bone transplantation, immunosuppression · Immunosuppression, bone transplantation

Introduction

Tumor, injury, and post-traumatic osteomyelitis are causes of large defects in long bones of the leg. In some cases, the currently available methods of reconstructive surgery are still unsatisfactory, particularly for young patients. Several techniques have been employed for bone replacement in orthopedic surgery. Large osseous allografts without a vascular pedicle from the bone bank have been implanted without immunosuppression [30]. These procedures have been accompanied by multiple complications, such as fractures of the graft and infection [10]. Original bone length has also been restored, either by the transfer of autologous, vascularized fibula [40] or a bone-lengthening procedure (Ilizarov's maneuver) [22]. However, these techniques are time-consuming and the patient is immobilized for weeks, unable to work or walk or to put any weight on the leg. These procedures often result in a high complication rate, and sometimes amputation of the leg cannot be avoided.

The original idea behind vascularized bone allografting was to bring living bone cells and a blood supply into the defect zone [43]. Chiron and coworkers performed the first transplantation of a human femoral diaphysis using arterial and venous anastomoses, but without immunosuppression [8]. Although there was initially good blood flow in the transplant vessels, long-term survival

of the graft was not reported. However, as early as 1984, it was shown in an experimental model that bone allograft transplantation with anastomoses requires the administration of cyclosporin A (CyA) as immunosuppressive therapy. CyA was the most effective immunosuppressant at that time, and it ensured survival and integration of the transplant [1]. Doi et al. were subsequently the first to perform vascularized transplantation of fresh, perfused, allogenic bone with postoperative immunosuppression (living related donation) [12]. However, this transplanted allogenic fibula failed because of insufficient immunosuppressive therapy [13]. Three years ago, we performed the first allogenic transplantation of a vascularized femoral diaphysis under immunosuppression [19, 26]. Here we report the first three cases.

Material and methods

Patients

From November 1994 to August 1995, femoral diaphyses were transplanted into three male patients (21, 50, and 54 years of age). Indications were post-traumatic osteomyelitis (patients 1, 2) and a chondrosarcoma grade 1 without recurrence for 3 years (patient 3). The length of the bone defects measured was 12, 14, and 33 cm, respectively. In the first patient, an infected fistula, resulting from osteomyelitis, had persisted for years with recurrent pain. The patient underwent more than 30 operations over a period of 10 years before he was referred to our clinic. The second patient had also had several operations because of recurrent osteomyelitis of the femur. After tumor resection of 33 cm of the femur, patient 3 received a nonvascularized graft, but twice developed a non-union at the proximal osteotomy.

Resection

In a first operating procedure, the corresponding bone segment was resected and the infection was cleared. An interlocking compression nail (ICN, Fa. Osteo, Selzach, Switzerland), in combination with a PMMA spacer, was inserted to bridge the defect until the transplantation was performed (Fig. 1). Angiography and phlebography were performed to map the topography of the vessels for the vascular anastomoses. Following this procedure, the patients were put on a transplant waiting list. In the meantime, the patients were able to move their knee and hip joint with the help of a physiotherapist. Due to the support provided by the nails, partial weight-bearing up to 20 kg was allowed.

Organ donation

We transplanted femoral diaphyses from brain-dead, multi-organ donors (MOD) who were allocated by the transplantation center of the University of Munich. The usual criteria for organ acceptance were respected. MODs older than 45 years or those who had had an accident involving the same leg were excluded from the pool of bone donors. Nor was any bone taken from MODs who had received either blood substitutes or fresh-frozen plasma while in the critical care unit.

