Curr Transpl Rep (2015) 2:276–283
DOI 10.1007/s40472-015-0073-6

Immunological Challenges in Vascularised Composite Allotransplantation

Emmanuel Morelon[1,2,3] · **Jean Kanitakis**[4] · **Palmina Petruzzo**[5,6] · **Lionel Badet**[3,5] · **Olivier Thaunat**[1,2,3]

Published online: 2 July 2015

Abstract Vascularised composite allotransplantation (VCA) is a new field in transplantation aiming to improve disabled patients' quality of life. Two tissues appear to play an important role in the immune response: the skin, which is highly immunogenic and the main target of T-lymphocyte-mediated acute rejection, and the vessels, which are targeted by the humoral arm of recipient's immune response, which lead to chronic rejection, as in solid organ transplantation. In preclinical models, transplantation of bone marrow is associated with mixed chimerism inducing and maintaining tolerance to allogeneic VCA. However, this is not the case clinically. Immunosuppression used in VCA patients is similar to that in solid organ transplantation with similar side effects and complications. However, as a life-enhancing transplant, the careful selection of recipients and a close follow-up cannot be overemphasised.

Keywords Composite tissue allotransplantation · Vascularised composite tissue allotransplantation · Hand and face allotransplantation · Acute rejection · Chronic rejection · Cellular and humoral immune response · Immunosuppression

This article is part of the Topical Collection on *Vascularised Composite Allografts*

✉ Emmanuel Morelon
emmanuel.morelon@chu-lyon.fr

Jean Kanitakis
jean.kanitakis@chu-lyon.fr

Palmina Petruzzo
petruzzo@medicina.unica.it

Lionel Badet
lionel.badet@chu-lyon.fr

Olivier Thaunat
olivier.thaunat@chu-lyon.fr

[1] Department of Transplantation, Nephrology and Clinical Immunology, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

[2] Université de Lyon, Lyon, France

[3] INSERM U 1111, Lyon, France

[4] Dermatology Department, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

[5] Department of Urology and Transplantation, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

[6] Department of Surgery, University of Cagliari, Cagliari, Italy

Introduction

Composite tissue allotransplantation (CTA) or vascularised composite allotransplantation (VCA) is defined as the simultaneous transplantation of several tissues including skin, muscles, tendons, nerves, vessels, bone marrow, cartilage and bones which are transplanted as a single functional unit from a deceased donor to a recipient. These allografts have recently become a clinical reality following the advances in microsurgery and immunosuppressive therapy. Hand and facial allografts are the most common examples of VCA. More than 60 patients have undergone upper limb—namely the hand, forearm and arm—allotransplantation, and 35 have received a face transplant [1] (IRHCTT, www.handregistry.com). Other VCAs such as larynx, femur, knee, abdominal wall, lower limbs and uterus have been performed. This article will focus on upper extremity and facial allotransplantations, the most commonly performed and with the longest follow-up.

The functional and aesthetic outcomes after limb transplantation allow amputees to regain manual dexterity to perform most daily activities. Functional recovery is based on

sensitivity and motion recovery. In our experience (six cases of bilateral hand allotransplantation) all recipients experienced adequate sensorimotor recovery (protective and tactile sensitivity and partial recovery of intrinsic muscles) and became able to perform the majority of activities of daily life allowing for a normal social life [2, 3•]. During the first post-graft year, all of them showed protective sensibility and partial recovery of tactile sensitivity, while discriminative sensitivity was absent. After 2 years, tactile sensibility improved and partial recovery of discriminative sensitivity was achieved. Motion recovery started between 3 and 6 months. Extrinsic muscle function allowed the patients to grasp large objects, while intrinsic muscle function started later, between 9 and 12 months, and increased during the first five post-transplant years. Muscular power was weak in all patients, ranging from a grip strength of 2.6 to 16 kg and from a pinch grip <0.5 kg to a pinch grip >2 kg. All recipients considered their quality of life significantly improved after transplantation. The recovery of sensitivity and motion was longer in the recipients with a more proximal level of amputation, although the final functional recovery was not different.

Complete functional restoration is conditioned by nerve regeneration, which has been shown to occur by immunohistochemical studies of the skin, electromyography and sensitive recovery tests. Functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation [4••, 5] showed that hand transplantation induces a global remodelling of the limb cortical map, reversing the functional reorganisation induced by the amputation.

The overall results so far highlight the importance of patients' compliance to immunosuppression and rehabilitation programme, which must be evaluated in detail prior to transplantation. As a life-enhancing transplant, it is important to evaluate the real possible functional recovery, the patients' expectations and motivations and their understanding of the undertaking.

