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Jean-Michel Dubernard
Earl Owen
Nicole Lefrançois
Palmina Petruzzo
Xavier Martin
Marwan Dawahra
Denis Jullien
Jean Kanitakis
Camille Frances
Xavier Preville
Lucette Gebuhrer
Nadey Hakim
Marco Lanzettà
Hary Kapila
Guillaume Herzberg
Jean-Pierre Revillard

First human hand transplantation

Case report

J.-M. Dubernard (✉) · P. Petruzzo · X. Martin · M. Dawahra
Service d'Urologie et de Chirurgie de la Transplantation, Hôpital Edouard Herriot, Lyon, France
Tel. + 33-4-72110581
Fax: + 33-4-72110582

E. Owen
Hand and Microsurgery Unit, Microsearch Institute, Sydney, Australia

N. Lefrançois
Service de Médecine de la Transplantation, Hôpital Edouard Herriot, Lyon, France

D. Jullien · J. Kanitakis
Service de Dermatologie, Hôpital Edouard Herriot, Lyon, France

C. Frances
Service de Médecine Interne, Hôpital de la Pitié Salpétrière, Paris, France

X. Preville · J.-P. Revillard
INSERM U 503, Hôpital Edouard Herriot, Lyon, France

L. Gebuhrer
Centre de Transfusion Sanguine, Lyon, France

N. Hakim
Transplantation Unit, St Marys' Hospital, London, UK

M. Lanzettà
Hand Surgery Department, Service de Chirurgie de la Main, Hôpital de Monza, Milan, Italy

H. Kapila
Hand Surgery Department, Canterbury Hospital, Sydney, Australia

G. Herzberg
Service d'Orthopédie, Hôpital Edouard Herriot, Lyon, France

Introduction

Hand transplantation is a model of composite tissue allografting (CTA). The immunological issues in CTA are extremely complex, as different tissues are involved, each of which has to be considered both individually and as a part of a unit while each has a different time and intensity of rejection. Cartilage, ligaments and fat present low antigenicity, and consequently they lead to a weak rejection; bone, muscles, nerves and vessels show a moderate rejection profile in spite of various degrees of immunogenicity; skin, a complex immunological structure, is the component that develops the most severe rejection, because of the abundance of dentritic cells within epidermis and dermis. Finally, bone marrow, a source of immunocompetent cells, is a major target for rejection, but also a source of contaminating donor T cells that could induce a graft-versus-host disease in a strongly immunosuppressed recipient, and a source of stem cells that might contribute to the development of a microchimerism. Limb transplantation, the most common experimental model of CTA, has met with varying degrees of success; however, results have improved with the introduction of new immunosuppressants, especially tacrolimus and mycophenolic acid [1–7].

In humans few cases of isolated muscle [8], bone, joint [9, 10], nerve [11] or vascular allografts [12] have been reported. The first vascularized human hand transplantation was performed on 23 September 1998 in Lyon, and it is described in the present report [13].

Case report

A 48-year-old male New Zealander suffered a traumatic circular saw amputation of his right forearm in 1984. This was initially replanted, but required reamputation in 1989 because of lack of function. The patient refused an aesthetic or functional prosthesis, preferring to explore the literature and make himself available to units contemplating limb transplantation. He was informed of all the potential risks, and he made the decision totally independently after psychological evaluation. He underwent routine pretransplantation investigations and specific tests, both morphological and functional, of the forearm stump. The donor was a 41-year-old man who had died of an intracerebral haematoma secondary to a skull fracture. He had the same blood group (0+) as the recipient and there were 6 HLA mismatches (A, B and DR); the cross-match was negative. The brachial artery was dissected free 3 cm above the elbow joint and cannulated, after which the limb was irrigated with 500 ml of University of Wisconsin (UW) solution at 4 °C before being amputated a few centimetres above the elbow.

Under general anaesthesia coupled with a brachial block, the recipient's stump was prepared by dissecting and identifying all available muscles and neurovascular structures. At the same time, the anatomical structures in the graft were dissected and tagged. Replantation consisted of sequential bone fixation, arterial and venous anastomoses (ischaemia time: 750 min), nerve sutures, muscle and tendon connection, cutaneous sutures.