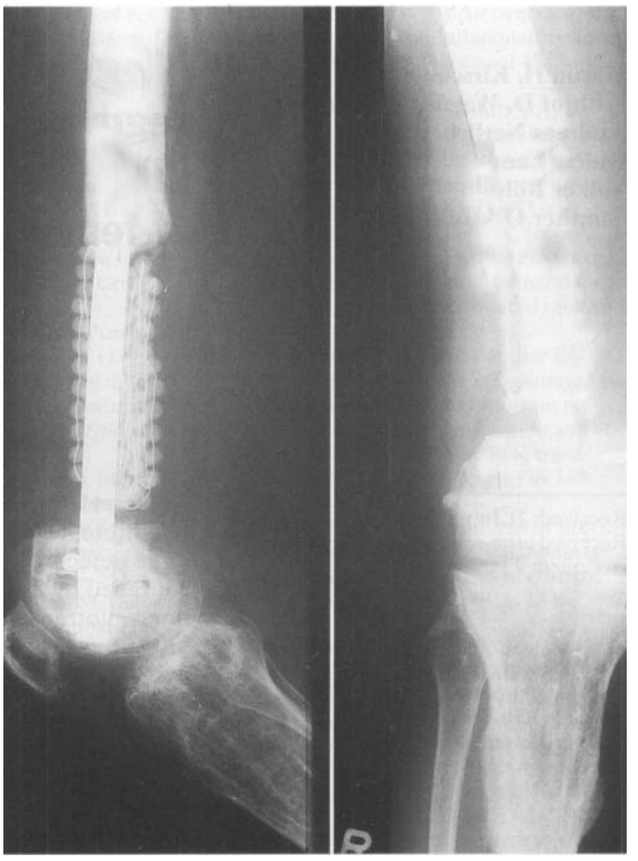

Fig. 1 Patient 1 (October 1994). Resection of a 14-cm bone segment after chronic osteomyelitis following supracondylar fracture of the right femur 10 years before (AO: 33-A3.3, open, II°). Bridging and stabilization were achieved with an interlocking compression nail with a temporary spacer (gentamycin-PMMA-chain)

Organ procurement

The parenchymatous organs and the femoral diaphyses were harvested in accordance with the general rules of organ procurement in multi-organ donation. Prior to transplantation, it is necessary to turn the graft 180° in order to present a long vascular pedicle for performing the anastomoses (Fig. 2a). In order to maintain the antecurvature of the femur, a graft for the right side was harvested from the donor's left side, or vice versa. After preparation of the abdominal organs, the corresponding external iliac artery was cannulated with a 14 Fr catheter. After starting perfusion of the abdominal organs, the corresponding leg was perfused separately with 4 l of University of Wisconsin (UW) solution at 4 °C. Subsequently, a skin incision was made in a frontal plane from the inguinal ligament to the center of the knee joint. The femoral artery and vein were dissected distally to the level of the lesser trochanter. The muscles were severed and the bone was sawed through at a level just below the lesser trochanter and just above the femoral condyles. Particular care was taken not to endanger the vascular pedicle. The harvested graft was stored sterile in three layers of plastic bags at 4 °C. The cold ischemia time was 16, 23, and 25 h for patients 1, 2, and 3, respectively.