Facial transplantation must be reserved to disfigurements, which cannot be corrected satisfactorily by conventional reconstructive surgery. Some complex deficiencies need numerous procedures to be reconstructed, with nevertheless uncertain outcomes; moreover, some disfigurements include muscles (such as the orbicular ones), which cannot be repaired by surgical reconstruction techniques. Face transplant recipients have shown aesthetic and functional recovery after transplantation. Indeed, in our experience, they regained the ability to speak, eat and swallow—functions that are often affected by disfigurement. Remarkable aesthetic results allow them to resume a social life [6–10]. In all cases, a return to body integrity considerably improves the patients' quality of life [11].

The major limit of VCA development is the need for a lifelong immunosuppression and the associated risks, which counterbalances the functional results seen so far. Herein, we will consider the various aspects and mechanisms involved in VCA rejection processes and the immunosuppression treatment used to prevent them.

Immunological Aspects of VCA Transplantation

Immune Responses in Organ Transplantation

For over 60 years, clinical and experimental solid organ transplantation has demonstrated that the allo-immune response uses every component of the immune system to reject vascularised grafts such as the kidney, pancreas, heart, lung or liver. Both humoral and cellular immune responses are involved in this complex process.

Basically, the cellular arm of the immune response includes $CD4^+$ T-lymphocytes and cytotoxic $CD8^+$ T-lymphocytes that are activated by donor dendritic cells in the secondary lymphoid organs. This activation leads to proliferation of donor-specific clones capable of recognising graft HLA molecules. The allogeneic $CD8^+$ T-lymphocytes then migrate into the graft, causing its destruction via cell lysis mediated by molecules such as perforin and granzyme. Graft biopsies show cell infiltration by $CD4^+$ and $CD8^+$ T-lymphocytes as well as monocytes, macrophages and neutrophils, which are involved in the non-specific inflammatory response.

The humoral arm of the immune response is mediated by donor-specific anti-HLA antibodies (DSA). The recent use of techniques identifying the specificity of anti-HLA antibodies has improved the understanding of the role of the humoral immune response in organ-transplant rejection. Antibody binding to the endothelial cell surface of the graft induces endothelium injury and graft dysfunction through classical complement pathway activation and recruitment of innate immune cells (antibody-dependent cell cytotoxicity, ADCC) leading to acute humoral rejection. In the absence of complement activation, ADCC can still promote a smouldering antibody-mediated lesions leading to chronic rejection epitomised by the development of allograft vasculopathy [12–14]. The humoral component of the allo-immune response is currently believed to be the primary cause of long-term graft loss in kidney transplantation.

The Role of the Cellular Response in VCA Rejection

The cellular arm of the immune response plays a major role in VCA transplantation. Despite intense immunosuppression involving induction therapy (ATG or anti-IL2 receptor antibody) combined with triple maintenance immunosuppression including tacrolimus, mycophenolate mofetil and steroids, over 75 % of hand or face transplant patients experienced at least one episode of acute skin cell rejection following transplantation [1], thus making the skin the main target for acute rejection. In our experience, at least one episode of acute skin

rejection occurred during the first year in all hand- and face-grafted patients following transplantation [3•] with subsequent acute rejections after the first post-transplant year [15]. The high incidence of acute rejection in patients despite an intense immunosuppressive therapy is due to the strong antigenicity of the skin [16–18]. Furthermore, the diagnosis of acute rejection of the skin is relatively easy because it manifests as a skin rash, which is macroscopically visible and does not depend on laboratory tests or organ dysfunction. Acute rejection manifests clinically with erythematous macules, diffuse erythema or asymptomatic scaly papules (Fig. 1a). The diagnosis of acute rejection must be confirmed by histological examination of a skin biopsy, showing characteristic, albeit non-specific, changes. The dermis and epidermis and even the hypodermis in cases of severe rejection are in the process. Several types of changes can occur [19–23]: (a) The earliest lesions include a perivascular lymphocytic inflammatory infiltrate in the superficial and middle dermis. This infiltrate, which is composed almost exclusively of T-lymphocytes, contains mainly $CD3^+$/$C4^+$ T-lymphocytes, with smaller proportions of $CD8^+$ T-lymphocytes, cytotoxic TIA-1^+ T-lymphocytes, $Fox3^+$ regulatory T-lymphocytes and occasional histiomonocytic cells. When this infiltrate becomes denser, it can fill the dermis, come into contact with the epidermis and give rise to exocytosis (passage of inflammatory cells in the epidermis). (b) The epidermis is initially not involved in the rejection episode. It can subsequently show exocytosis and contain necrotic keratinocytes in the basal cell layer. This is frequently accompanied by vacuolar degeneration (vacuolisation) of basal keratinocytes. Spongiosis (inter-keratinocytic oedema) may rarely be seen. In some cases of persistent or inadequately treated rejection, lichenoid lesions may also appear, showing pathologically a generalised thickening of the epidermis (orthokeratotic hyperkeratosis, hypergranulosis, acanthosis) with vacuolisation of the basal layer and presence of colloid bodies (apoptotic keratinocytes) in the epidermis. The lymphocytic infiltrate is dense and forms a horizontal subepidermal band, infiltrating the basal layer of the epidermis. These changes are very similar to those observed in cutaneous (lichenoid) graft-versus-host disease. (c) In the case of severe rejection, the epidermis (and its appendages, hair follicles and sweat glands) may contain necrotic areas. The dermal infiltrate becomes dense, more or less diffuse; it can invade the hypodermis and may contain eosinophils.

