REVIEW

# Fundamental Immunology of Skin Transplantation and Key Strategies for Tolerance Induction

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**Abstract** Transplantation of allogeneic or xenogeneic skin grafts can evoke strong immune responses that lead to acute rejection of the graft tissues. In this process, donor-derived dendritic cells play crucial roles in the triggering of such immune responses. Both the innate and acquired host immune systems participate in graft rejection. At present, the rejection of skin grafts cannot be well-controlled by ordinary systemic immunosuppression therapy. Although several strategies for the long-term survival of allogeneic or xenogeneic skin grafts have been demonstrated in animal models, the induction of long-term tolerance to skin grafts is still a great challenge in clinical settings. In this article, we review the progress in the understanding of immune responses to skin grafts and discuss the possible methods that can decrease the immunogenicity of graft tissues and improve the survival of skin grafts, especially those included in preoperative pre-treatments.

**Keywords** Skin transplantation · Immunology

## Introduction

The successful transplantation of allogeneic organs and tissues has significantly improved the outcomes for patients with severe organ dysfunctional diseases and is one of the greatest surgical and immunological achievements of the twentieth century.

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The transplantation of allogeneic skin grafts is an important therapy for patients with major burns or chronic refractory wounds. However, allogeneic skin grafts evoke potent immune responses. Following the transplantation of skin onto a healthy MHC-mismatched recipient, a quick immune rejection process will lead to the failure of the graft within 10–14 days (Richters et al. 2005) with characteristic skin tissue destruction. Allogeneic skin grafts are rejected in a short time period of approximately 20–30 days, even if grafts are placed over the fully excised burn wound base (Bravo et al. 2000; Hansbrough et al. 1997). This process could hardly be controlled by currently available immunosuppressive agents (Gardner 1995). To date, allogeneic skin grafts serve mainly as temporary biological wound dressings that provide a suitable environment for the survival and expansion of autologous small patch or micro skin grafts (Kreis et al. 1989). In the clinic, autologous skin transplantation is the only viable means of achieving permanent closure of massive wounds and the restoration of skin function.

Although great achievements have been made in the understanding of immune responses to allogeneic and xenogeneic skin grafts, the induction of long-term tolerance to of skin grafts is still a grand challenge for immunologists. In this review, we introduce the latest perspectives on skin transplantation immunology and investigate the potential new technologies and methods that offer the prospects of donor-specific tolerance induction to skin grafts.

## The Characteristics of Skin Graft Immunology

Due to the unique tissue structure and cell constitution of the skin, allogeneic skin transplantation leads to different immune responses than solid organ transplantation.

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The rejection of allogeneic skin grafts is considered primarily to be a model of acute graft rejection. As the skin is not a primary vascularised graft, hyperacute rejection, which is a rapidly occurring reaction induced by pre-existing alloreactive antibodies in blood, rarely occurs (Colletti et al. 1994). Once placed on the wound, the skin grafts must establish their own blood vascularisation. The skin grafts initially survive through the use of oxygen that is present in the wound bed. The primary trauma and tissue injury result in an inflammatory process with the infiltration of inflammatory cells and accumulation of angiogenic factors around the graft. Over the course of several days, angiogenesis will lead to the ingrowth of vessels (Dresel et al. 2002).

Following the vascularisation of the graft, antigen-presenting cells (APCs) from the donor can migrate out of the skin graft through the lymphatic vessels and infiltrate the draining lymph nodes of the recipient. These cells can present donor antigens directly to the host alloreactive T cells (i.e., direct antigen-presentation) or through the host APCs (i.e., indirect antigen-presentation), both of which can lead to the activation of host T cells (Benichou et al. 2011) (Fig. 1a).

Once activated, cytotoxic CD8$^+$ T lymphocytes (CTL) and CD4$^+$ T helper (Th) cells become effector cell if specifically recognised alloantigens are encountered. CTL activity, mediated by the perforin–granzyme and Fas–Fas ligand pathways, induces the antigen-specific lysis and apoptosis of donor alloantigen-expressing target cells in the graft, leading to an acute rejection of the graft. Th cells aid the activation and function of CD8$^+$ T cells, which, in turn, cause chronic transplant rejection (Benichou et al. 2011; Binet and Wood 2003).

