Hand Transplantation

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Abstract

Composite tissue allotransplantation originated during World War II, when skin allografts were first used to provide a soft-tissue coverage for severely burned patients. Composite tissue, and in particular skin allotransplantation, was limited by the stringent immune response towards the skin and reconstructive transplantation only became a clinical reality after more powerful immunosuppressants were developed in the 1980s and 1990s and after large animal trials indicated that graft survival was plausible under use of high-dose multidrug immunosuppression. Reconstructive transplantation has provided the successful restoration of partial and complete faces, hands, forearms, arms, abdominal walls, larynx, diaphyseal bone, bone-joint complexes, flexor tendon constructs, and, in one case, a penis. The surgical procedures are time-intensive and technically demanding and postoperative care and monitoring require a high degree of compliance. Hand transplantation has been the most frequently performed human composite tissue allotransplantation, with more than 50 upper extremity based transplants done worldwide. The functional and cosmetic outcome as well as patient satisfaction has been convincing in most cases, but skin rejection occurs in the majority of cases and requires adequate topical and systemic treatment. The intense immunosuppressive therapy usage in reconstructive transplantation has also resulted in loss of one patient after a combined bilateral hand and face transplantation as well as a high morbidity with metabolic side effects, infections, and malignancies.

Recent advances in transplant immunology are shifting the focus from immunosuppression towards immunoregulation, making reconstructive transplantation with novel and less toxic immunosuppressive regimens a possibility. While early results are promising, further experimental and clinical trials are needed for better definition of suitable immunomodulatory protocols in reconstructive transplantation, and careful oversight and individualized screening procedures will be required as patients seeking improved quality of life through human composite tissue allotransplantation come to accept a certain level of risk in these experimental procedures. In summary, reconstructive transplantation offers to advance transplant medicine and reconstructive surgery, but more patients and science are needed before complex tissue defects can routinely be treated with composite tissue allografts.

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Introduction

Surgical reconstruction of complex tissue defects, e.g. after surgical excision of tumors, accidents, or to treat congenital malformation, is in high demand. While small tissue defects can often be treated by transfer of autologous tissue, large and complex tissue defects leave the patient with significant deficits in function, body integrity, and appearance. Reconstructive transplantation (RT) using allogeneic tissue from a brain-dead organ donor represents a valid solution and has been performed in over 100 patients worldwide with success[1-9] (www.a-s-r-t.com).

The first attempt to transplant a human hand was carried out in Ecuador in 1964[10]. After rejection and re-amputation of the hand, the second attempt was only carried out in 1998 in Lyon, France[11]. A series of small and large animal studies indicated the efficacy of modern immunosuppression in limb transplantation and led us to believe that hand loss could now be prevented[12-14]. And this turned out to be the case. Over 60 hand transplants have since followed and one-year graft survival has exceeded 90%. The satisfactory functional outcome also encouraged surgeons to proceed with forearm, arm, and face transplants.

Despite good return of function and high patient satisfaction, episodes of acute skin rejection were observed in most patients and some hands were lost when immunosuppression was withdrawn or reduced in an uncontrolled fashion upon non-compliance or lack of funding[14-19]. Monoclonal or polyclonal antibodies were used for induction, and tacrolimus, prednisone, and mycophenolic acid comprised maintenance immunosuppression in the majority of patients[14-19] (www.handregistry.com). Side effects of immunosuppression included viral, bacterial and fungal infections, diabetes mellitus, nephrotoxicity, osteonecrosis, leucopenia, hypertension, posttransplant lymphoproliferative disease, and hyperlipidemia[1-7,20] and the need for long-term immunosuppression limited its wider application in hand transplantation. We herein summarize the achievements in human hand transplantation and highlight interesting findings in basic science.

Hand transplantation – Clinical aspects

Immunosuppression in reconstructive transplantation

Vascularized composite tissue allografts with a skin component are considered an immunological challenge as the skin is highly immunogenic[21]. Immunosuppressive regimens used in RT are in conformity with protocols used in solid organ transplantation. Most hand and face transplant patients received either polyclonal (anti-thymocyte globulins, ATG) or monoclonal (alemtuzumab, basiliximab) antibody infusions for induction followed by tacrolimus, mycophenolate mofetil (MMF) and steroids triple-drug combination for maintenance[20]. Further, steroid-sparing protocols, mammalian target of rapamycin (mTOR) inhibitors, and use of topical steroids and tacrolimus ointments have been applied with success. The treatment used is efficient in preventing graft loss, but was not sufficient to prevent acute skin rejection the majority of cases.