The classification of rejection in VCA is scored using the Banff VCA classification, which includes the following grades (Fig. 2) [24]:

- Grade 0. *No rejection*. No inflammatory infiltrate (or minimal dermal infiltrate)
- Grade I. *Mild rejection*. Sparse dermal inflammatory infiltrate, epidermis intact
- Grade II. *Moderate rejection*. Moderate to dense perivascular dermal inflammatory infiltrate. Involvement

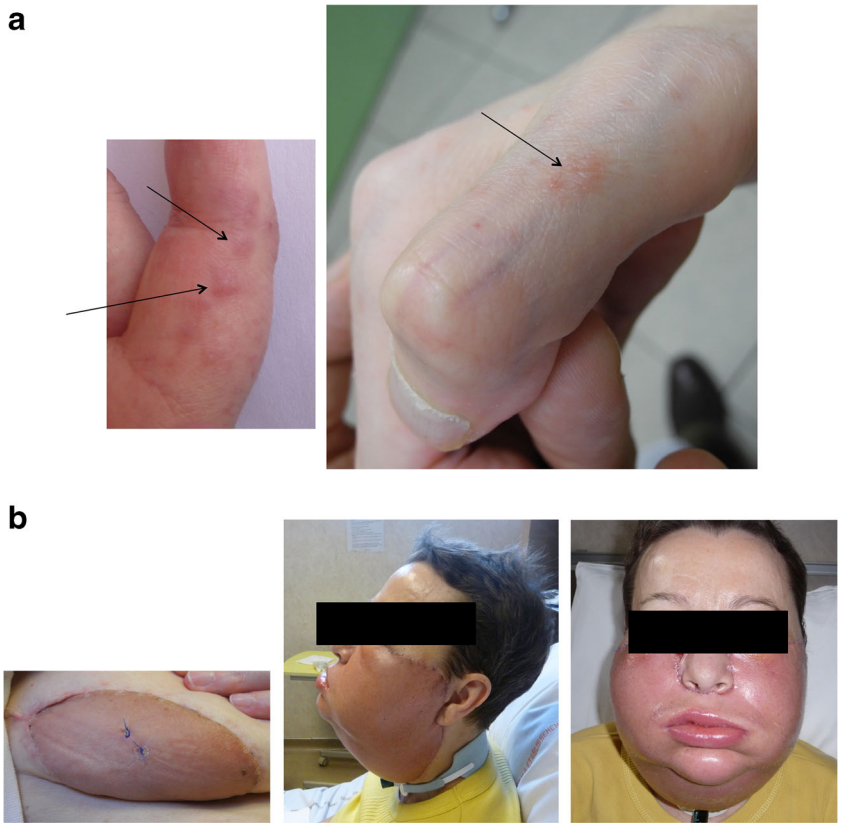

Fig. 1 Clinical aspect of acute rejection episodes aspect in vascularized composite tissue allotransplantation. **a** Skin acute rejection in bilateral hand transplantation: focal erythematous macules (*arrow*) developed in the donor skin. **b** Skin acute rejection in face transplantation. Acute facial graft rejection manifests with edema and intense erythema of the facial graft (*right panel*). Diffuse erythematous rash in the sentinel skin flap concomitant to face inflammation (*left panel*)