Although the rejection of allogeneic skin transplants is principally a T cell-dependent process, inflammation mediated by the innate immune network also contributes to graft-specific immune responses. Transient inflammation at the early stages of transplantation has a marked effect on allograft rejection that is associated with the infiltration of natural killer (NK) cells (Habiro et al. 2005). Conversely, the alloimmune response enhances inflammation injuries. Sensitisation by alloantigens can facilitate innate tissue inflammation and organ injury via a non-specific CD154-dependent mechanism (Shen et al. 2010). In alloresponses, keratinocytes may be activated and recruited as non-professional APCs, leading to an amplification of the inflammatory response that may be associated with the susceptibility of skin to immune rejection (Binet and Wood 2003; Haw 1995).

Another important immune cell type involved in the rejection or tolerance of skin grafts is the NK cell. In the conventional theory, NK cells participate in transplantation rejection as effector cells, which can mediate cytolytic effects against allogeneic and xenogeneic cells via the antibody-dependent cell-mediated cytotoxicity mechanism (Kumagai-Braesch et al. 1998) and produce a variety of proinflammatory cytokines that promote the maturation of APCs and indirectly amplify T cell activation (Kroemer et al. 2008a). Kroemer et al. (2008b) reported that NK cells could reject skin allografts when stimulated with interleukin (IL)-15 in the absence of adaptive immune cells. However, recent studies have demonstrated that NK cells can kill allogeneic graft-derived APCs and prolong the survival of skin grafts through the inhibition of direct T cell priming or the elimination of activated alloreactive T cells in a perforin-dependent mechanism (Beilke et al. 2005; Yu et al. 2006). Thus, it is possible that NK cells have bidirectional effects on skin graft rejection and tolerance.

## The Immune Effects of Dendritic Cells in Skin Grafts

In the immune rejection of skin grafts, the Langerhan's cells (LCs) are important APCs that can trigger a T cell response. LCs are a special and major subpopulation of dendritic cells (DCs) that reside in the epidermis and are most prominent in the stratum spinosum regions. LCs are distinguished from other DCs by the expression of the haematopoietic marker CD45 (LY5), the non-MHC class I molecule CD1a, the intercellular adhesion molecule E-cadherin (CDH1), and the novel C-type lectin Langerin (Merad et al. 2008). Along with this unique phenotype, LCs are estimated to have much longer lifespans and stronger proliferative capacities compared with other DCs (Hemmerling et al. 2011; Vishwanath et al. 2006). These features empower LCs with a potent capacity for antigen presentation and a strong immunogenicity in allotransplantation. Further research confirmed that the immunogenicity of skin grafts is correlated with the distribution and density of LCs (Chen and Silvers 1983). Therefore, it has been widely accepted that donor-derived LCs play an important role in immune responses to allogeneic skin grafts.

Although LCs are the most numerous DC subtype in the skin, other donor-derived APCs in the graft, including dermal DCs and macrophages, can also participate in graft rejection (Benichou et al. 2011; Morelli et al. 2005). The encounters of these APCs with alloreactive recipient T cells result in direct alloantigen recognition, which can mediate acute immune responses to allogeneic skin grafts (Kazansky 2008).

Alloantigen recognition and graft-specific T cell activation occur by three mechanisms: the direct, the indirect and the semi-direct pathways (Jiang et al. 2004). Although the indirect pathway is the most common manner by which T cells detect antigens and is sufficient to induce graft

rejection, the direct pathway plays an important role in immune responses to skin grafts. In the direct recognition pathway, donor-derived APCs present intact donor MHC molecules but not peptide fragments to host T cells and induce a potent polyclonal T cell response in which up to 10 % of the entire peripheral T cell repertoire is activated, leading ultimately to the rejection of MHC-mismatched grafts (Benichou et al. 2011). Thus, the epidermal LCs and dermal DCs, as the major donor-derived APCs in skin graft, have been shown to be crucial for acute immune responses in skin transplants.