Donor bone marrow cell infusions have been added to the induction regimens in hand

transplantation in one center. The early outcome suggests that bone marrow infusion is safe and enhances the chance of reducing maintenance immunosuppression[22-24]. Data also indicate that implementation of cell-based therapies could help to induce immunomodulation subsequent to composite tissue allotransplantation (CTA) and thus further optimize the outcomes.

Reconstructive transplantation rejection

Intrinsic differences make RT rejection both interesting and challenging. Per definition of composite tissue allografts, they comprise a set of different tissues conjoint to build a functional, but not immunological, unit. Experimental work has indicated that the skin might be the immunologically most challenging tissue and it has long been believed that human skin allotransplantation cannot be performed successfully under conventional immunosuppression. Early work investigating the immune response to RT has revealed an interesting phenomenon: the sum of tissues comprising a RT induces a weaker immune response that the individual tissues transplanted separately[25]. The mechanisms responsible for this, however, have not been adequately analyzed and it hence remains speculative if the quantity or the quality of tissue has a prominent role in RT. A similar phenomenon has been described in combined transplantation of solid organs.

Rana, et al. recently found that combined simultaneous transplants of heart, liver, and kidneys protect and are themselves protected from rejection. Also, this analysis revealed that a higher antigen load, such as in double-lung or double-kidney transplantation, also reduces the risk of rejection[26].

Rejection of the individual tissues

External location and ease of sampling allow for close monitoring of skin rejection in RT. Skin rejection can present either in a focal or diffuse pattern of maculopapular lesions of diverse intensity[3,5-8,18]. In atypical cases of rejection, specially reddening of the palm and changes of the nails have been described[27] Histopathological and immune cellular characteristics of skin rejection in RT have been well characterized and a uniform Banff grading system has been developed[3,28,29]. Grading of rejection also reflect interesting dynamics of cellular trafficking. Rejection first appears in the perivascular space of the dermis. If rejection progresses, the infiltrate spreads to the interphase between dermis and epidermis and/or adnexal structures[3,28]. A cellular infiltrate within the epidermis is typical for moderate rejection, with the immunologic response reaching the outermost layer. When progressing further, necrosis of single keratinocytes can be observed, resulting in focal dermal-epidermal separation and finally necrosis and loss of the epidermis[3,28].

Immunohistochemical markers for cellular infiltration and adhesion molecule expression in human hand transplant skin rejection were analyzed in a set of 174 skin biopsies from five human hand transplant recipients[29]. Acute rejection was characterized by a predominantly $CD3^+$ cell infiltrate that began in the perivascular areas and spread from the dermis to the epidermis as severity of rejection increased.

Among the $CD3^+$ cells, $CD8^+$ cells are more prominent than $CD4^+$ cells. Ten to 50% of cells stain positive for CD68 (histiocytic/macrophage linage). Very few cells are positive for CD20.

Antibody mediated rejection

Analysis of C4d depositions has been used as the prime tool in the search for antibody mediated rejection (AMR) in RT. Complement staining, however, is an unspecific and indirect diagnostic marker for AMR and it remains to be proven if absence or insignificance of C4d staining indicates absence of antibody mediated processes in RT.

In a recent systematic analysis of AMR in a rat limb transplant model, an increase in antibody against third party following multiple rejections was observed, but no clear evidence of an antibody mediated alloresponse was found[30]. This is in agreement with most case reports, where mild and unspecific C4d staining at the vascular endothelium was found in some biopsy specimens in presence and absence of cellular rejection. The significance remains unclear, this but did not correlate with rejection in any of the patients investigated[6-8].

Deposits of C4d were found in capillaries not only accompanying cellular rejection, but also in the absence of clinical rejection.

