Vascularized composite allotransplantation: towards tolerance and the importance of skin-specific immunobiology

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Purpose of review
Vascularized composite allotransplantation (VCA) is increasingly utilized in the restoration of complex injuries and tissue loss. Acute skin-targeted rejection episodes are common and concerns remain regarding the risks of conventional immunosuppression. We review current immunosuppressive regimens for VCA, progress with immunomodulatory and tolerance protocols, and highlight recent advances in cutaneous immunobiology which will have significant implications for future development in the field.

Recent findings
Advances in induction protocols have demonstrated effective prevention of early graft loss in hand transplantation, although long-term outcomes are still pending. Furthermore, recent findings in leukocyte populations within the skin and their mechanisms of communication reveal that considerable numbers of resident T-effector memory cells, including a T-regulatory subset, exist, and that epidermal Langerhans' cells communicate with these cells, mediating both immunity and tolerance to maintain skin homeostasis.

Summary
The majority of VCA centers utilize antibody-mediated induction, followed by double or triple-agent maintenance immunosuppression. A clinical trial of a minimal-immunosuppression protocol based on bone marrow infusion reports encouraging interim results, but long-term follow-up will be required. Skin remains the primary target of rejection in VCA. New data demonstrate extensive T-cell memory resident in skin, and complex interactions between these cells and epidermal Langerhans' cells will have implications for VCA rejection and tolerance, and warrant further investigation in the allogeneic setting.

Keywords
immunosuppression, skin immunobiology, tolerance, transplant, vascularized composite allotransplantation

INTRODUCTION

The transplantation of vascularized composite allografts is an increasingly common approach to the restoration of complex injuries and extensive tissue loss or destruction, particularly in cases involving the specialized anatomical and functional units of the craniofacial area or upper extremity, where replacing like with like by conventional, autologous reconstructive techniques is not possible. Whereas there has been some variation between institutions, the current immunosuppressive standard for preventing vascularized composite allotransplantation (VCA) rejection typically includes antibody-based induction therapy (e.g. T-cell depletion by antithymocyte globulin) followed by maintenance with tacrolimus, mycophenolate mofetil, and prednisolone [1]. To date, such regimens have proven effective, with no reported cases of graft loss to acute rejection in compliant patients; however, concerns remain regarding the side-effect profile of current immunosuppressive regimens including infection, metabolic disturbance and malignancy, and the potential long-term sequelae of acute rejection episodes, which have been common. In comparison to solid organ transplantation,

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KEY POINTS

- Conventional immunosuppressive protocols achieve 96% hand transplant survival at 1 year, but 85–90% of patients experience at least one episode of acute skin rejection during this period.
- Mixed chimerism effectively induces kidney transplant tolerance in animal models and clinical trials, but similar protocols in VCA models, have until recently, commonly resulted in split tolerance with skin rejection.
- Recent studies have demonstrated that skin contains large numbers of resident T_{EM} cells and FoxP3$^+$ T_{REG} cells.
- Epidermal Langerhans' cells are capable of functional polarity, stimulating a protective antimicrobial response by T_{EM} cells in the presence of pathogen, but contributing to immune homeostasis by activation of skin resident T_{REG} cells in steady-state conditions.
- The complex and specific interactions between Langerhans' cells and skin-resident T_{EM} and T_{REG} cells may contribute to the susceptibility of the skin component of VCAs to rejection, but further studies are required to define the function of the skin immune system following allotransplantation and the role of these mechanisms in VCA tolerance protocols.

the incidence of acute rejection episodes during the first year is high, with approximately 85% of patients experiencing at least one episode [1], of which the primary target has been the skin [2].