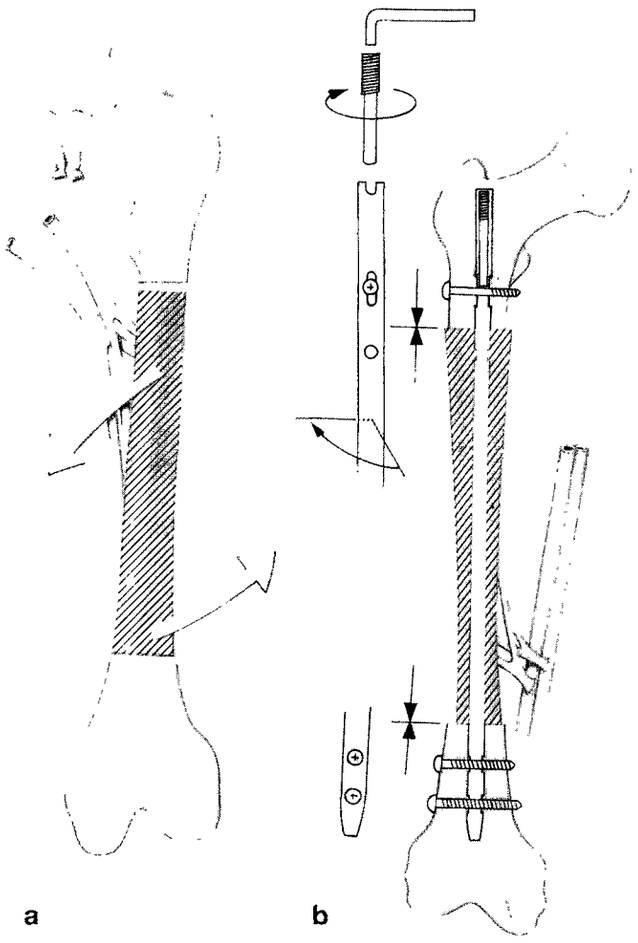

Fig. 2a, b Principles of harvesting and transplantation of femoral diaphyses: **a** Explantation: The graft is harvested from the contralateral side and turned sagittally 180°. As a result of this maneuver, the graft vessels lie distally and medially for the operating procedure; **b** The rotated graft is fit into the defect (step 1) and the ICN is inserted (dynamically interlocked with three interlocking screws). The compression screw provides dynamic pressure on the transplant (step 2). The anastomoses are performed in the classical way (i.e., in an end-to-side technique; Step 3). The nail is drawn schematically at a 90° angle

Back-table preparation

During the back-table preparation, the femur was carefully freed from the muscles and connective tissue. The vessels of the muscles were ligated while the vessels of bone were preserved with particular care. Finally, the graft artery was flushed with methylene blue in order to assure that there was adequate perfusion of the graft. In two preparations, there was no reflux over the graft vein at the end of back-table-preparation and the specimens had to be discarded.

Transplantation

At first, a lateral approach to the thigh was chosen. After the removal of the PMMA spacer, the nail was partially pulled back in order to perform the osteosynthesis with exactly the same nail. The newly formed connective tissue was resected, the graft was sawed to the size needed to fit precisely, and then it was placed in the defect. All osteosyntheses were performed according to the principles of internal fixation with intramedullary devices using interlocking compression nails (ICN; Figs. 2b, 3). The ICN provides active compression for the osteotomy site, leading to a highly stable connection between the graft and the recipient femur (Fig. 4). Using this technique, the patients were able to stand up on the 1st postoperative day. Particular care was taken to rotate the femur sagittally 180° in order to keep a long vascular pedicle for the anastomoses. In a second step, an incision was made from a medial approach. Generally, the graft vessels were connected to the femoral artery and vein in an end-to-side technique after preparation of a muscular tunnel from the adductor canal to the femur graft (Fig. 2b).

Immunological aspects and immunosuppressive drugs

All transplantations were performed with ABO-compatible grafts (0- > A, A- > A, A- > AB) without regard to HLA matches. Cross-matches were made prior to transplantation and were negative in all three cases. Immunosuppression was started immediately after reperfusion of the graft. Initially, quadruple induction therapy was administered. This consisted of CyA (1.5 mg/kg BW i.v. daily), azathioprine (2 mg/kg BW i.v. daily), cortisone (methylprednisolone 250 mg i.v. daily), and ATG (4 mg/kg BW daily). After 7 days the patients were switched to an oral double maintenance therapy of CyA and azathioprine. The patients were discharged with an oral CyA monotherapy (dose according to blood levels).

Early postoperative management

Heparin was administered intravenously for 3 days (PTT ranging from 60 to 80 s), followed by subcutaneous administration. The patients were mobilized on the 1st postoperative day. They were able to walk immediately. The patients received physiotherapy until they were discharged from the hospital.

Postoperative monitoring

Clinical signs, such as local inflammation of the leg and fever, were indicators of early infection under immunosuppression or rejection of the graft. In addition, the differential white blood cell count and sedimentation rate were measured to confirm the clinical diagnosis. CyA levels were checked every 2nd day during the 1st week post-transplantation and twice a week thereafter. X-rays were used to visualize bone healing and to control the position of the nail and screws. On the 1st postoperative day and whenever clinical problems occurred, angiograms (DSA) were made to confirm blood flow in the transplant artery. Duplex sonography was performed daily during the 1st week. ^{99m} Tc-MDP scintigrams were regularly made to demonstrate perfusion, metabolism, and viability of the graft. Moreover, single photon emission computed tomography (SPECT) was used to exclude the tracer uptake from the overlying soft tissues. Bone biopsies were taken whenever open operating procedures became necessary. In patient 3, a percutane-