The adhesion molecules most significantly associated with severity of rejection were intercellular adhesion molecule (ICAM)-1 and E-selectin. An E- and P-selectin inhibitor, efomycine M, administered in the subcutaneous tissues of the graft, facilitated allograft acceptance in a rat RT model when combined with ATG and tacrolimus[29]. These studies demonstrate the feasibility of investigating early markers of rejection and testing their functional significance in rodent RT models.

Cell adhesion and trafficking, however, only represents one of many mechanistic elements of skin rejection. After invasion of alloreactive cells, a complex cascade of cell communication and interaction can be monitored during rejection. Similar to solid organ rejection, some of these markers might be interesting targets for intervention (manuscript in preparation).

Effect of allograft mass

Little is known about the relevance of tissue mass on the alloimmune response. In a recent experimental trial, Ususal, et al. demonstrated an advantageous effect of a larger vascularized skin tissue mass on velocity and severity of rejection[31]. The effect, however, was much less dramatic when compared with the effect of transplanting tissues as a composite. This phenomenon was first described in a landmark article by Lee, et al. in 1991[25]. In an attempt to investigate the relative antigenicity of tissues, different timing and intensity of the rejection of individual tissues was observed. More importantly, the whole-limb allograft elicited less immune response than did the individual components. This evidence, however, derived from systematic analysis in animal models in which no or little immunosuppression was used. When RT in humans was initiated under conventional immunosuppressive regimens, early observations in individual cases seemed to confirm the theory of a diverse immune response to the individual tissue components. Individual case reports and cumulative evidence from the International Registry on Hand and Composite Tissue Transplantation indicate that the vast majority of patients experience one (> 85%) or multiple (56%) episodes of skin rejection. Tissues other than the skin remained unaffected or were affected to a lesser extent[2-8,20,32]. Probably the best individual example was obtained from the first hand transplant of this series, where the patient requested re-amputation of the transplant after progressive skin rejection[32]. The systematic histopathological assessment of the hand revealed erosive and necrotic areas over the skin, but only mild inflammation in muscles and tendons and no changes in bones and joints. Additional evidence from deep-tissue biopsies performed occasionally confirmed a dominance of the immune response towards the skin, but also revealed cellular infiltrates in muscle and connective tissue. The predominant role of the skin in RT rejection was also observed in large animal trials in which survival of all tissues except the skin was achieved after immunomodulation with donor cells. In summary, there is good evidence that a composite of vascularized tissue induces less immune response than the individual components, convincing data supporting the concept that the skin elicits the strongest immune response, but only weak

evidence that tissue mass has a major impact on allograft rejection. Furthermore, little is known about the difference in the mechanisms of rejection in tissues other than the skin. In particular, it has not been analyzed if dynamics and trafficking patterns, such as those described for the skin, exist in other tissues.

Chronic rejection

While chronic rejection – or composite tissue vasculopathy and degeneration – has not been an eminent threat in human hand or face transplantation, proliferation and/or degenerative processes after RT remain a concern. A high rate of acute rejection episodes as frequently seen in RT was speculated to be myointimal proliferation at the graft vessels, but little evidence has risen from the case reports published. It remains unclear at this point if (i) skin rejection correlates with vasculitis and endothelial cell damage, (ii) if the vessels of a hand or face are equally susceptible to vascular changes as e.g. coronary arteries, and (iii) if mechanisms other than an alloimmune response may contribute to myointimal proliferation.

Recently, a transplanted hand was lost at nine months after transplantation in Louisville as a result of myointimal proliferation in allograft vessels and subsequent malperfusion and necrosis of the transplanted hand[33]. So far, this is the only report on chronic rejection in RT; however, long-term follow-up and evaluation for vascular endothelial proliferation and myointimal thickening as a risk factor for graft failure and graft loss are still needed.

Transplant vasculopathy has not been systematically investigated in a rat RT model[30]. After multiple treated episodes of acute rejection, significant vascular lesions along with skin and muscle atrophy, upregulation of profibrotic gene expression and fibrosis was observed. The bone became sclerotic, weak and prone to malunion and nonunion. While muscle atrophy with macrophage infiltration was seen early after transplantation, vasculopathy was only observed later.