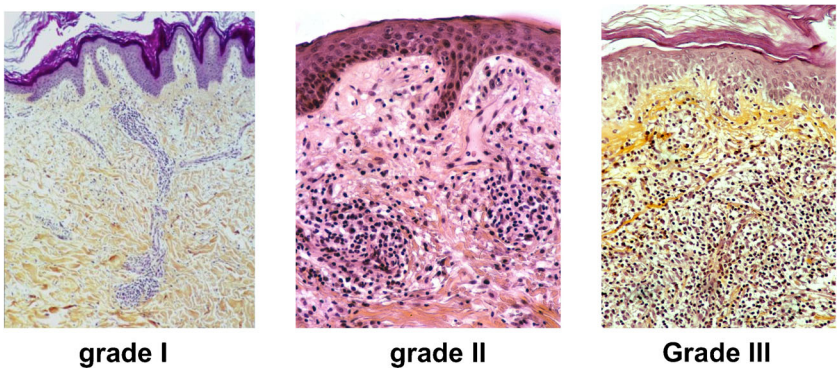

Fig. 2 Pathology of skin rejection in bilateral hand transplantation. Three grades of acute rejection episodes are displayed: The earliest lesions include a perivascular lymphocytic inflammatory infiltrate in the superficial and middle dermis (*grade 1*). When this infiltrate becomes denser, it can fill the dermis, come into contact with the epidermis and give rise to exocytosis (*grade 2*). In the case of severe rejection, the dermal infiltrate becomes dense, more or less diffuse (*grade 3*) and the epidermis (and its appendages, hair follicles and sweat glands) may contain necrotic areas

of the epidermis or appendages absent or mild (spongiosis or exocytosis), no necrosis or apoptosis of keratinocytes

- Grade III. *Severe rejection*. Dense, perivascular dermal inflammatory infiltrate, involvement of the epidermis or appendages with apoptosis, dyskeratosis or keratinocyte necrosis
- Grade IV. *Very severe/acute necrotising rejection*. Obvious necrosis of the epidermis or other skin structures

Acute facial graft rejection often manifests clinically with severe inflammation of the facial graft, manifesting with edema and intense erythema (Fig. 1b). In hand allotransplantation acute rejection episodes often manifest as focal erythematous macules. This difference could be due to the different venous and lymphatic vascularisation of the face. For facial grafts, we used a sentinel vascularised donor skin graft [6, 7], taken from underneath the breast or the abdomen. This was placed on the recipients' thorax or abdomen and enabled us to perform skin biopsies from these sentinel grafts, thus avoiding facial biopsies that could induce visible and potentially unsightly visual scars. For the three patients who underwent transplants by our group, the rejection episodes consistently involved concomitantly the sentinel and the facial grafts. Moreover, when the second patient presented with facial erythema due to HSV-1 infection, the sentinel graft skin remained normal. The sentinel graft can therefore be used for positive and differential diagnosis of acute rejection without the need for a facial skin biopsy.

In the case of facial allografts, oral mucosa biopsies displayed similar alterations, which were regularly more severe than those observed simultaneously on facial skin or the sentinel graft skin [25]. The reason for this difference is currently unclear. It could be due to the greater density in the mucosa of cells (dendritic and endothelial) involved in immune reactions, which raises the question of what tissue should be for a reliable diagnosis of rejection in the absence of obvious clinical signs.

Few histological studies in VCA have been performed on underlying tissues and have shown that the skin is the main target in acute rejection. The changes observed in other tissues (such as lymphocytic perivascular infiltration in the muscle, tendons and bones) are less severe compared to those observed in the skin [19].

One of the apparent paradoxes of VCA transplantation is the high incidence of acute skin rejection contrasting with the low incidence of chronic (skin) rejection. Skin fibrosis has not been observed in our cohort of hand- or facial-transplant patients undergoing immunosuppressive therapy [26••], despite a high incidence of acute rejection in some of them. Conversely, chronic skin rejection was observed in one patient with a facial graft, in whom immunosuppressive therapy had been considerably reduced because of severe immunosuppression-related complications [27]. This patient developed permanent T-lymphocyte-mediated allo-immune reaction, which triggered fibrous retraction of the facial graft over a period of 2 years, demonstrating for the first time the association between chronic rejection and the cellular component of the immune response in VCA [28].

It can therefore be hypothesised that the skin has the capacity to regenerate itself without fibrous scarring when acute rejection is controlled by immunosuppressive therapy. However, if adequate immunosuppression cannot be maintained, the cellular immune response may trigger sclerosis of the skin similar to that observed in cases of chronic graft-versus-host disease following haematopoietic stem cell transplantation. If this hypothesis is correct, VCA transplantation could be similar to liver transplantation in that tissue regeneration following immune aggression could prevent fibrous sequelae more

frequently following acute rejection. In the case of renal transplantation, however, acute rejection lesions may cause irreversible interstitial fibrosis.

The Role of Humoral Response in VCA Rejection

The role of the humoral response, which has been the subject of discussion over many years in the transplantation field, is becoming clearer in the context of VCA.

