Minimization of Immunosuppression and Tolerance Induction in Reconstructive Transplantation

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Abstract Vascularized composite allotransplantation (VCA) is an innovative reconstructive modality for patients sustaining complex injuries not amenable to conventional treatment. Advances in immunosuppression have made VCA a clinical reality and a valid reconstructive option for such patients. The requirement, however, for multi-drug high-dose immunosuppressive regimens with their numerous side effects has hindered widespread clinical application of VCA. There is thus a need for novel immunologic modalities to minimize or even obviate the need for immunosuppression (tolerance induction) while still preserving the allograft and preventing rejection. Recent advances in targeted immunotherapy and cell-based protocols were able to achieve tolerance in selected cases of solid organ transplantation. This paved the way for

innovative immunomodulatory protocols now also applied to VCA that aim for minimal immunosuppression or for induction of donor-specific tolerance. These concepts and novel protocols will be discussed in this review.

Keywords Vascularized composite allotransplantation · Hand transplantation · Face transplantation · Immunosuppression minimization · Tolerance induction · Bone marrow · Chimerism · Reconstructive transplantation

Introduction

Reconstructive transplantation using vascularized composite allografts represents an innovative strategy for optimal restoration of anatomy, function and appearance in select cases of devastating tissue loss. Despite initial skepticism, vascularized composite allotransplantation (VCA) has become a clinical reality. Numerous hand and face transplants have been performed worldwide with highly encouraging early and intermediate outcomes. These achievements were built on many years of basic and translational research aiming largely at establishing the immunobiology of VCA. This type of transplantation is unique and carries complex immunologic challenges distinctly different from solid organ transplantation. It consists of various heterogeneous tissue types and components of different antigenicity including skin, vasculature, muscle, cartilage, tendon, nerve, bone, and vascularized bone marrow (BM). The high immunogenicity of the skin necessitated the use of multi-drug immunosuppressive regimens, sometimes administered at high doses. Adverse events from high-dose long-term immunosuppression in the setting of a non-life saving procedure such as VCA are obviously of great concern, and continue to hinder

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widespread clinical application of these novel types of transplants. There is thus a critical need for innovative strategies aiming at minimizing or even obviating the need for long-term high-dose multi-drug immunosuppression, while preserving the allograft and preventing rejection, by inducing immune tolerance. This will not only avoid the numerous adverse effects of immunosuppression, but will also promote wider application of VCA. Therefore, minimization of immunosuppression and inducing immune tolerance constitute one of the primary goals of global research in this field.

In this review, we will discuss the different immunomodulatory approaches used to minimize immunosuppression and induce tolerance in reconstructive transplantation, taking into consideration the unique immunobiology of VCA.

Conventional Immunosuppressive Strategies Used in VCA

Conventional immunosuppressive protocols warrant life-long immunosuppression in VCA where allografts are derived from genetically non-identical, cadaveric donors. After the failure of the immunosuppressive regimen (azathioprine and prednisone) used in the first single hand transplant performed in 1964 [1], VCA remained dormant for the next two decades. In 1985, Black et al. [2], however, demonstrated that long-term graft survival in a rodent hindlimb transplantation model can be achieved across major histocompatibility barriers using the calcineurin inhibitor cyclosporine A thereby leading to a regained interest to move forward with clinical reconstructive transplantation.

Further research in immunosuppressive strategies and the introduction of new agents such as tacrolimus and mycophenolate mofetil (MMF) in the 1990s provided evidence that VCA can be successfully performed in large animal translational models with reproducible results. These advances suggested also that VCA would be a viable option for reconstructive surgery with possible attainment of rejection free survival. These developments and continued success rates in solid organ transplantation ultimately initiated the modern era of hand and face transplantation.