Basic science in reconstructive transplantation

As outlined above, over the past decade RT such as hand and face transplantation has become a clinical reality and a clear treatment option for those patients in need, suffering from complex tissue injuries or defects not amenable to conventional reconstruction. Despite the fact that early and intermediate functional outcomes are highly encouraging, rejection and the need for high-dose multidrug immunosuppressive treatment continues to be the bane of vascularized composite allotransplantation (VCA), preventing wider clinical application. Thus, any reconstructive measures to improve these non-life-threatening conditions must address a delicate balance of risks and benefits.

Therefore, research in this novel field needs to focus on both basic and translational studies related to the unique immunological features of VCA, to investigate the basic mechanisms of an alloimmune response in VCA as well as to develop novel strategies and protocols for immunomodulation and tolerance induction after VCA without the need for long-term immunosuppression. Another major scientific emphasis currently is centered around the question of how to establish new treatment options to improve and enhance nerve regeneration and hence functional outcome in VCA.

Animal models

To date, preclinical experimental animal models have substantially propelled VCA research and paved the way to study basic immunology, functional recovery, and technical feasibility of RT procedures. Orthotopic hindlimb transplantation in the rat has been the

preferred rodent model ever since; however, novel super-microsurgical techniques[34,35] facilitated VCA in even smaller species like the mouse[36]. In addition, different swine[37] and primate[38] models have been applied to study acute and chronic rejection as well as tolerance induction on the basis of different immunosuppressive protocols.

In 1984, cyclosporine (CsA) monotherapy was successfully used to prevent hind-limb allograft rejection between major histocompatibility complex (MHC)-mismatched Buffalo and Lewis rats[39] and several years later the combination of both CsA and MMF was shown to be synergistically effective[40]. Since orthotopic limb transplantation is a rather traumatic operative procedure, requiring the removal of the limb at the recipient site to gain room for an allograft, non-functional heterotopic hind-limb transplant procedures have been developed to overcome the surgical and technical difficulties of orthotopic transplantation[41]. Accordingly, these models were mainly used to investigate tolerance-inducing immunomodulatory protocols in RT[42]. In this regard, it has been shown that the administration of CTLA4-Ig or CD40-Ig, two co-stimulatory blocking agents, significantly reduced rejection episodes, and the combination of both agents led to an additional prolongation of allograft survival when compared to their sole application[43].

Recently, it has been brought up that the induction of donor-specific chimerism is capable of inducing alloantigen-specific immunological tolerance in VCA[44]. In this context, it has been demonstrated that chimerism levels of more than 60% are required to induce and maintain tolerance in irradiated and T-cell depleted hind-limb recipients[45]. Further investigations provided evidence that the triple combination of antilymphocyte globulin (ALG), tacrolimus (FK506) and CD-28 co-stimulatory blockade on top of a 300 cGy total body irradiation before bone marrow transplantation (100 x 10^6 T-cell depleted bone marrow cells) established stable chimerism and induced tolerance to vascularized composite allografts[46]. In addition, Prabhune, et al. showed that transplantation of donor bone marrow cells into conditioned hosts immediately after hind-limb transplantation combined with tacrolimus and MMF resulted in stable mixed chimerism and limb allograft tolerance[47].

Posited by the striking homology between the mouse H2 and the human HLA system, the widespread availability of genetically defined inbred and knockout strains and the applicability of specific monoclonal antibodies, murine orthotopic and heterotopic transplant models have been developed only recently[48]. Similar to the rat models, orthotopic murine hind-limb transplant models seem to be best suited to study functional outcome and nerve regeneration in VCA, whereas the technically less demanding heterotopic cervical model may be used to investigate basic immunology and clinically relevant questions related to acute and chronic rejection as well as ischemia and reperfusion injury in RT[36].

Apart from small animals, preclinical swine and primate models of VCA have been introduced to the RT world in the recent past. Ren, et al. developed an osteomyocutaneous forearm flap model in swine for the evaluation of effective immunomodulatory protocols[37]. The induction of indefinite graft survival to VCA with hematopoietic stem-cell infusion was furthermore tested in an MHC-disparate miniature swine model. The authors concluded that tolerance to the myocutaneous elements of the graft is possible across an MHC barrier and that stable chimerism may not be necessary for functional tolerance[49].