The high incidence of acute rejection episodes has been attributed to the considerable antigenicity of skin, which has long been recognized to pose a stringent test of immunosuppressive or transplant tolerance protocols [3]. The potent immune response to skin has classically been attributed to the mode of transplantation [4], the presence of skin-specific antigens [5,6], and the presence of immunologically active cells and extracellular elements associated with its role as an immunologic barrier [7]. However, more recently, there has been increasing appreciation of the role of the skin as an immune organ in its own right, with increased appreciation for the complexity of the 'skin immune system' [8]. Recent studies have demonstrated the presence of large numbers of resident memory T cells within normal skin [9▪▪], and suggested a more complex role for Langerhan's cells, potentially including contribution to immune homeostasis and activation of resident T-regulatory (T_{REG}) cells [10▪▪]. Thus, whereas skin undoubtedly poses many challenges in the context of transplantation, these recent advances significantly contribute to increased understanding of the immunobiology of skin and to the development of novel strategies to simultaneously reduce the incidence and severity of skin-rejection episodes while minimizing exposure of VCA recipients to the burden of side effects associated with conventional immunosuppressive regimens.

In this study, we will review current insights into skin immunobiology, discuss the implications of these findings in terms of VCA, and summarize the current status of immunomodulatory and tolerance protocols for VCA. Finally, we will summarize current research aimed at defining the role of the skin immune system in VCAs, with particular emphasis on the skin immune system in the context of mixed chimerism-based tolerance protocols.

CURRENT PRACTICE AND PROGRESS TOWARD TOLERANCE PROTOCOLS

Immunosuppressive protocols for VCA have thus far largely been adapted from those used in solid organ transplantation, with the overall immunosuppressive requirement to sustain graft survival comparable to, or perhaps slightly greater than, that for renal transplantation [11]. Whereas there has been center-to-center variation, and a standardized protocol does not exist, the majority of VCA patients have received antibody-based induction therapy with either polyclonal (antithymocyte globulin) or monoclonal (alemtuzumab or basiliximab) agents, followed by triple-drug maintenance therapy with tacrolimus, mycophenolate mofetil, and steroids [1,12]. Amongst numerous, mostly minor, variations on this theme, the most common (8 of the 33 patients covered by the latest report of the International Registry on Hand and Composite Tissue Transplantation, all of whom received tacrolimus initially) was conversion from tacrolimus to sirolimus, an mTOR inhibitor, in order to achieve better glycemic control, reduce nephrotoxicity, and potentially mitigate chronic vasculopathy [11]. Regimens of this type have proven highly effective at preventing graft loss to acute rejection, particularly in upper extremity transplantation, which has to date been the most frequently performed type of VCA, with patient and graft survival at 1 year of 100 and 96%, respectively, an outcome not yet reported in any other branch of transplantation [12]. However, the efficacy in protecting against acute rejection episodes has a dramatically different picture, with 85–90% of patients experiencing at least one episode during the first year after transplant (in contrast to rates of approximately 10% following renal transplantation) [13,14]. Although the high incidence of acute rejection may reflect some degree of bias due to the uniquely accessible nature of VCAs, it nonetheless carries with it morbidity for the patient, and is a cause

for concern in the long term in light of recent findings from animal models suggesting a potential correlation between acute rejection episodes and later chronic allograft vasculopathy in VCAs [15].

Transplant tolerance, defined as a state of specific unresponsiveness to donor antigens permitting life-long acceptance of tissues or organs transplanted without maintenance immunosuppression, offers the possibility of VCA without the burden of side effects associated with current immunosuppressive regimens, and potentially free from the spectre of chronic rejection.