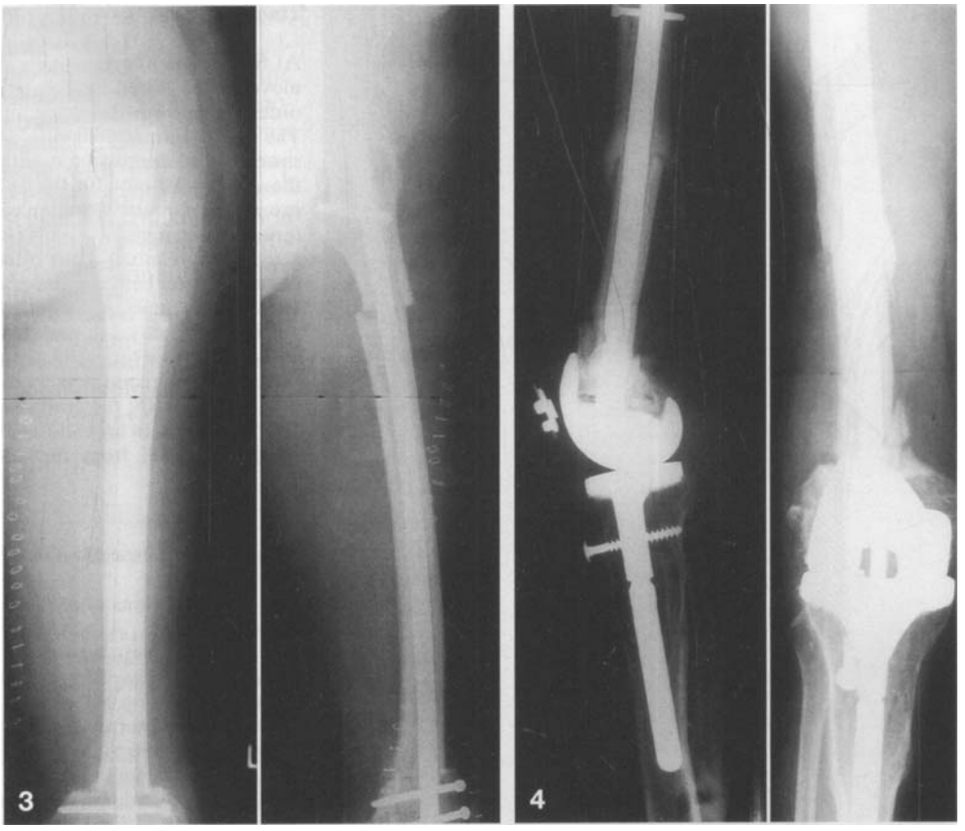

Fig. 3 Patient 3 (November 1995). Allogenic vascularized transplantation of a fresh and perfused femoral diaphysis employing an intramedullary nail (diameter 12 mm, length 440 mm, interlocked dynamically)

Fig. 4 Patient 1 (2.5 years post-transplantation of 14-cm vascularized femoral bone). X-rays show the combination of a newly designed prosthesis with the femur transplant

ous biopsy of the soft tissue was taken to exclude a rejection crisis with an examination of the surrounding muscle tissue. After discharge, the clinical follow-up was done weekly in the patients' hometown by their practitioners. In addition, the patients were routinely monitored every 2 months in our surgical outpatient department.

Results

Case 1

In the first patient, complications arose during the operation. First, the donor vessels were led from the lateral side over the bone graft medially. This procedure had to be carried out for anatomical reasons. In this case, a femur graft from the donor's right side was taken and transplanted to the recipient's right femur. Consequently, the vessels were lying in a lateral, instead of a medial, position. Second, a medial approach was not chosen for the anastomoses. The vessels were prepared instead from the lateral side. During this maneuver, the recipient's femoral vein slipped behind the medial condyle and had to be ligated due to severe bleeding. Subsequently, the venous anastomosis had to be performed in end-to-end technique.