In 2005, Cendales, et al. reported a preclinical VCA model in nonhuman primates using a sensate osteomyocutaneous radial forearm flap[38]. One major limitation to the use of nonhuman primates is the difficulty in achieving therapeutic levels of immunosuppression

without serious toxicity[50]. Accordingly, subtherapeutic immunotherapy caused alloantibody development and delayed graft rejection with a marked dermal lymphocytic infiltrate, comparable to those observed in human hand transplant recipients.

The technical aspects of VCA are no longer the factors limiting the widespread application of this treatment modality in the clinical setting. Animal models of VCA are in crucial demand to provide immunologists a playground that facilitates preclinical testing of a variety of immunosuppressive protocols, aiming to minimize long-term immunosuppression or even create donor-specific tolerance to ultimately eliminate toxic side effects of high-dose immunosuppressants.

Targeted therapy in reconstructive transplantation

Experience gained from experimental studies as well as clinical observations revealed that the skin component of a vascularized composite allograft is most prone to rejection[1-9]. It was shown that first histopathologic signs of rejection include a perivascular inflammatory infiltrate in the dermis, which then starts to traffic towards the epidermis[3,28,32]. This leads to dermal-epidermal separation and necrosis of the epidermis in more advanced stages of rejection. To better understand the mechanism of skin rejection, skin biopsies of human hand allografts were intensively investigated by immunohistochemistry[29]. A set of adhesion molecules was identified to be upregulated upon skin rejection, including adhesion molecules LFA-1, ICAM-1, E-selectin and P-selectin. Targeting molecular markers involved in leukocyte trafficking holds great potential for treatment of skin rejection and might help to minimize systemic immunosuppression.

In a series of experimental studies using a rat hind-limb transplant model, adhesion molecule blockers were administered subcutaneously (s.c.) into the allograft to prevent rejection. For systemic immunosuppression, low-dose tacrolimus (0.30 mg/kg/day) for 50 days and induction with antilymphocyte serum on days 0 and 3 were given.

Local administration of efomycine-M

One of the very early steps upon an inflammatory response is mediated by the interaction of selectins with their ligands on immune cells[14]. Targeting E- and P-selectin showed an effect in preclinical models of psoriasis as well as in human trials[15]. Efomycine-M, a small molecule inhibitor of E- and P-selectin, was tested for its efficiency on skin rejection in the rat transplant model. Animals received efomycine-M s.c. into the graft once a week in combination with systemic immunosuppression (mentioned above). After weaning tacrolimus on day 50, animals rejected within 10 days. Additional treatment with local efomycine-M resulted in long-term (150 days) allograft survival in five out of six animals. Histology on day 150 showed a mild lymphocytic infiltrate in the dermis and only single vacuolated keratinocytes in the epidermis. Local efomycine-M alone resulted in rejection by day 9, which was similar to untreated animals.

Local administration of anti-ICAM-1 and anti-LFA-1

The integrin LFA-1 and the Ig-superfamily-molecule ICAM-1 on endothelial cells are important for tight adhesion of the leukocytes to enable infiltration into the dermis[51]. Application of efalizumab, a humanized LFA-1 blocking antibody, was sufficient in the treatment of inflammatory skin diseases[52,53]. Blocking the interaction between LFA-1 and ICAM-1 was addressed in the experimental rat hind-limb transplant model to assess its effect on allograft survival. Anti-LFA-1 and anti-ICAM-1

were injected s.c. once a week together with a short-term course of systemic immunosuppression (see above). Graft survival was significantly prolonged in animals receiving local migratory inhibitors when compared to controls. In three out of four animals, long-term graft survival was achieved (unpublished data).

Local administration of Bβ15-42

In another attempt to address local inhibition of adhesion molecules, the fibrin derivative Bβ15-42, which blocks vascular endothelial cadherin, revealed a statistically significant prolongation of hind-limb allograft survival in the rat. Specifically, subtherapeutic doses of tacrolimus (0.1 mg/kg) in conjunction with weekly s.c. injections of Bβ15-42 lead to prolongation of limb survival from seven days (controls) to 17 days. When local treatment with Bβ15-42 was then combined with an induction with IL-2 Fc and a short course of CsA, long-term allograft survival with significant reduction of CD4 and $CD8^+$ T-cells was achieved (manuscript in preparation).