The immunological effects of hematopoietic cell transplantation (HCT) have been extensively studied and the ability of mixed hematopoietic chimerism to induce robust tolerance across major histocompatability complex (MHC) barriers is well established, and has been reviewed elsewhere in detail [16,17]. Uniquely amongst the multiple protocols capable of inducing donor-specific tolerance in small animal models, mixed chimerism has made the translation to clinical practice, with successful induction of renal transplant tolerance in clinical trials at a number of centers [18,19▪]. However, whereas tolerance of kidneys and other solid organ transplants has been achieved in large animal models by such protocols [20–22], tolerance of VCAs has remained elusive, as previously reviewed [23▪]. Success using HCT to induce stable mixed chimerism and VCA tolerance has been achieved across minor antigen barriers in canine models [24]; however, across MHC disparities the most common result has been split tolerance, with acceptance of musculoskeletal elements but rejection of the skin or epidermis [25]. Additionally, the use of HCT raises the concern of graft-versus-host disease (GvHD), and it has been demonstrated that co-transplantation of mesenchymal stem cells (MSCs) with VCA and bone marrow can mitigate the otherwise lethal effects of GvHD in some models [26]. Whereas these studies reinforce the challenges of successfully transplanting skin and the development of true tolerance-inducing protocols, recent findings in our laboratory demonstrate the induction of tolerance of all components of VCAs across MHC barriers by simultaneous establishment of stable multilineage mixed chimerism, with immunosuppression-free acceptance of VCAs throughout the period of experimental follow-up (>400 days) [27].

Interestingly, the ongoing development of tolerance protocols through use of HCT has further illustrated a stark difference between VCAs and other transplanted organs. Whereas long-term indefinite acceptance of VCAs in stable (macro) mixed chimeras can be achieved across MHC barriers, protocols in which infusion of donor bone marrow failed to achieve engraftment and stable chimerism [either due to insufficient conditioning (unpublished data) or administration of intentionally low doses of cells] [28] fail to achieve VCA tolerance, suggesting a requirement for continual donor hematopoietic stem cell contribution in the maintenance of tolerance. In contrast, clinical protocols reporting successful induction of tolerance of transplanted kidneys have achieved both transient [18] and stable [19▪] mixed chimerism. Taken together, these data would seem to support the classical finding that skin has greater antigenicity and poses a more significant challenge to transplantation than a kidney [3].

Whereas transient chimerism has not been demonstrated to provide for long-term VCA tolerance, the immunomodulatory properties of donor bone marrow have been translated to a clinical immunosuppression-minimization protocol for upper extremity transplantation during induction phases. This trial remains active, and long-term follow-up will be required to fully determine the efficacy and safety of this protocol, but interim results are encouraging with a rate of acute rejection episodes comparable to conventional immunosuppression and no reported cases of chronic rejection while significantly reducing the overall levels of immunosuppression required [29▪]. However, this protocol does not achieve detectable chimerism (either macro, or micro), nor is there in-vitro evidence for persisting abrogation of the antidonor immune response; therefore despite the impressive reduction in immunosuppressive burden and the encouraging clinical results to date, there is no evidence to suggest induction of tolerance, or to support weaning of these patients from immunosuppression altogether.

Interestingly, one patient in this trial experienced an acute rejection episode following a scald injury to the transplanted hand, and the majority of rejection episodes experienced by patients on this protocol have been successfully treated using local, topical administration of tacrolimus and clobetasol, in some cases without additional systemic immunosuppression [30▪▪], illustrating the importance of a thorough understanding of skin immunobiology, both in identifying risk factors for and designing immunosuppressive or tolerance strategies to prevent skin rejection.

STATE OF THE ART: SKIN IMMUNOBIOLOGY

The relative difficulty in achieving acceptance of skin across allogeneic barriers in comparison with kidney and other organ transplants has long been

recognized, although the mechanisms contributing to this observation have never been fully elucidated. Classically, this finding has been attributed either to skin's unique mode of transplantation, or to the presence of skin-specific alloantigens [7]. Presumably, the prolonged period of relative ischemia and subsequent ischemia–reperfusion injury following secondary establishment of circulation contribute to a local inflammatory milieu and presentation of donor antigen to the immune system in a manner which strongly promotes rejection. However, the susceptibility of the skin component of primarily vascularized VCAs to acute rejection episodes in both experimental and clinical settings suggests that a factor intrinsic to the skin itself, rather than the mode of transplantation, also contributes to its immunogenicity.