On day 34 post-transplantation, the patient developed pain at the transplantation site, with a decreased range of motion in the hip and knee joint. Within 24 h the patient's temperature had increased from 37.3 °C to 39.5 °C. Although leukocyte counts were low at that time, clinically a rejection crisis was diagnosed. Subsequently, 250 mg methylprednisolone per day was administered intravenously for 3 days. An angiogram was made on day 38 post-transplantation. Although the patient received antirejection therapy immediately after diagnosis, no blood flow was detected in the transplant artery.

On day 36 post-transplantation, Clonab testing for CMV became positive for the first time. Because the clinical symptoms had disappeared and the temperature had decreased under antirejection therapy, no further therapy was given. Within the next 4 days the patient again developed a fever over 39 °C and leukocyte counts increased as well. CMV infection was diagnosed and Cymeven was consequently administered for 10 days (gancyclovir 3 mg/kg BW) after immunosuppression had been stopped. On day 40 post-transplantation, CMV IgM (CMV IgM, IMX test, Abbott, Wiesbaden, Germany) became positive and leukocyte counts showed values of nearly 20000/µl. The patient also

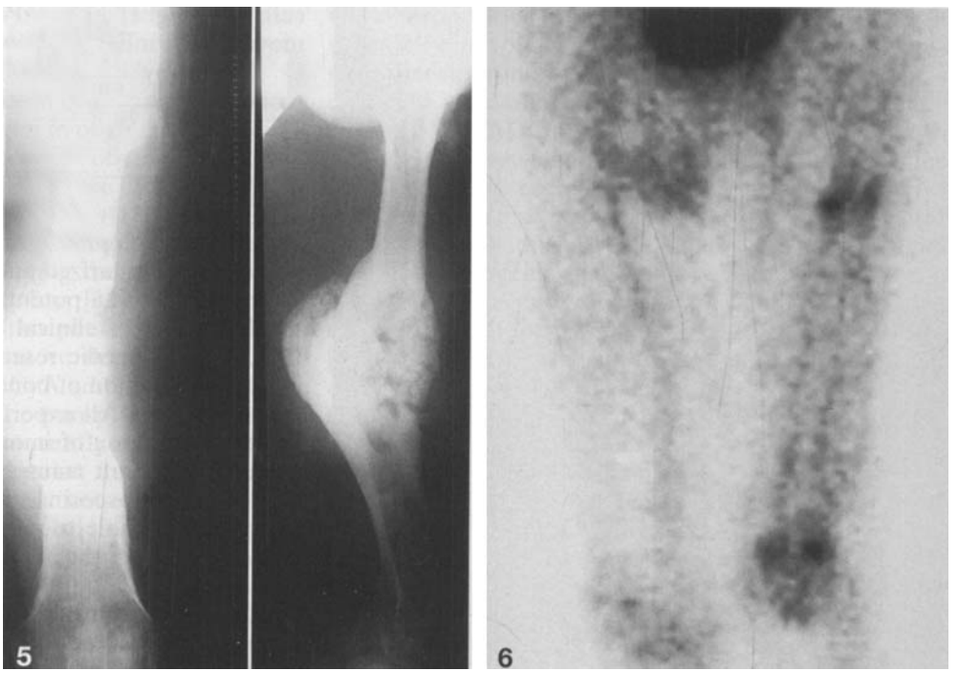

Fig. 5 Patient 2 (2 years post-transplantation). X-rays show the zone of the femur where spongiosa was inserted after the removal of the graft. In this state, the leg is stable. The patient had no recurrent infection, is full weight bearing, walks without pain, and is able to participate in sports

Fig. 6 Patient 3 (16 months post-transplantation; left leg). ^{99m}Tc-MDP scintigram shows adequate tracer uptake along the whole bone graft and increased bone metabolism at the osteotomy sites due to bone healing. A blank zone in the middle of the transplant remains due to the osteosynthesis performed before using an intramedullary nail

showed signs of a moderate hepatitis with increased levels of γGT and transaminases and decreased pseudocholinesterase levels. When antiviral therapy was stopped, leukocyte counts, γGT, and transaminase levels reached normal values and pseudocholinesterase returned to values over 4000 U/l.