In patients without preformed anti-HLA antibodies, only very few B-lymphocytes are present in skin infiltrates [29, 30]. It is believed, therefore, that acute skin rejection in non-sensitised patients is more often mediated by T-lymphocytes. One team recently reported an antibody-mediated rejection episode in a patient who underwent a facial graft for burns. He presented with donor-specific antibodies (DSA) prior to transplantation related to allogeneic skin grafts used to treat his burns. The humoral rejection episode was controlled by intense treatment against the B-lymphocyte response [31•]. Another team reported a probable acute rejection episode involving the humoral arm of the immune response. This occurred late after hand transplantation and was linked to non-compliance [32]. Experimental limb transplant models in animals previously immunised against the major histocompatibility complex show that acute rejection is accelerated in animals with preformed anti-donor antibodies. However, contrary to the experience in kidney transplantation, acute rejection in these immunised animals did not result in the loss of the transplanted limb and was mediated by T-lymphocytes [33]. The low incidence of antibody-mediated acute rejections reported to date is probably due to the small number of transplanted patients and to the different sensitivity of the tissues to complement activation. VCA tissues could be less sensitive to complement than the kidney. Donor-specific antibodies may only be simple markers of a memory T-lymphocyte response promoted by B-lymphocytes functioning as antigen-presenting cells.

VCA and Chronic Rejection

The issue of long-term changes in VCA and, consequently, the clinical and histological presentation of chronic rejection have become increasingly important as procedures have developed over time. The 2007 Banff classification did not include chronic rejection because at that time it was established that no relevant data were available due to a rather short follow-up of patients with VCA. The involvement of epidermal appendages and nails, as well as mucosal, cutaneous and muscular atrophy and allograft vasculopathy, could be expected in VCA by analogy with solid organ transplantation [24]. Such lesions have been observed in VCA rat transplantation models in which a succession of acute rejection episodes was triggered by repeatedly stopping and resuming immunosuppression [34]. Allograft vasculopathy was a late finding in these rejection models.

Although the majority of compliant patients on triple immunosuppressive therapy did not develop chronic rejection lesions in the long term [26••], published data and our own experience have begun to highlight aspects indicative of chronic rejection in some patients.

As described previously, a patient who underwent facial transplantation and in whom the allo-immune cell reaction was not controlled developed chronic graft rejection manifesting clinically and pathologically as skin sclerosis. This patient also showed depigmentation of the graft skin without obvious clinical involvement of the appendages (for example, his beard continued to grow), although microscopically sweat glands became atrophic. Retraction of the facial graft reduced mouth opening resulting in a decrease in graft function [28].

Some hand-grafted patients have developed allograft vasculopathy [35••, 36]. The vascular lesions reported in these patients were similar to those observed in solid organ transplantation, with intimal proliferation of smooth muscle cells. The vascular lesions triggered arterial thrombosis causing, in some cases, allograft loss from 9 months up to 11 years after transplantation. However, allograft vasculopathy has not yet been described in patients undergoing facial transplantation.

Up to now, the mechanisms of allograft vasculopathy in VCA have not been fully elucidated. The majority of patients developing these lesions do not comply with treatment or have had an excessive reduction in immunosuppression because of minimisation protocols [35••]. Surprisingly, whereas the role of anti-HLA antibodies in the development of allograft vasculopathy (kidney, heart) has been clearly highlighted clinically and in experimental models [14], some patients who developed typical vasculopathy following hand allotransplantation did not have detectable circulating anti-HLA antibodies. Even if the latter are not present in all patients, their involvement in VCA remains probable. Anti-HLA antibodies could be present but not detectable in the circulation because they are bound to the graft. This is consistent with data published in renal transplantation [37] and a recent work from Louisville group, in which DSAs were detected 2 days after amputation of hand allograft [35••]. Alternative hypotheses are that vasculopathy lesions are due to non-HLA antibodies or that they are the consequence of cell-mediated damages (mediated by T-lymphocytes and/or innate immune effectors).

Interestingly, in our experience, the patient who experienced thrombosis associated with allograft vasculopathy did not present concomitantly major lesions indicative of skin rejection. This suggests that the absence of severe skin involvement does not rule out simultaneous ongoing rejection of vascular compartment. This finding suggests that specific vascular monitoring tools should be developed for VCA monitoring. In that perspective, high-frequency ultrasonography which allows measuring intimal media thickness in medium-size arteries such as radial and ulnar arteries might be interesting [35••].