The recognition that skin provides more than a simple, mechanical barrier between the body and the environment and possesses discrete immunologic functions is not new. The identification over 30 years ago of Langerhan's cells as bone marrow-derived antigen-presenting cells found primarily within the epidermis [31], and the ability of epidermal cells to activate and direct differentiation of T cells through secretion of cytokines and chemokines [32], suggested a model where skin could serve as a site of immune sampling and effector immune function. This network of multiple immunologically active cell types suggests that similar to other tissue-specific coordination between leukocytes and resident cells, the 'skin immune system' is another unique and distinct component of the overall immune system [8].

The appreciation of the scale of the skin immune system changed significantly, however, with the recent enumeration of the T-cell content of normal human skin; for a normal adult the presence of approximately 1×10^6 T cells/cm^2 equates to almost twice the number of T cells circulating in blood. Furthermore, T cells isolated from skin were demonstrated to express the skin-homing addressins cutaneous lymphocyte-associated antigen (CLA) and CCR4, and 80% lacked expression CD62L and CCR4, a phenotype consistent with T-effector memory (T_{EM}) cells. Interestingly, cells that did express CD62L and CCR4 co-expressed CLA and CCR4, suggesting a population with the ability to cycle between skin and draining lymph nodes [9▪▪]. The presence of such a significant population of T_{EM} cells in normal skin stands in contrast to the conventional model where T_{EM} cells remain primarily in the circulation until recruited to the tissues by inflammation. Recent extensions of this study, in both human skin and murine models, have demonstrated that these skin-resident T_{EM} cells do not recirculate, persist for a long term, and provide distributed cutaneous immunity which appears to accumulate over time following repeated infections [33▪,34▪].

T-regulatory cells have also been identified in human skin, where they represent between 5 and 10% of the resident T-cell population [35,36]. $CD4^+$ $FoxP3^+$ T_{REG} cells have also been identified in the skin of hand transplants for several years after transplant [37], although the origin of these cells remains to be defined. A series of in-vitro and in-vivo studies have demonstrated that human skin T_{REG} cells undergo antigen-dependent proliferation when cultured in the presence of IL-15 and dermal fibroblasts [35], and that these cells proliferate *in vivo* under inflammatory condition [38] and may serve to control the inflammatory response *in situ* [39], and following migration to the draining lymph node by interfering with dendritic cell-naïve $CD4^+$ cell interactions [40]. Skin-derived T_{REG} cells have also been shown to contain a $CD25^{hi}$ fraction expressing higher levels of IL-10, transforming growth factor (TGF)-β, and cytotoxic T-lymphocyte antigen-4 than lymph node-resident T_{REG} cells, suggesting potent regulatory activity [41]. Interestingly, it has recently been demonstrated that treatment with corticosteroids induces expansion of T_{REG} cells, through up-regulation of TGF-β secretion by Langerhans' cells [42], suggesting a potential mechanism through which topical application of clobetasol may have been acting in the treatment of acute rejection episodes in patients.

The suggestion of a regulatory function for Langerhans' cells is at odds with their classical description as the first line of defense against invasion of exogenous pathogens [43]. However, recent findings from a murine model of contact hypersensitivity [44] and more recently, studies investigating antibacterial immunity using a human in-vitro model system [45▪] support the hypothesis that Langerhans' cells contribute to immune regulation. In contrast, other recent studies support the classical model demonstrating potent stimulatory activity by Langerhans' cells, including the induction of naïve $CD4^+$ T-cell differentiation into Th2 [46], Th17 [47], and Th22 [48] phenotypes, and priming and cross-priming of naïve $CD8^+$ T cells [49]. This dichotomy may seem counterintuitive, but a recent study investigating the in-vivo function of Langerhans' cells in the presence and absence of pathogen (*Candida albicans*) offers a plausible explanation, demonstrating functional polarization of Langerhans' cells (LC) function, whereby at steady state, LCs were found to selectively induce activation and proliferation of skin-resident T_{REG} cells, but following exposure to pathogen, these cells preferentially activated and induced proliferation of resident T_{EM} cells [10▪▪].