During the following weeks, the patient developed a deep venous thrombosis of the operated leg. Although there was almost no detectable perfusion, the transplant was clinically incorporated and X-rays showed callus formation. Within the following months, clinical signs of post-traumatic arthritis were observed on the knee joint of the operated leg. A special knee joint prosthesis was developed and implanted 2 years post-transplantation (Stryker, Kalamazoo, Mich., USA). A combination of a surface substitute and an interlocking nail allowed us to combine a prosthesis with the bone transplant (Fig. 4). Since this last operation, the patient has been able to walk without pain. Today, he is able to move his knee joint with a range of motion of 0–0-100.

Case 2

In the second patient, occlusion of the graft vessels was detected by duplex sonography and angiograms within 24 h post-transplantation. The patient was operated on immediately. Reoperation presented thrombosis of both the artery and the vein of the graft. After thrombectomy, an arteriovenous fistula was created and the vessels remained open. However, scintigrams made 4 and 7 days post-transplantation showed a reduced tracer uptake and a large, cold area in the late scans. Within the next days, a local infection in the deep wound bed developed that had to be operated on and cleared several times. Swabs taken during the operations demonstrated *Enterococcus faecalis*, which had been responsible for the former osteomyelitis. Due to local infection with the risk of anastomotic rupture, graft vessels were ligated 4 weeks post-transplantation. The patient had to be operated on several times and developed a fever over 40 °C. Knowing that there was no tracer uptake on the bone scans, immunosuppression was stopped 6 weeks post-transplantation. When the infection seemed to be cured, the patient was discharged and he returned to work.

Sixteen months post-transplantation, the patient was readmitted to the hospital due to an infected fistula. It was now decided to remove the graft, which had already induced strong callus formation. The remaining defect was filled with autologous spongiosa (Fig. 5). Two years after the first operation, the patient was free of infection, full weight bearing, and he went back to work.

Case 3

The third patient did not show any complications during the operation or the postoperative stay in the clinic. However, after discharge, the patient was readmitted

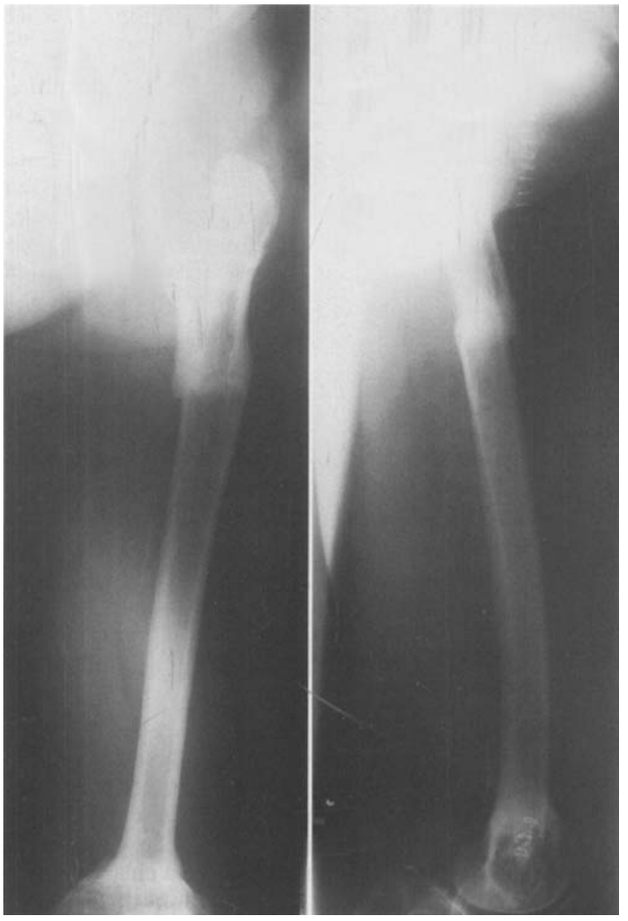

Fig. 7 Patient 3 (21 months post-transplantation). X-rays show a stable and incorporated transplant following removal of the nail. These pictures show evidence of bone healing at both osteotomy sites. There was neither local tumor recurrence nor a systemic relapse of the malignant disease

with pain in the operated leg but with no symptoms of infection or rejection. Eight weeks post-transplantation, X-rays showed a fracture of the nail but an open arterial transplant vessel. With a minimally invasive operating procedure, the nail was changed without touching the graft or the anastomoses.