Immunosuppression in Hand Transplantation

Most of the conventional immunosuppressive agents utilized in VCA to date block the cellular alloimmune response in a nonspecific manner. The majority of hand transplant patients received either polyclonal (anti-thymocyte globulin, ATG) or monoclonal (alemtuzumab,

basiliximab) antibody preparations as induction agents, followed by maintenance therapy with a high-dose triple-drug combination including a calcineurin inhibitor (tacrolimus), an antiproliferative agent (mycophenolate mofetil, MMF), and corticosteroid (prednisolone) [3, 4]. Most centers advocate maintaining high tacrolimus trough levels (10–15 ng/ml) during the first 3 months and then tapering it down to 5–10 ng/ml. Similarly, prednisone doses are rapidly tapered in the early post-transplant period to be maintained at lower doses (5–15 mg/day) for 6–12 months in the majority of hand recipients. Of the 39 recipients who received 57 upper extremity transplantations (18 bilateral and 21 single transplantations) included in the most recent report of the international registry [4], 40.5 % received induction therapy with ATG, 32.4 % with alemtuzumab and 27.1 % with basiliximab. Maintenance therapy at 3 months consisted of tacrolimus ($n = 39$), MMF ($n = 34$), and steroids ($n = 32$). During the follow-up period, ten patients were converted from tacrolimus to the mTOR inhibitor sirolimus, to minimize renal side effects, improve glycemic control and potentially avoid chronic vascular changes (myointimal hyperproliferation) and neurotoxicity [5]. In ten cases steroids were completely withdrawn; in five cases, MMF were withdrawn; and two recipients received low-dose tacrolimus and sirolimus [6, 7]. In patients who did not receive induction therapy ($n = 2$) or were not started on triple immunosuppressive regimens ($n = 2$), topical steroid and tacrolimus ointments were applied in addition to systemic immunosuppressive medication.

Immunosuppression in Face Transplantation

Most of the face transplant centers have reported the use of induction therapy with polyclonal antibodies (ATG) or anti-interleukin 2 receptor antibodies (daclizumab, basiliximab), followed by triple drug maintenance protocol consisting of tacrolimus, MMF and steroids (similar to solid organ and hand transplantations). Maintenance therapies have been adjusted by dose reduction to minimize adverse effects while preventing graft rejection. In some recipients, topical ointment of calcineurin inhibitors and steroids was applied to avoid or treat skin rejection [8, 9].

Immunologic Outcome of Conventional Immunosuppressive Protocols

As outlined above, the vast majority of VCA recipients are currently being maintained on immunosuppressive protocols similar to those of solid organ transplants. However, there is no established standard protocol, and it is still difficult to claim the superiority of one regimen over another, since no randomized clinical trials have been

performed to date—mainly due to the small patient numbers. Nevertheless, such conventional protocols have resulted in a 100 % patient and 96 % graft survival at 1 year for hand transplantation in patients compliant with immunosuppressive medications. This patient and graft survival data is more encouraging than any solid organ transplant series to date [3•]. For face transplants, overall survival rate is 87 % due to two patient losses out of the 15 face transplants that were included in the latest published report [4•]. The cause of death of the Chinese patient remains elusive, and that of a French patient, who received also concomitant bilateral hand transplantation, was attributed to a cardiac event secondary to severe sepsis. To date, however, no graft loss, hyperacute chronic graft rejection or graft-versus-host disease were reported [6, 10].

As of now, induction therapy followed by maintenance immunosuppression with at least a dual-drug combination at optimum doses is considered the most widely used treatment regimen for VCA. Such conventional regimens were sufficient to prevent early immunological graft loss, but were not able to prevent acute rejection. Indeed, 85–90 % of hand transplant recipients and 54.5 % of face transplant recipients experienced at least one episode of acute rejection in the first post-transplant year regardless of their induction or maintenance regimen. All patients transplanted to-date continue to remain on maintenance immunosuppression.