In addition to the vigorous ability to activate T cell responses, in transplantation, the donor LCs can also induce a special immune response that is independent of costimulatory signalling and the nuclear factor (NF)-κB pathway. The full activation of naïve T cells requires stimulation through both the T cell receptor (TCR) and the costimulatory molecule CD28. These events trigger the activation of several intracellular signalling pathways that lead to T cell proliferation and clonal expansion, cytokine secretion, cell survival, differentiation, and memory cell generation. Signalling through the canonical NF-κB pathway is critical for proper T cell activation. In certain cases, inhibition of either the costimulatory signal or the NF-κB pathway could successfully abrogate the acute allograft rejection process (Finn et al. 2001).

However, a recent study showed that LCs can induce T cells to reach an activation threshold that is sufficient for transplant rejection in the absence of a costimulatory signal or under the condition of impaired NF-κB pathway, which indicates that LCs can prime T cells through a bypass signal pathway (Molinero et al. 2008; Rulifson et al. 2002). This finding may explain why certain immune inhibitors, such as rapamycin, which target NF-κB, do not display the expected effects in skin transplantation models that have abundances of LCs (Sun et al. 2008).

Although donor-derived LC-mediated antigen recognition and rejection have been widely accepted, LCs may also participate in transplantation tolerance. In a recent study, Obhrai et al. (2008) showed that in a transgenic LC-ablation skin transplantation mouse model, the complete absence of LCs could abrogate the long-term survival of minor antigen-mismatched skin grafts, which indicates the possibility that donor LCs are essential for the maintenance of graft tolerance. Thus, the exact contribution of LCs to immune reactions against skin grafts is still a matter of discussion.

## Skin Graft Pre-Treatment

Currently available systemic immunosuppressive agents cannot effectively prevent the rejection of skin grafts.

Moreover, because the majority of skin graft recipients have defects in skin barrier function, immunosuppressive therapy can significantly increase the risk of infection. Therefore, pre-operative treatment of skin allografts to decrease immunogenicity is considered to be a putative solution to improve skin graft survival or even induce long-term tolerance.

### Depletion of Donor-Derived DCs

Because of the crucial role of donor-derived DCs in the rejection of skin grafts, it was hypothesised that the depletion of these cells in grafts or a blockade of the direct antigen recognition process may improve the survival of skin grafts.

9,10-Dimethyl-1,2-benzanthracene (DMBA) was the first chemical agent observed to effectively deplete LCs from the epidermis, as determined by ultrastructural examination (Halliday and Muller 1986). When transplanted onto MHC-mismatched recipients, DMBA-treated mouse skin grafts had a prolonged survival time compared to control skin grafts (Odling et al. 1987b). However, because DMBA is a potent chemical carcinogen, the application of DMBA-treated skin grafts to human beings is impractical.

Other than chemical agents, short wavelength ultraviolet light (UV) irradiation can successfully eliminate LCs in skin grafts. Odling et al. (1987a) demonstrated that UVB irradiated skin grafts were depleted of LCs and had significantly longer survival times. We have also used UVC as a tentative pre-treatment for cadaver grafts. In this study, UVC pre-treatment significantly lowered the numbers of APCs in skin samples and thus induced longer survival times of alloskin grafts on patients with full skin thickness burn wounds when combined with an anti-β2 microglobulin monoclonal antibody (β2 mAb) preincubation (Wu et al. 1996). The mean survival time of skin graft survival was 37 ± 8 days in the UVC with anti-β2 mAb group, which was approximately 50 % greater than that observed for the group that was pre-treated with anti-β2 mAb alone.

However, the LC-free cultured allogeneic epidermal sheets showed only a slightly prolonged survival time (4–5 days) when used as a wound coverage in humans (Aubock et al. 1988), which raises doubts that the depletion of LCs in skin grafts might be clinically viable.