VCA: a Vascularised Bone Marrow Allotransplantation Model

One of the major differences between VCAs and organ transplants is that VCA grafts contain bone marrow, which might be involved in inducing donor-specific central tolerance through the establishment of mixed chimerism. Murine models have indeed shown that lower limb transplants triggered thymo-dependent chimerism with tolerance of the transplanted limb [38–42]. Because transplanted limbs contain donor bone marrow and the stromal environment needed to induce chimerism, VCA recipients do not need prior conditioning in experimental model. Unfortunately, there is still no evidence to support extrapolation of these experimental murine model results in the clinical setting. Patients who underwent VCA are not spontaneously tolerant: the experience of the first hand-grafted patient [19], and of the Chinese patients in whom immunosuppression was stopped after the first year, shows that without immunosuppression VCAs are rejected within a few months. Moreover, donor chimerism has never been observed on the long term in the peripheral blood of patients who underwent hand, forearm or even arm transplantation, not even in patients who received infusion of donor haematopoietic stem cells during hand-transplant surgery [43]. In one of our face-grafted patients who received also the mandible combined with an infusion of haematopoietic stem cells, chimerism was transiently detected only in the $CD34^+$ T-cells of the recipient's bone marrow. Furthermore, this patient developed chronic rejection following drastic reduction in maintenance immunosuppression [28]. These results might be partially explained by the fact that, in adult patient, the bone in the transplanted VCA does not contain enough haematopoietic stem cells to trigger a sustainable combined chimerism. Alternatively, the donor bone marrow could have been rejected.

Immunosuppression and VCA

The initial fear of acute and irreversible skin rejection of VCA grafts has encouraged centres to initiate intense immunosuppression, using depleting antibodies in most cases, or IL2 anti-receptor antibodies, combined with maintenance immunosuppression comprising a combination of tacrolimus, mycophenolate mofetil and low-dose corticosteroids [1]. This immunosuppressive therapy is generally well tolerated because the patients are usually young with no concomitant diseases. The same side effects as in organ transplantation are nevertheless encountered, in particular an increased risk of infection and malignancies [3•, 27]. Given the very small patient cohorts, it is impossible to perform randomised studies, and our ability to rationally optimise patient immunosuppression is therefore limited. Immunosuppression is based on experience obtained with organ transplantation, the experimental studies and the performed successful clinical cases. Our team chose to use rabbit antithymocyte globulin (Thymoglobulin) combined with a triple maintenance immunosuppression based on calcineurin inhibitor drugs. However, given the lack of clinical experience in terms of patient follow-up, it is impossible to formulate any long-term predictions about this strategy or to establish the incidence of chronic rejection. The initial immunosuppression is modulated according to the incidence of rejection, signs of over-immunosuppression and side effects, particularly the development of chronic kidney failure which could lead to replacement of the calcineurin inhibitor by a mammalian target of rapamycin (mTOR) inhibitor [7]. mTOR inhibitor can be added to the treatment regimen by reducing the dose of tacrolimus so as to prevent acute skin rejection in patients who developed repeated episodes of acute rejection under triple therapy. The Boston team discontinued corticosteroids after facial allotransplantation following the introduction of Thymoglobulin in conjunction with tacrolimus and mycophenolate mofetil, whereas the Pittsburgh team chose to use alemtuzumab induction combined with infusion of haematopoietic stem cells and tacrolimus maintenance monotherapy [43]. Given the lack of comparative data, it is impossible to establish the optimum benefit-risk ratio for these new immunosuppressive regimens.

Acute skin rejection can be treated with topical immunosuppressants, corticosteroids or tacrolimus, but usually warrants high doses of intravenous corticosteroids. Rejection therapy with depleting antibodies, Thymoglobulin or alemtuzumab has been necessary for a small number of patients when rejection has proven refractory to high doses of corticosteroids. The earlier the treatment is introduced, the more effective it is [1].

Conclusions

VCAs are a new therapeutic option for patients with severe physical (in case of limb) or social (after disfigurement) disabilities. An immunosuppressive treatment, as in solid organ transplantation, is required to ensure graft survival, which despite its bone marrow content and promising experimental data from murine models, is not spontaneously tolerated in patients. Similar to organ transplantation, the current strategy for patients receiving a VCA remains based on (i) careful evaluation of the benefit-risk ratio of the procedure, (ii) optimisation of immunosuppression and (iii) close follow-up by multidisciplinary teams to detect and treat early acute rejection episodes, but also the potential life-threatening complications due to immunosuppression.

Acknowledgments The authors thank to the surgical teams from CHU Amiens (University Hospital Centre) and especially Professors Bernard Devauchelle and Sylvie Testelin who performed the face allotransplantations and the hand surgery teams from the Clinique du Parc in Lyon, particularly Dr. Aram Gazarian. These complex grafts could not have been carried out without their involvement. We are also grateful to all members of the staff involved in the follow-up of these patients including the medical team involved in patients' follow-up and the Roman Ferrari team involved in their functional rehabilitation. Last, but not least, we wish to thank Céline Dagot for organising the follow-up procedure and managing the clinical data.