Minimization of Immunosuppression in VCA

Immunologic Challenges in VCA

When aiming to design novel immunosuppressive regimens for reconstructive transplantation utilizing minimal medications, both drug-specific requirements and unique biological features of VCA need to be taken into account. However, these unique immunologic features pose certain challenges, but also opportunities to transplant immunologists. Indeed, no other organ or tissue transplant matches VCA in its complex immunogenicity as it involves multiple heterogeneous tissue types, such as skin, muscle, tendon, bone, cartilage, fat, nerves, blood vessels and vascularized BM [11]. The skin in particular, being highly antigenic and constituting a major component of the graft, represents the main immunologic barrier. This is in part due to skin-specific antigens in the epidermis as well as a high proportion of potent antigen-presenting cells (Langerhans cells and other dermal dendritic cells). Furthermore, keratinocytes express major histocompatibility complex (MHC) I constitutively, in addition to MHC II, intercellular adhesion molecule 1 (ICAM)-I, and various proinflammatory cytokines upon stimulation [12, 13].

Following skin, muscle, bone, cartilage, blood vessels and nerves predictably induce a relatively lower immune response [14].

Favorable Characteristics of VCA

While VCA carries complex immunologic challenges, it also offers unique opportunities for basic and translational research aimed at minimizing immunosuppression and inducing tolerance.

Visible Graft

The most immunogenic component of VCA, skin, can be constantly and directly monitored for signs of rejection, providing live and real-time feedback on the efficacy of the immunomodulatory regimen being utilized. This permits prompt diagnosis of acute rejection episodes (through clinical and histologic analysis). Hence, treatment of acute rejection can be initiated early in its course potentially minimizing the incidence of chronic rejection.

Composite Versus Individual Tissue Antigenicity

Lee et al. [15] have shown that in VCA the skin is not rejected as stringently as when skin or other tissues are transplanted individually. In this seminal study, the concept of tissue antigenicity was challenged demonstrating that an entire hindlimb transplant is actually less immunogenic than its individual components. The various tissue components interact with the host immune system in a complex but predictable pattern with variable timing and intensity. This led to a change in the paradigm that the skin component is considered an insurmountable hurdle to broad application of VCA.

Bone Marrow and Vascularized Bone Marrow Niche

Most VCA include immunocompetent elements such as BM and a vascularized BM niche, which were shown in animal models to modulate the immune interaction between donor and recipient. These elements can function as a vascularized BM transplant by themselves [16–18], and provide a continuous source of donor-derived stem cells. The potential consequences of this phenomenon, however, can be either a favorable slowdown of rejection or an unfavorable risk factor for graft-versus-host disease (GvHD)—therefore, a dynamic balance of the two needs to be maintained for optimal outcomes. In pre-clinical models, this balance has been achieved and attributed to the BM component in conjunction with specific immunomodulatory protocols and myeloablative as well as non-myeloablative conditioning regimens, which allow BM

engraftment and provided sufficient immunosuppression to prevent rejection [19]. If these findings are translated to the clinical setting, they will pave the way for novel immunomodulatory strategies minimizing immunosuppression and inducing tolerance to VCA.

Tolerance Induction

Bone Marrow-Based Protocols

Several approaches have been attempted in the search for the most favorable BM-based protocol that would facilitate induction of donor-specific tolerance and long-term immunosuppression free survival of allotransplants. Such protocols have shown encouraging results in solid organ transplants as well as VCA [20, 21, 22•] through mechanisms such as the establishment of macro- and micro- chimerism, and exhaustion and deletion of the donor-specific alloreactive T cell clones. Although myeloablative protocols provide sufficient immunosuppression to prevent VCA rejection and allow for engraftment of donor hematopoietic cells, they carry high risks and toxicities related to myeloablation as the conditioning regimen [19]. This is why, in the setting of VCA (a non-life saving procedure), myeloablative protocols are not a feasible or clinically relevant option.