### Inhibition of Donor DC Antigen Presentation

As increasing numbers of research studies indicated that donor-derived DCs contribute to the induction and maintenance of allograft tolerance (Jung et al. 2011; Obhrai et al. 2008), blocking the antigen presentation process in DCs was considered to be a possible and potentially effective therapy for the induction of skin graft tolerance.

McMinn et al. (1990) reported that gliotoxin, a member of the epipolythiodoxopiperazine family of fungal metabolites, could significantly reduce epidermal LC densities and alter the morphology and function of LCs. Skin grafts that were pre-treated with gliotoxin showed prolonged survival times on MHC-mismatched recipients. When placed onto haplotype-mismatched recipients, gliotoxin pre-treated skin grafts were accepted permanently and were able to induce donor-specific tolerance (McMinn et al. 1990). Other research demonstrated that gliotoxin pre-treatment could prolong the survival of thyroid grafts after transplantation into allogeneic recipients (Sutton et al. 1995). The successes of these grafts demonstrate the potential for gliotoxin as an immunomodulatory agent in organ transplantation. However, a recent study indicated that gliotoxin could cause cell necrosis and apoptosis, especially of Kupffer cells (Anselmi et al. 2007), which raises concern about risks associated with gliotoxin pre-treatment.

Norcantharidin (NCTD), the demethylated form of cantharidin, is a water-soluble synthetic small molecule with immunomodulatory functions, especially of cell lineages that originate from the bone marrow, such as DCs (Chen et al. 2008). NCTD modulates the activity of DCs and inhibits the expression of CD1a, DC-SIGN, and CD83 in a dose-dependent manner. NCTD-treated DCs also stimulate the proliferation of allogeneic CD4$^+$Foxp3$^+$ regulatory T cells, which might contribute to the induction of donor-specific tolerance. NCTD treatment also resulted in significantly prolonged skin allograft survival in a mouse model ($18.2 \pm 2.3$ days) in comparison to the non-treated group ($11.6 \pm 0.9$ days) (Hsieh et al. 2011).

Although the above-mentioned agents appear to be good prospects in the promotion of skin engraftment, the toxicities of these agents might restrict their clinical applications to a certain extent. Photodynamic therapy (PDT) promises to be a novel therapeutic procedure for immune regulation without obvious adverse effects (Mroz and Hamblin 2011). PDT significantly downregulated the expression of MHC and the costimulatory molecule B7 (60–90 % reduction) on LCs, and this treatment effectively suppressed the ability of LCs to stimulate alloreactive T cells to proliferate. An experiment showed that the pre-treatment of skin grafts with low-dose PDT could significantly prolong the survival of allografts in a mouse model, from $9.3 \pm 2.2$ to $16.9 \pm 1.7$ days (Obochi et al. 1997). Furthermore, an upregulation of IL-10 protein expression (up to 2.85-fold) was observed in PDT-treated keratocytes in vitro (Byun et al. 2009), which may also contribute to the protective effects in skin allografts.

Prevention of Oxidative Damage

During allograft rejection, graft-infiltrating inflammatory cells produce high levels of reactive oxygen species (ROS).

These potent oxidants can damage cell membranes and proteins, which are involved in the rejection of allogeneic grafts. Antioxidant therapy has been documented to suppress allograft rejection through the prevention of inflammation and ischemic reperfusion injuries (Ma et al. 2008).

As the skin is a non-primary vascularised graft, more time is required to establish blood flow. The ischemia–reperfusion process produces an abundance of ROS, which aggravates damage to the grafts (Baines and Shenkin 2002). A synthetic superoxide dismutase and a catalase mimetic have been proven to protect grafts from ROS injury (Gianello et al. 1996). Tocco et al. used complexes of salen manganese (Salen-Mn), a metal scavenger of ROS that exhibits superoxide dismutase and catalase activities, to inhibit the rejection of fully MHC-mismatched allogeneic skin grafts in mice. The pre-treatment of C57BL/6 donor skin with Salen-Mn complexes strongly impaired the CTL-mediated alloresponse and significantly delayed allograft rejection (Tocco et al. 2006), which indicates that ROS scavengers are promising candidates for the improvement of skin graft survival.