The patient (90 kg body weight) was given 500 units of heparin s.c. on the 1 day and low-molecular-weight heparin (fraxiparine 300 units) for 10 days, then aspirin 150 mg/day. A broad-spectrum antibiotic therapy was administered for 10 days. The induction immunosuppressive protocol consisted of antithymocyte globulins (Thymoglobuline 75 mg/day for 10 days), anti-CD 25 monoclonal antibody (Simulect) on day 26 and day 100; tacrolimus (Prograf) to maintain blood levels between 10 and 15 ng/ml during the first month, mycophenolate mofetil (Cellcept) 2 g/day; and steroids (prednisone) 250 mg on the 1 day, tapering rapidly to 20 mg/day. One 90. Maintenance therapy included tacrolimus (serum levels between 5 and 10 ng/ml), mycophenolate mofetil (2 g/day), and prednisone (20 mg at 3 months). Sulfadoxine pyrimethamine (fansidar) was used for prevention of *Pneumocystis carinii* pneumonia. Physiotherapy was started 10 h postoperatively and performed twice a day for the entire follow-up period. Psychological support was offered once a day during the first 3 weeks, then twice weekly. Skin biopsies were taken once a week from several areas, and more frequently when rejection was suspected. No surgical complications were encountered and wound healing was satisfactory, as was the take of skin autografts. The blood supply was excellent, as demonstrated initially by the PO_2 saturation values in all fingers and later by scintigraphy. The patient's general conditions remained satisfactory throughout. An episode of hyperglycaemia requiring insulin administration followed by the intake of oral hypoglycaemic agents was observed; this coincided with the initial high doses of steroids and tacrolimus. Serum creatinine increased when tacrolimus levels were high and returned to normal values with drug dose reduction.

A herpesvirus (HSV-1) infection occurred 2 months after the transplant and was successfully treated with aciclovir. At 8 weeks after the transplant, following a decrease in tacrolimus plasma levels the skin demonstrated a mild disseminated erythema, and histological examination revealed a major perivascular dermal infiltrate of mononuclear cells consistent with rejection. On days 57, 63 and 77 the inflammatory infiltrate was made up mostly of CD3+/CD4+/CD25 lymphocytes, with only occasional CD3+, CD8+ cells; it formed well-defined perivascular nodules and gave rise to a mild exocytosis in the epidermis. The infiltrating cells originated from the recipient, as shown by the strong expression of the HLA-A24 antigen. Doses of both tacrolimus and prednisone were increased (from 20 to 40 mg/day); in addition topical immunosuppression was started with tacrolimus and clobetasol (Dermoval) ointment twice a day. This regimen kept the rejection episode under control, and subsequent skin biopsies showed near-normal dermis. Although no microchimerism was demonstrated in the peripheral blood by DNA typing on day 85 and day 100, Cd1 a-positive Langerhans cells of the recipient (expressing the recipient's own MHC class 1 HLA-A24 antigen) were demonstrated by double immunohistochemical labelling within the graft epidermis and hair follicles of the grafted forearm from day 77 onward. At day 85 after the graft, the density of the recipient's Langerhans cells reached 10% of the total number of Langerhans cells within the graft.

The rehabilitation programme consisted in passive and active exercise schedules and early sensory re-education. At 1 day after the transplantation passive finger and wrist mobilisation was started, and active mobilisation was begun 3 weeks postoperatively. At 6 weeks passive mobility of all joints below the elbow was achieved, and active mobility was possible with and without visual control.

By 110 days after the transplantation, flexion of wrist and fingers was satisfactory, extension of the wrist was weak (20°) and the index-thumb pinch was satisfactory; the patient could write with a pen and grip a glass securely. Physiotherapy was interrupted between days 110 and 155 and resumed on 1 March 1999 to restore the grip and the extension of the wrist. At present, the patient can hold bottles and drive vehicles. Motivation has returned. On day 110, Tinel's sign, which tests sensitivity, improved to 21 cm in the median nerve and 20 cm in the ulnar nerve; it reached 30 cm on day 210 and 36 cm on day 210; the Semmes Weinstein test, another sensitivity test, showed palmar deep pressure sensations (6.65 g/mm^2 microfilament) and wrist light touch sensations (1.65 g/mm^2 microfilament) on day 180. On day 240, sensitivity was present in the palm and at all fingertips for light and deep touch, temperature and pain.

Discussion

Advances in microsurgical techniques suggest that the technical problems can be favourably overcome, as demonstrated by successful hand reimplantations in man (autografts) [14]. However in this case the rapidity and quality of nerve regeneration was surprising. It can be explained by the clean-cut amputation in the donor, the use of UW preservative solution and the relatively short ischaemic time (12.5 h). Furthermore, tacrolimus has been demonstrated to accelerate functional recovery and nerve regeneration in experimental models by increasing synthesis of the axotomy-induced growth-associated protein (GAP) 43 [15, 16].

The single most important obstacle currently preventing clinical application of limb transplantation is not the inability to restore function but rather the risks of rejection and the risks associated with efficient immunosuppressive therapy. In this case, we assumed that the major risk for the composite structure of the graft was rejection. Therefore, we decided to provide the patient with the most potent immunosuppressive regimen pre-