These data indicate the potential of leukocyte migration blockers to prevent skin rejection in a rat VCA model.

Tolerance protocols including cell therapy

Donor antigen-specific immunological tolerance without the need for long-term maintenance immunosuppressive therapy still remains the ultimate attainable clinical goal in transplantation. In fact, such tolerance-inducing strategies might be particularly appealing in RT due to some unique biological and immunological features of this particular type of transplant. In particular, for the first time in the history of transplantation, we are able to continuously monitor an allograft by simple visual inspection of the skin component of a VCA, which is considered to be the main target of rejection. This allows for directed biopsies and unbiased pathological confirmation of the earliest stages of acute rejection and subsequent timely intervention, treatment, and precise adjustments of immunosuppression on an individualized basis. Furthermore, when treated adequately and effectively, acute rejection does not seem to impair graft function or long-term survival. Therefore, novel strategies to minimize/avoid immunosuppression or to induce immune tolerance are potentially most likely to be realized in RT. Studies from our own group demonstrated that a whole limb allograft elicited a less intense alloimmune response than did allografts of each of its individual components, thereby challenging the relative scale of tissue antigenicity[25]. In addition, VCA contains immunocompetent elements such as bone marrow and lymph nodes that may hasten the rejection processes or result in graft versus host disease (GvHD). These factors not only govern the immune reactivity of these allogeneic tissues, but also define potential immunomodulating strategies that are different from those currently used in solid organ transplantation[54,55].

When considering development of novel therapeutic concepts for tolerance inductions following reconstructive transplantation, cell-based protocols including donor bone marrow or stem cells are promising candidates due to the unique nature of VCA. This trend is further fueled by recent innovative advancements in solid organ transplantation, where both cell-based therapies and non-cell-based protocols have resulted in reduction or elimination of long-term immunosuppression[56-59]. Some type of VCA, in particular limb transplants or Le-Fort III based facial tissue transplants, include bone marrow and might thereby function as a vascularized bone marrow transplant by itself[60,61]. Such a graft represents a continuous source of donor antigen and stem cells, which have been demonstrated in experimental

models to favorably modulate the host immune response[61]. In these models, induction of donor-specific tolerance was attributed to this bone marrow component and to specific immunomodulatory protocols permissive for bone marrow engraftment[62]. Bone marrow is furthermore critical to establish macro-, micro- or mixed chimerism following organ transplantation, which is known as a prerequisite for donor-antigen-specific tolerance induction[63,64]. Bone marrow or hematopoietic stem cells have also been identified to possess inherent tolerogenic properties and have become the backbone of a number of protocols aiming for tolerance induction in transplantation[65,66].

Bone marrow-based therapeutic principles have thus been intensively investigated in animal models and preclinical trials. Such protocols have consistently shown a beneficial effect of supportive cellular therapy on organ as well as VCA survival[67,68]. Underlying mechanisms that have been studied include, for example, effects such as macro- and microchimerism, and clonal exhaustion and deletion of alloreactive T-cells. These insights now help to refine treatment protocols aiming to support long-term graft survival on minimal maintenance immunosuppression[69]. One remaining challenge of transplanting a graft with functional immune effector cells, though, is the potential for these mature allogeneic T-cells to attack the host, resulting in GvHD[70]. However, it is now widely accepted that high doses of bone marrow cells infused in the absence of recipient conditioning or myoablation do not induce GvHD and although an obstacle in VCA, GvHD has not been observed in any reconstructive transplant recipient to date[71].

Thus, the idea of donor bone marrow cell infusion to either induce chimerism or for the intensification of clonal exhaustion and deletion of alloreactive T-cells is appealing. Nevertheless, the combination of such a concept with high-dose multidrug immunosuppression might be counterproductive because such regulatory mechanisms require the persistence of a certain degree of immune response to be successful in a clinical setting[72]. However, the implementation of cell-based therapies in VCA that integrate and unify the concepts of immunoregulation and tolerance induction with those of tissue/nerve regeneration will certainly help to fine-tune current immunomodulatory approaches and further optimize the outcomes of these reconstructive modalities in the future.