Compliance with Ethics Guidelines

Conflict of Interest Emmanuel Morelon, Jean Kanitakis, Palmina Petruzzo, Lionel Badet and Olivier Thaunat declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Petruzzo P, Lanzetta M, Dubernard JM, et al. The international registry on hand and composite tissue transplantation. Transplantation. 2010;90(12):1590–4.
2. Gazarian A, Abrahamyan DO, Petruzzo P, et al. Hand allografts: experience from Lyon team. Ann Chir Plast Esthet. 2007;52(5):424–35.
3.• Petruzzo P, Gazarian A, Kanitakis J, et al. Outcomes after bilateral hand allotransplantation: a risk/benefit ratio analysis. Ann Surg. 2015;26(1):213–20. **This monocentric retrospective study provides functional outcomes and complications of immunosuppressive therapy in six bilateral hand transplant patients**.
4.•• Giraux P, Sirigu A, Schneider F, Dubernard JM. Cortical reorganization in motor cortex after graft of both hands. Nat Neurosci. 2001;4(7):691–2. **This pioneer study demonstrated for the first time the cortical reorganisation in motor cortex in bilateral hand transplantation, a mandatory step for functional recovery after the graft**.
5. Vargas CD, Aballéa A, Rodrigues EC, et al. Re-emergence of hand-muscle representations in human motor cortex after hand allograft. Proc Natl Acad Sci U S A. 2009;106(17):7197–202.
6. Devauchelle B, Badet L, Lengelé B, et al. First human face allograft: early report. Lancet. 2006;368(9531):203–9.
7. Dubernard JM, Lengelé B, Morelon E, et al. Outcomes 18 months after the first human partial face transplantation. N Engl J Med. 2007;357(24):2451–60.
8. Lantieri L, Meningaud JP, Grimbert P, et al. Repair of the lower and middle parts of the face by composite tissue allotransplantation in a patient with massive plexiform neurofibroma: a 1-year follow-up study. Lancet. 2008;372(9639):639–45.
9. Siemionow M, Papay F, Alam D, et al. Near-total human face transplantation for a severely disfigured patient in the USA. Lancet. 2009;374:203.
10. Pomahac B, Pribaz J, Eriksson E, et al. Three patients with full facial transplantation. N Engl J Med. 2012;366(8):715–22.
11. Jensen SE, Butt Z, Bill A, et al. Quality of life consideration in upper limb transplantation: review and future directions. J Hand Surg [Am]. 2012;37(10):2126–35.
12. Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. Nat Rev Nephrol. 2012;8(6):348–57.
13. Valenzuela NM, McNamara JT, Reed EF. Antibody-mediated graft injury: complement-dependent and complement-independent mechanisms. Curr Opin Organ Transplant. 2014;19(1):33–40.
14. Thaunat O. Humoral immunity in chronic allograft rejection: puzzle pieces come together. Transpl Immunol. 2012;26(2-3):101–6.
15. Schneeberger S, Landin L, Jableki J, ESOT CTA Working Group, et al. Achievements and challenges in composite tissue allotransplantation. Transpl Int. 2011;24(8):760–9.
16. Murray JE. Organ transplantation (skin, kidney, heart) and the plastic surgeon. Plast Reconstr Surg. 1971;47(5):425–31.
17. Lee WP, Yaremchuk MJ, Pan YC, Randolph MA, Tan CM, Weiland AJ. Relative antigenicity of components of a vascularized limb allograft. Plast Reconstr Surg. 1991;87(3):401–11.
18. Thaunat O, Badet L, El-Jaafari A, Kanitakis J, Dubernard JM, Morelon E. Composite tissue allograft extends a helping hand to transplant immunologists. Am J Transplant. 2006;6(10):2238–42.
19. Kanitakis J, Jullien D, Petruzzo P, et al. Clinicopathologic features of graft rejection of the first human hand allograft. Transplantation. 2003;76(4):688–93.
20. Kanitakis J, Petruzzo P, Jullien D, et al. Pathological score for the evaluation of allograft rejection in human hand (composite tissue) allotransplantation. Eur J Dermatol. 2005;15(4):235–8.
21. Kanitakis J. The challenge of dermatopathological diagnosis of composite tissue allograft rejection: a review. J Cutan Pathol. 2008;35(8):738–44.
22. Hautz T, Zelger B, Grahammer J, et al. Molecular markers and targeted therapy of skin rejection in composite tissue allotransplantation. Am J Transplant. 2010;10(5):1200–9.
23. Hautz T, Zelger BG, Weissenbacher A, et al. Standardizing skin biopsy sampling to assess rejection in vascularized composite allotransplantation. Clin Transplant. 2013;27(2):E81–90.
24. Cendales L, Kanitakis J, Schneeberger S, et al. The Banff 2007 working classification of skin-containing composite tissue allograft pathology. Am J Transplant. 2008;8(7):1396–400.
25. Kanitakis J, Badet L, Petruzzo P, et al. Clinicopathologic monitoring of the skin and oral mucosa of the first human face allograft: report on the first eight months. Transplantation. 2006;82(12):1610–5.
26.•• Petruzzo P, Kanitakis J, Badet L, et al. Long-term follow-up in composite tissue allotransplantation: in-depth study of five (hand and face) recipients. Am J Transplant. 2011;11(4):808–16. **This retrospective analysis suggests that the incidence of chronic rejection is low in hand and face recipients compliant with triple immunosuppressive therapy**.
27. Conrad A, Brunet AS, Hervieu V, et al. Epstein-Barr virus-associated smooth muscle tumors in a composite tissue allograft and a pediatric liver transplant recipient. Transpl Infect Dis. 2013;15(5):E182–6.
28. Petruzzo P, Kanitakis J, Testelin S, et al. Clinical and histological findings of chronic rejection in a patient with face allotransplantation. Transplantation. 2015 [In press].
29. Kanitakis J, McGregor B, Badet L, et al. Absence of C4d deposition in human composite tissue (hands and face) allograft biopsies: an immunoperoxidase study. Transplantation. 2007;84(2):265–7.
30. Landin L, Cavadas PC, Ibañez J, Roger I, Vera-Sempere F. CD3+-mediated rejection and C4d deposition in two composite tissue (bilateral hand) allograft recipients after induction with alemtuzumab. Transplantation. 2009;87(5):776–81.