Fourteen months post-transplantation, routine duplex sonography detected no blood flow in the transplant vessels, and immunosuppression was stopped immediately. However, scintigrams still showed a good tracer uptake (Fig. 6). Bone biopsies from the shaft were taken 21 months post-transplantation when the nail and screws were removed. The histological evaluation of these specimens revealed sclerotic spongy bone with viable osteocytes and focally visible normal bone lining cells. The marrow space contained few lymphoid cells and slight fibrosis. Postoperative X-rays after removal of the material showed complete bone healing at the osteotomy sites (Fig. 7). The patient is now walking without pain and is full weight bearing.

Discussion

After the development of suitable microsurgical techniques for vascularized autografts [40], the discovery of cyclosporin A as a potent immunosuppressant [5], and the beginning of clinical organ transplantation under CyA [7], orthopedic research focused on vascularized allotransplantation of bone with immunosuppression in animal models. All experimental results indicated that the administration of immunosuppressive drugs in vascularized allograft transplantation was necessary [2, 4, 6, 14, 35]. Cyclosporin A was found to be the most potent drug available to prevent rejection in such procedures [3, 37]. In the two clinical case reports dealing with vascularized bone allotransplantation in humans, the transplantations failed due to insufficient immunosuppression. In the last case, CyA levels were well below the levels used in organ transplantation [9, 12, 13]. Moreover, antibodies against HLA class I and II antigens, even in recipients of nonvascularized bone allografts, were found to be a possible target of rejection [21]. The next logical step, therefore, was to transplant allogenic bone as an organ with the accompanying immunosuppression. We decided to operate on three patients who had had many unsuccessful operations on the thigh in their longlasting history or who were likely to lose their leg by amputation [19, 24].

Complications during and following the operations were comparable to those after transplantation of parenchymatous organs. In the first patient, deep venous thrombosis occurred, apparently due to the ligated femoral vein and the missing blood flow in the transplant vein after its occlusion. Since the initial operating technique was obviously insufficient, we chose the combined approach for further operations. Osteosynthesis was subsequently performed from the lateral side and vascular anastomosis from the medial side (Fig. 2b). Using this technique, no technical complications occurred during the operating procedure. After the initial transplantation, this patient suffered from post-traumatic arthritis. However, as a result of the allogenic bone transplantation, the infected bone was cured so that the patient could be fitted with a prosthesis. This combined procedure of first fighting the infection by bone resection and of second performing a knee arthroplasty led to a satisfactory situation for the patient. This technique had been performed successfully before in 13 patients, as reported by Harris and coworkers [17]. Although patient 1 had some severe complications, his ability to walk has greatly improved and he is free of infection.

Fortunately, no recurrence of infection occurred in the two patients with osteomyelitis. Nevertheless, compared to the long-term results of Mankin et al. [31], who reported a 10% infection rate, these results are preliminary.

Graft thrombosis is well known in organ transplantation and is one major reason for failure of pancreatic as well as kidney transplants [23, 33, 41]. Renal graft thrombosis, for example, usually occurs within 7 days post-transplantation, mostly during the 1st 48 h, and it almost always results in graft loss [32]. Some factors that could be responsible for this phenomenon include cold ischemia time (CIT) of more than 24 h [16, 33], sex (female donors) [33], and CyA administration itself [23, 36]. In renal and pancreatic transplantation, vascular graft thrombosis is most frequently related to surgical problems [38, 41]. In particular, anatomical problems, such as multiple renal donor vessels [36], discrepancy in donor and recipient vessels [16], vessel torsion, compression, or stenosis of the transplant vessels, seem to be responsible for early organ failure [32].