SKIN-SPECIFIC IMMUNOBIOLOGY IN VASCULARIZED COMPOSITE ALLOTRANSPLANTATION TOLERANCE

The experiments contributing to our recently increased knowledge of the scale and complexity of the skin immune system have almost exclusively utilized autologous models of inflammatory skin disease or antimicrobial immune responses. The role and significance of these cellular networks in the context of VCA remain largely unknown, and warrant considerable further research. It is easy to conceptualize that an exchange of cells will occur immediately following transplant, whereby not only would host-derived leukocytes migrate into the VCA, but resident cell populations from the VCA may traffic into the blood and interact with the patient's systemic immune system.

Current work in our laboratory aims to identify the resident and migratory T cells within VCAs, and to identify the mechanisms at play within the skin immune system that dictate the balance between VCA rejection and tolerance, specifically in the context of mixed chimerism protocols. We have recently demonstrated the induction of VCA tolerance, including the critical epidermal component, across MHC barriers in a miniature swine model (Leonard, in submission). In this model, resident populations of T cells, including FoxP3^{+} cells, and antigen-presenting cells of both donor and host origin, can be identified in tolerized VCAs up to 1 year from cessation of immunosuppression by immunofluorescence microscopy. Isolation and analysis of these cells by flow cytometry has confirmed the presence of both chimeric T cell and LC populations (unpublished data). These findings, taken together with previous studies of VCA in which stable mixed chimerism was not achieved and skin was ultimately rejected, might suggest that failure of appropriate signaling between donor and recipient elements within the skin immune system may contribute to the challenge of successful skin transplantation.

In this context, development of clinically applicable tolerance protocols for VCA may necessitate reconsideration of the importance of MHC matching between donor and recipient. It is well established that human leukocyte antigen (HLA) matching correlates with improved outcomes in solid organ transplantation [50]. In contrast, evidence for the significance of HLA matching in VCA is so far lacking and remains largely anecdotal; the recipient of the first successful face transplant was reported to share 5/6 HLA loci with her donor, but she has nonetheless required conventional doses of immunosuppression to prevent rejection [51]. In our VCA tolerance protocol, transplantation was performed between haploidentical donor–recipient pairs; in other words, they shared a single haplotype at both MHC class I and class II. It is conceivable that this haplotype sharing facilitates interaction between Langerhans' cells and T cells of both donor and host origin within the skin, allowing them to contribute to skin tolerance by the same mechanisms through which they contribute to immune homeostasis and control of inflammation in the autologous setting.

CONCLUSION

Immunomodulatory and tolerance protocols are an active area of research for VCA, driven by the need to reduce the morbidity and risk associated with conventional immunosuppressive agents in the context of procedures that are life-enhancing rather than life-preserving. Current regimens effectively prevent early graft loss to rejection; however, the incidence of acute skin-rejection episodes remains high. Introduction of an immunosuppression-minimization protocol for hand transplantation, which, to date, has achieved outcomes comparable to conventional protocols with considerably reduced overall immunosuppression requirements, is encouraging. However, the inherent complexity of VCAs and in particular the considerable immunogenicity of skin has so far precluded introduction of a true tolerance protocol to clinical application.

Recent advances in our understanding of the skin immune system include the identification of large numbers of T cells, both resident memory and T_{REG} phenotype, as well as the expanded role for Langerhans' cells in both homeostatic and regulatory functions mediated by interaction with these resident lymphocyte populations. The functional significance of these cellular networks in the context of VCA warrant further research, and may be of considerable significance in addressing the challenges of skin transplantation and the development of tolerance protocols for VCA.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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