Nerve regeneration

In addition to overcoming the immunogenicity, optimizing nerve regeneration is key to success in reconstructive transplantation. Unlike solid organ transplants that are immediately functioning following revascularization, a VCA is viable after revascularization of the graft but not functional. The recipient nerves/axons have to re-grow and replace the donor nerves, which serve as temporary scaffold, and finally re-innervate the motor end plates and sensory end organs within the graft. Thereby, the nerve undergoes a chimeric state, which is progressively replaced by host tissue. Although peripheral nerve regeneration is essential for the function of a VCA, there is very limited data on nerve regeneration in this context. Nerve regeneration after RT represents a unique challenge since, in addition to functional loss caused by lack of innervation, changes occur along the entire route of the nerve from the target end organ to the central nervous system, which might have important implications on recovery and outcome[73].

The backbones of conventional immunosuppressive protocols applied to VCA are still calcineurin inhibitors, of which tacrolimus can be considered the “gold standard” used for immunosuppression in VCA. Tacrolimus, apart from potent T-cell inhibitory effects, has also been demonstrated to have neuro-regenerative capacity. Thereby, pathways and mediators independent of calcineurin inhibition,

such as FK binding protein 52 (FKBP52), growth associated protein 43 (GAP43) or heat-shock proteins, have been shown to be responsible for the neuro-regenerative properties of tacrolimus[74]. Studies evaluating the enhanced neural regenerative effects of tacrolimus in isolated nerve allograft transplantation have also established that timing, dosing, and combinations of immunosuppressive therapies affect and are critical for nerve regeneration[75].

Schwann cells (SC) surround axons and are key players during the process of axonal regeneration. Schwann cells are, on the one hand, vulnerable to immune rejection, while on the other hand are stimulated to migrate during limited bouts of rejection, leading to enhanced nerve regeneration. In this regard, both recipient and donor SC migration and viability are critical. In isolated nerve allotransplantation, allograft survival depends on proximal and distal SC migration. However, in VCA, critical distal host SC are lacking[76]. Therefore, it is essential that sufficient immunosuppression be given to prevent loss of donor SC and subsequent demyelinization, which might result in permanent or irreversible functional impairment[77].

Several therapeutic agents have been added to the immunosuppressive protocols used in RT and are currently being studied in cadaveric peripheral nerve allografts for their ability to enhance nerve regeneration. In particular, studies in small and large animal models have suggested the use of autologous SC in conjunction with nerve allotransplantation as a potential means to enhance nerve regeneration[78,79].

Schwann cells are known to support and promote nerve regeneration *in vivo* by myelinating regenerating axons, producing neurotrophic factors, and increasing synthesis of cellular adhesion molecules such as N-cadherin[80]. In addition, autologous cultured SC were shown to be viable after injection and to permit axonal regeneration without causing SC-derived tumors or iatrogenic nerve injury in rodent models[78]. These studies all confirm the integral role of SC in nerve regeneration. As a therapeutic agent, SC can by successfully and safely harvested, cultured, and reintroduced into peripheral nerves to promote nerve regeneration[78]. However, the exact mechanisms of SC to enhance nerve regeneration as well as their feasibility and potential to improve functional outcome in clinical VCA are yet to be elucidated.

An intriguing concept that has been recently more and more pursued is the use of stem cells in RT to combine immunoregulation and nerve regeneration. In the recent past, more and more emphasis has been placed on exploring various cell sources, in particular bone marrow and adipose-derived stem cells or progenitor cells that are easily accessible, rapidly expandable *in vitro*, and capable of survival and integration within the host tissue to be added to the armamentarium of treatment protocols used in VCA.

In particular, mesenchymal stem cells (MSC) have been identified as a promising tool to further enhance not only the beneficial immunoregulatory effect of bone marrow cell infusion, but also improve nerve regeneration. This most interesting concept has been investigated in large animal trials for solid organ as well as VCA survival.