31.• Chandraker A, Arscott R, Murphy GF, et al. The management of antibody-mediated rejection in the first presensitized recipient of a full-face allotransplant. Am J Transplant. 2014;14(6):1446–52. **This is the first reported case of acute humoral rejection in face transplant patient who received face transplantation despite preformed DSA**.

32. Weissenbacher A, Hautz T, Zelger B, et al. Antibody-mediated rejection in hand transplantation. Transplant Int. 2014;27(2):e13–7.

33. Wu S, Xu H, Chen B, et al. Sensitized recipients exhibit accelerated but not hyperacute rejection of vascularized composite tissue allografts. Transplantation. 2011;92(6):627–33.

34. Unadkat JV, Schneeberger S, Horibe EH, et al. Composite tissue vasculopathy and degeneration following multiple episodes of acute rejection in reconstructive transplantation. Am J Transplant. 2010;10(2):251–61.

35.•• Kaufman CL, Ouseph R, Blair B, et al. Graft vasculopathy in clinical hand transplantation. Am J Transplant. 2012;12(4):1004–16. **This paper reports for the first time transplant vasculopathy in the VCA setting**.

36. Kanitakis J, Karayannopoulou G, Lanzetta M, Petruzzo P. Graft vasculopathy in the skin of a human hand allograft: implications for diagnosis of rejection of vascularized composite allografts. Transplant Int. 2014;27(11):e118–23.

37. Bachelet T, Couzi L, Lepreux S, Legeret M,,Pariscoat G, Guidicelli G, Anies G, Merville P, Taupin JL. Kidney intragraft DSA detection implements the definition of antibody-mediated lesions and is associated with a poor prognosis of the graft. Am J Transplant. 2013.

38. Siemionow MZ, Izycki DM, Zielinski M. Donor-specific tolerance in fully major histocompatibility major histocompatibility complex-mismatched limb allograft transplants under an anti-alphabeta T-cell receptor monoclonal antibody and cyclosporine A protocol. Transplantation. 2003;76(12):1662–8.

39. Siemionow M, Izycki D, Ozer K, Ozmen S, Klimczak A. Role of thymus in operational tolerance induction in limb allograft transplant model. Transplantation. 2006;81(11):1568–76.

40. Siemionow M, Klimczak A, Unal S, Agaoglu G, Carnevale K. Hematopoietic stem cell engraftment and seeding permits multilymphoid chimerism in vascularized bone marrow transplants. Am J Transplant. 2008;8(6):1163–76.

41. Rahhal DN, Xu H, Huang WC, et al. Dissociation between peripheral blood chimerism and tolerance to hindlimb composite tissue transplants: preferential localization of chimerism in donor bone. Transplantation. 2009;88(6):773–81.

42. Huang WC, Lin JY, Wallace CG, Chuang WY, Wei FC, Liao SK. Vascularized bone grafts within composite tissue allotransplants can autocreate tolerance through mixed chimerism with partial myeloablative conditioning: an experimental study in rats. Plast Reconstr Surg. 2010;125(4):1095–103.

43. Schneeberger S, Gorantla VS, Brandacher G, et al. Upper-extremity transplantation using a cell-based protocol to minimize immunosuppression. Ann Surg. 2013;257(2):345–51.