In addition, a potential disadvantage of transplanting a graft with functional immune effector cells and renewable sources of donor-derived stem cells is the likelihood of these cells to mature into allogeneic T and NK cells, potentially resulting in GvHD. Although such adverse events have not been reported in clinical cases of VCA and BM infusion, the concern of GvHD has led to explore new avenues of research for selecting optimal-cell- and stem-cell-based protocols combined with non-myeloablative induction regimens for inducing donor antigen-specific tolerance in VCA. Furthermore, novel biologic agents that target key pathways during the initiation of an alloimmune response can aid in immune tolerance. One such example is costimulation blockade, which has demonstrated potent immunomodulatory effects in translational large-animal models of VCA. These selective and targeted approaches would significantly reduce undesirable complications of induction regimens while still enabling rejection free graft survival [23].

Cell-Based Protocols, Principles and Applications

The aim of cell-based protocols is to ultimately facilitate the development of either central or peripheral tolerance. Acquired immunologic tolerance can result from clonal deletion of donor-reactive T cells during development in the thymus (central tolerance) and the induction of regulatory T cells (T-regs) that suppress the alloreactive T cells escaping intrathymic deletion (peripheral tolerance). This concept of

donor antigen-specific immunological tolerance, also referred to as operational tolerance, was achieved in a small number of solid organ transplant recipients. Recent data from living related kidney-transplant recipients demonstrated that BM and stem-cell-based therapies enabled reduction and elimination of immunosuppressive medication [24, 25, 26•, 27]. This limited case series provided a ray of hope for overcoming histocompatibility barriers in VCA, not by means of immunosuppression but by immunoregulation. Still, VCA is different from traditional solid organ transplants due to its unique structure that includes multiple diverse tissue components; hence, strategies proven successful in the setting of renal transplantation might not necessarily translate into the field of VCA.

Operational tolerance refers to a state that is achieved when the immune response towards the transplant is exhausted because specific immune cell clones mediating rejection are deleted or controlled either by activation-induced or programmed cell death, or by the suppressive effect of T-regs. In experimental transplant models, this is often the result of an exhausted host-versus-graft immune response mediated by donor-derived hematopoietic cells. Small numbers of donor leukocytes that persist in the recipient long-term (microchimerism) can furthermore facilitate maintenance of immunosuppressive-free graft survival [28]. Such mixed donor/recipient chimerism gives rise to a new T cell pool that is tolerant to both recipient and donor, and might subsequently facilitate immunoquiescence and the ability to wean or even withdraw immunosuppressive medication. Potential regulatory effects have also been shown for induction regimen with poly- or monoclonal antibodies (e.g., thymoglobulin, alemtuzumab) by deleting alloreactive T effector cells while preserving T-regs, thereby favoring immune regulation and operational tolerance [29, 30].

Taking into consideration the specific and unique immunological features of vascularized composite allografts, multiple small- and large-animal studies have shown encouraging results with regard to the ability to minimize immunosuppression or even to completely avoid the need for long-term maintenance immunosuppression [31, 32]. The strategies and protocols applied in these models included the use of total lymphoid irradiation, costimulatory blockade (CD28/B7 and CD154/CD40 pathways), selective depletion of alloreactive recipient T and B cells (e.g., $\alpha\beta$ -T cell and CD20-specific monoclonal antibodies), inhibition of lymphocyte trafficking, infusion of CD4⁺CD25⁺Foxp3⁺ T-regs and tolerogenic APCs, as well as donor BM infusion and chimerism induction [20, 32–39].

A translational, clinically relevant protocol was recently developed by Mathes et al. [40] in a MHC-matched canine model that has led to tolerance to all components of the VCA, including skin using a hematopoietic mixed chimerism

protocol with non-myeloablative induction. Furthermore, indefinite graft survival without long-term immunosuppression could also be achieved with the use of an immunomodulatory protocol employing donor BM cells in the more stringent setting of a complete MHC-mismatched swine heterotopic hindlimb transplant model [41, 42]. Barth et al. [43•], using a nonhuman VCA primate model of facial segment allotransplantation, recently demonstrated a critical role of vascularized BM to enable for prolonged and rejection-free allograft survival. In their elegant study the group showed that facial segment VCA including vascularized BM, demonstrated increased rejection-free survival as compared to VCA without vascularized BM that experienced early rejection episodes and graft loss.