Mesenchymal stem cells are capable of differentiation along multiple mesenchymal lineages into osteocytes, chondrocytes, myocytes, adipocytes, and SC, thereby emerging as a promising tool for tissue engineering and cell therapy[81]. They have been reported to have significant potential for improving the neurological outcomes after stroke and traumatic brain injury. In addition, MSC have shown phenotypical, biochemical and functional properties similar to SC and promote functional recovery of peripheral nerves when introduced at the site of nerve injury[82]. The mechanisms of MSC-induced nerve regeneration include *in vivo* trans-differentiation into

neural phenotypes as well as paracrine effects on SC via released cytokines and growth factors[83]. Although potent MSC-enhanced nerve regeneration has been demonstrated *in vitro* and by local administration *in vivo*, systemic application as would be required for immunomodulation has not been tested. Such ongoing studies will yield important insights towards minimizing immunosuppression and improving functional outcomes after RT.

Recently, bone marrow-derived MSC, apart from their capacity for multi-lineage differentiation and neuroprotection, have also been identified to have potent immunosuppressive properties to inhibit the activation and proliferation of immune cells. Numerous *in vitro* studies have reported that MSC are immunoregulatory and can alter differentiation, maturation, and cytokine secretion profiles of dendritic cells (DC), B-cells, NK-cells as well as T-cells[84]. It has also been shown that autologous and allogeneic MSC have comparable immunosuppressive capacity and most importantly that MSC are considered to be immunoprivileged by their low immunophenotype[85]. The MSC offer some potential advantages over conventional immunosuppressive agents by specifically enhancing immuno-inhibitory effects that could prevent rejection, and minimize the systemic complications of non-specific immunosuppressants in the setting of VCA[86]. The addition of MSC to a particular immunosuppressive regimen might also allow reducing or minimizing the dose of conventional immunosuppressive drugs without affecting the overall efficacy of the therapy. Studies have shown that injection of allogeneic MSC prolonged skin-graft survival in primates[86]. The utilization of MSC for clinical purposes in CTA takes advantage of their described poor immunogenicity *in vitro* as well as in preclinical and human studies, which supports the possible use of MSC obtained from allogeneic donors. The ideal cellular immune treatment for hand transplantation should be able to provide both systemic and local therapeutic effects. Transplant experiments in nonhuman primates have shown that MSC are able to spread to many tissues after intravenous administration, but seem to preferentially home to the site of injury, where they support functional recovery[87]. However, there are several factors that need to be carefully considered when regarding MSC for cellular immune therapy in organ and composite tissue transplantation or in combination with bone marrow cell infusions. One of the most critical questions concerning the clinical application of MSC is the source of these cells, whether using MSC of autologous or allogeneic origin. Other factors that need to be taken into consideration are the route of administration and migration of MSC as well as optimal dose and timing in relation to the transplant. Overall, available current data indicate that although MSC were first proposed for use in regenerative medicine, their therapeutic effect can result from the immunosuppressive activity of MSC. This may provide a novel promising tool for minimizing immunosuppression or tolerance induction following systemic injection. The underlying effect seems to depend on the capacity of MSC to inhibit proliferation of immunocompetent, alloreactive cells following antigenic stimulation and maintaining them in a quiescent state. In addition, MSC may enhance and improve nerve regeneration and promote engraftment of bone marrow cells as supportive cells within the stem cell niche, which makes them particularly attractive for novel treatment regimes in RT.

Conclusion: challenges and opportunities

The functional outcome and patient satisfaction after hand transplantation at the wrist and distal forearm level has been highly encouraging. Peripheral nerve regeneration and cortical reintegration of the graft feed into return of upper extremity motor and sensory function of various degrees. Further increasing the ve-

locity and quality of peripheral nerve regeneration and cortical integration may further enhance the functional outcome and seems critical for transplantation at a level above the elbow.

The most limiting factor in hand transplantation remains the risk posed by high-dose immunosuppression. While individual dose adaption is facilitated by the ability to visually monitor the graft and respond promptly to rejection, immunosuppression as applied in the majority of cases is associated with a set of side effects well known from solid organ transplantation. While the current challenge poses some limitations to rapid expansion of the field, the early results from current clinical trials as well as the increasing activity in RT basic science research indicate that significant progress towards reduction of immunosuppression and/or the induction of tolerance towards a limb allograft is to be expected. In particular, cell-based treatment protocols in combination with novel immunosuppressive or immunomodulatory agents, together with local and targeted treatment of skin rejection, seem promising and may lead the way to widespread application of RT for treatment of complex tissue defects.

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