In our opinion, the problem with transplanting femoral diaphyses is the lack of information about the exact topography of vascular anatomy of the femur. In particular, very few details are known about the periostal blood supply of the femoral shaft. Therefore, it is quite likely that we did not adequately prepare the graft according to the actual course of the femur's blood vessels. Two facts support this hypothesis of "anatomical organ failure": (1) in two cases, no reflux was detected over the graft vein after back-table preparation, and (2) the largest femur graft had the best outcome. In whole-diaphysis transplantation, it is possible to ensure the graft's original blood supply. In segmental bone grafting it is questionable because the graft has to be shortened [27]. All other reasons for acute graft failure (CIT, donor sex, CyA) were unlikely in our cases. CIT was less than 24 h in the second patient but more than 24 h in the third patient, who had the better outcome. In patient 2 the graft was harvested from a male, in patient 3 from a female donor. CyA was administered in equal doses in all patients.

Ultimately, the course of the second patient was also satisfactory. Unfortunately, graft thrombosis and reinfection occurred and necessitated the removal of the "organ", but no major operation was necessary to save the limb. The graft acted osteoinductively and subsequently led to a situation where it was possible to perform autologous bone transplantation. With this operation the leg became fully stable. In the end it was a "part-time transplantation" that helped to clear the infection and restore normal function. Now the patient is able to work and to run again.

The transmission of CMV infection by transplanted organs is a major problem in transplantation [39]. In the first patient, the femur graft was harvested from a CMV-seropositive MOD and was transplanted into a seronegative recipient. The clinical course with positive Clonab testing and IgM levels clearly indicated that vascularized bone allotransplantation may be a potential pathway for CMV transmission [25]. It has been shown for renal allotransplantation in humans that CMV infection is not only correlated with a lower graft survival rate, but that it also leads to a 10% reduction in the graft survival rate within 1 year [20, 34]. In experimental models of allogenic lung and heart transplantation in the rat, it was recently shown that CMV infection induces MHC class II expression on vascular endothelial cells [42, 44]. These results and the observation that class II expressing cells are increased during both CMV infection and rejection in human liver transplantation strongly suggest that CMV infection is able to trigger acute graft rejection [29, 44]. From the clinical course of patient 1, it is highly likely that acute graft rejection was associated with CMV infection. The presence of antibodies against class II antigens in human bone transplantation lends support to this suggestion [21]. However, CMV infection must be accepted as a severe complication of vascularized, allogenic bone transplantation.

In the third patient, the material was removed after a clinical course with one single minor complication [18]. The patient now walks normally without help and has no pain. It remains to be seen, however, whether patients with vascularized bone transplants will show better results in terms of their fracture rate, which is about 19% in larger series [17], and/or in terms of their mechanical properties than recipients of nonvascularized bone transplants [15].

Other techniques, such as revascularization of bone allografts and bone tissue engineering, seem to be promising experimental approaches [11, 28]. To date, they have not been established in humans. However, in the future, these new techniques must demonstrate that they are as effective as nonvascularized and vascularized bone allotransplantation in humans.

Our experience leads us to conclude that vascularized bone allotransplantation under immunosuppression can be a limb-saving operation. Compared to nonvascularized bone transplantation, these grafts are biological substitutes with living bone cells and blood vessels. They have the potential to remain viable for long periods of time if immunosuppression is administered. CyA and azathioprine have proved to be clinically effective and must be used until the graft is incorporated. However, the clinical course of our first three patients demonstrated that while the administration of these immunosuppressive drugs is necessary, it can only be temporary. When immunosuppression is stopped after a period of less than 2 years, the graft can still be perfused, even though no flow is detected on the vascular pedicle, as shown in our third patient. Moreover, vascularized

bone allografting is a time-saving procedure compared to alternative therapies such as Ilizarov's maneuver.

Once again, our first three cases demonstrate that allogenic vascularized bone transplantation under immunosuppression in humans has the potential to become an alternative treatment for patients with large bone defects. It is still in an experimental stage and should be performed at specialized clinics with experience in both transplant and orthopedic surgery.

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