sently available, associating antibodies (anti-human thymocyte globulins, anti-CD 25 monoclonal antibodies), a calcineurin inhibitor (tacrolimus), a purine synthesis inhibitor (mycophenolate mofetil) and steroids. This drug association proved to be efficient in the short term, as shown by the clinical outcome of the patient. Thymoglobulins were selected for induction therapy because we needed a potent lymphoablative agent with a rapid action in view of the high risk of rejection [17]. Following a 10-day course at the standard dosage, there was a complete disappearance of peripheral blood T cells in 1 month, with progressive recovery during the 2nd month, characterized by a predominance of NK cells (300/μl) and low CD4+ (50/μl) and CD8+ (150–200/μl) cell counts. The patient did not produce antibodies against rabbit ATG, but free antilymphocyte antibodies in the patient's plasma decreased rapidly within a week after treatment. For this reason, we decided to use a CD25 antibody to ensure further protection. The risks associated with lymphoablative therapies such as ATG, Cdw 52, and total-body irradiation are excessive destruction of peripheral T cell pools with defective reconstitution of naive T cells in adults and loss of heterogeneity in the T cell repertoire. The precise dose-effect relationship and optimal risk/benefit ratios according to ATG dosage remain to be determined. The risks inherent in use of CD25 antibody are the transient expression of IL-2 Rα chain by activated T cells and the possible activation of CD25 cells, resulting in a lack of efficacy of the antibody. One of the main difficulties with CTA is the lack of known criteria for acute rejection episodes. We relied on clinical symptoms: serum levels of C reactive protein and skin biopsies. Only the latter proved to be a reliable indicator of dermal rejection, and we cannot exclude the possibility that unnoticed low-grade or localized rejection episodes may have developed in other tissues. Foci of hyperfixation observed on bone scintigraphy at 3 months may reflect minor focal rejection. The only episode of skin rejection was observed between 8 and 9 weeks after transplantation, when the serum tacrolimus concentration dropped as a consequence of a dose reduction implemented because of renal toxicity. The rejection episode was reversed by an increase in steroid doses (from 20 to 40 mg/day) and topical application of immunosuppressive creams (tacrolimus, clobetasol). The contribution of local versus systemic treatment is difficult to ascertain retrospectively. The main advantage of local drug administration is that a sufficient amount of immunosuppressive drugs is delivered locally without increasing their serum levels and consequent toxicity [18]. Other investigators developed several CTA models of immunosuppression, such as intra-arterial infusion and liposomes [19]. The decision to use a potent immunosuppressive treatment was also based on data reported in the literature. In rodents, the first cases of long-term, rejection-free survival of rat limb allografts were reported when high-dose cyclosporine A was administered [20]. In contrast, other authors have noted early or delayed skin rejection in CSA-treated animals [21]. In addition, discontinuation of CSA administration resulted in rapid rejection of vascularized muscle allografts, peripheral nerve allografts and vascularized bone allografts in rats [21]. The use of more modern immunosuppressants has only modestly improved the results. High oral doses of tacrolimus allowed long-term allograft survival [22], and mycophenolate mofetil was shown to both prevent and reverse acute rejection with concomitant drug-induced side effects [1, 4]. Long-term graft survival was also obtained by a combination of low-dose cyclosporine A and low-dose mycophenolate mofetil, with less toxicity [3]. In nonhuman primates, long-term partial or total hand allograft survival required high and toxic doses of cyclosporine A, steroids and monoclonal antibodies [5, 6].

Besides efficacy, safety is the main goal of all immunosuppressive treatments in clinical transplantation. In this patient, the toxic drug side effects seen were hyperglycaemia and increased serum creatinine. The hyperglycaemia required several days of insulin therapy and was then well controlled by oral antidiabetic drugs and the concomitant reduction in tacrolimus and steroid doses. On two occasions, serum creatinine increased and returned to normal values when the tacrolimus concentration in the serum declined. Infections and malignancies are the most severe complications of immunosuppression. In this case, a herpesvirus infection occurred 2 months after transplantation, but was easily reversed by aciclovir treatment. The most common malignancies induced by immunosuppression are lymphoid tumours and skin cancers [23]. The risk of EBV-associated B cell lymphoid tumour development is difficult to predict when a combination of new immunosuppressive drugs is associated with polyclonal or monoclonal antibodies. In the CTR, Opelz and Henderson reported a 0.28% incidence of lymphomas in patients treated with cyclosporin A and azathioprine [23]. The risk of skin cancer is dramatically higher in Australia, where it reaches up to 40% of patients after 10 years of immunosuppressive treatment. Restricted sun exposure and use of ultraviolet filter creams plus monitoring to ensure early detection and treatment of any lesions are adequate prevention and therapy for this severe complication.

Although chronic administration of immunosuppressive agents is accepted in visceral organ transplantation, the risks associated with such treatments in patients requiring a functional nonvital part of the body should be discussed on ethical bases. In our opinion, the recipient is the only person able to make an appropriate decision after being given detailed and comprehensive information on the risks of surgery and the risks of immunosuppression. Our patient took the ini-

tial decision autonomously, while he was in good mental health and able to balance an improvement in the quality of life against the potential risk of morbidity and mortality.

In conclusion, in the absence of further rejection the functional prognosis of this graft should be similar to if not better than that reported in large series of autoreconstructions. The potential human applications of CTA are numerous after traumatic forearm amputation and also for functional and anatomical reconstruction of many peripheral tissue defects following traumatic injuries, major burns, oncologic diseases or birth abnormalities.

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