Based on such encouraging concepts and extensive preliminary large-animal data the joint team at Johns Hopkins University School of Medicine and the University of Pittsburgh has recently implemented the first clinical cell-based immunomodulatory protocol for upper extremity transplantation [44•]. As part of their protocol, recipients are pre-treated (1–2 h prior to transplantation) with the monoclonal and CD52 antibody alemtuzumab (Campath 1-H for lymphocyte depletion and tacrolimus monotherapy commenced thereafter. In addition, all patients receive an unmodified donor BM cell infusion 2 weeks post-transplantation. This clinical trial is currently ongoing, but has demonstrated so far that the protocol is safe, efficacious and well tolerated, allowing upper extremity transplantation to be performed with low-dose tacrolimus monotherapy. The underlying hypothesis of this protocol is that depletion of circulating T cells with alemtuzumab followed by low-dose maintenance immunosuppression and donor BM cell augmentation in the early post-transplant period would increase the intrinsic tolerogenic properties of the allograft and potentially lead to a state of immunoquiescence [44•, 45].

Conclusions and Future Directions

Ever since the seminal experiments by Billingham, Brent and Medawar more than half a century ago, cell-based immunotherapy has been regarded as the most promising approach for immunomodulation and to induce donor antigen-specific tolerance. With the insight gained in the immunobiology of vascularized composite allografts over the past decade, there is now also growing interest in utilizing cell-based protocols to prolong allograft survival and to minimize/avoid immunosuppression in the field of reconstructive transplantation. However, despite the many pre-clinical studies that have demonstrated the beneficial immunoregulatory role of the vascularized BM component in VCA and have succeeded in performing VCA with minimal or no immunosuppression by using different types

and numbers of donor-derived cells in combination with established conventional as well as novel biological immunosuppressive agents, there remains the concern of the risk of GvHD of such concepts.

In this regard, alternative sources of cells with potent immunoregulatory and tolerogenic properties but reduced risks of GvHD are warranted. Mesenchymal stem cells (MSCs) were shown to fulfill this premise and have recently gained great attention for their use in solid organ transplantation [46, 47]. In addition, MSCs are currently applied to prevent and treat GvHD after BM transplantation and to prevent acute rejection episodes after solid organ transplantation [48]. Kuo et al. [49•] have shown prolonged allograft survival of vascularized composite allografts by treatment with BM-derived MSCs in a swine hindlimb transplantation model. MSC treatment thereby correlated significantly with an increase in the percentage of CD4⁺/CD25⁺/FoxP3⁺ T- cells in both peripheral blood and intra-graft tissues [50].

Additionally, a novel approach using a bioengineered mobilized cellular product enriched for hematopoietic stem cells (HSCs) and tolerogenic graft facilitating cells (CD8⁺/TCR⁺, FCs) combined with non-myeloablative conditioning in kidney transplantation has promoted engraftment, durable macrochimerism and tolerance induction in recipients with highly mismatched related and unrelated donors without occurrence of GvHD or anti-donor antibody [26•]. Exploring the tolerogenic role of FCs in VCA would be an interesting approach since these cells have demonstrated potential to induce FoxP3⁺/CD4⁺/CD25⁺ T-reg cells in vitro [51] and potent antigen-specific immune regulation in vivo [52]. Thus FCs may be a future attractive source to address the primary challenge in translating cell-based therapies to the clinic: maintaining tolerogenic features after transplantation while avoiding GvHD [26•].

In any case, it will be an exciting but long and winding road for the field of VCA to implement reliable and safe protocols for these life-changing transplants that will allow minimizing or ultimately avoiding the requirement for maintenance immunosuppression.

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