Modification of Xenogeneic Skin Grafts

It is well-known that allogeneic skin transplantation is an effective protective dressing for patients with massive skin defects; however, the gap between the increasing demand for graft tissues and the limited sources necessitates the discovery of a substitute for cadaver skin. Xenogeneic skin grafts have advantages in both biological activity and availability compared with artificial skin or tissue-engineered autologous skin. Among the mammals, porcine skin has a strong resemblance to human skin with regards to the dermal tissue structure, collagen arrangement, and follicle density (MacLeod et al. 2004). Research has also shown that porcine split-skin grafts are easily prepared, stored and preserved with regard to biological activities (Chiarini et al. 2007). Therefore, porcine skin has been considered as a possible substitute for cadaver skin. However, despite similarities between allogeneic and xenogeneic skin grafts, immune rejection is still a huge obstacle to the use of porcine skin grafts in the long-term coverage or replacement of skin.

At one time, the hyperacute rejection that was mediated by a natural antibody reaction to the Galα1, 3Gal oligosaccharide on porcine endothelial cells was commonly described as the greatest barrier to the induction of immune tolerance to porcine xenografts (Vaughan et al. 1994). However, unlike vascularised solid organ grafts, the remaining vessels in partial-thickness skin grafts are not often anastomosed with the recipient vessels. A further

study also confirmed that xenogeneic skin grafts did not elicit stronger Gal-mediated rejection when compared to allogeneic grafts (Gock et al. 2004). Therefore, the rejection of porcine skin grafts is a predominantly cell-mediated response. Based on this premise, many bioengineering or genetic modification strategies have been developed to reduce the vulnerability of porcine grafts to acute rejection (Fig. 1b–g).

Bioengineering Modifications of Skin Graft

Skin grafts are isolated from vascular circulation when first implanted with a corresponding delay in revascularisation. This delay was traditionally thought to result in ischemic injuries to skin grafts. However, the delay may provide a window of opportunity in which to block the encounters between immune cells and graft antigens or to interrupt the immune effector cell cascade through bioengineering modifications to the grafts.

In recent years, an acellular matrix membrane has been widely studied as a bioengineering modification technique for the improvement of allogeneic and xenogeneic graft acceptance. These acellular membranes, which are mainly composed of an array of laminin, vitrogen, fibronectin, and type IV collagen, can provide an apical surface that remains non-thrombogenic and non-immunogenic. This network of molecules, which comprises the membranes, functions as a barrier to antigen recognition and immune cell migration while simultaneously permitting the free diffusion of nutrients and oxygen (Stubenitsky et al. 2009).

As the vascular endothelial cells are major targets for immune responses, this novel technique was first applied to the vasculature of solid grafts, such as kidney grafts. Pretreatment of a kidney graft with a nano-membrane provided ubiquitous coverage of approximately 90 % of the vascular luminal surface, including both small and large blood vessels, which significantly delayed the onset of rejection without apparent adverse effects on renal function (Brasile et al. 2010).

When used as an artificial interface between skin allografts and wound surfaces, nano-membranes also significantly prolonged graft survival times (28 $\pm$ 3.8 vs. 6.8 $\pm$ 1.5 days) without immunosuppression. Recent studies have implied that these nano-membranes might also serve as drug delivery systems to further improve graft outcomes (Brasile et al. 2011; Stubenitsky et al. 2009). These results demonstrated that the nano-membrane might be a promising method with which we can protect skin grafts from acute rejection.

Genetic Modification of Skin Grafts

Gene transfer can provide cells with an expressible cloned DNA sequence that encodes a functional protein, with the intent to achieve a therapeutic effect. In gene transfer therapy for the induction of transplantation tolerance, two strategies have been pursued: modification of the graft or modification of the recipient.

Due to the risk of complications associated with the use of viral vectors, the use of gene therapy for patients in the clinic is still years away. Thus, the modification of candidate genes that alter the immune functions of the host, including BAFF, PDL1 and Foxp3, are not viable, although these genes have been proven effective in the improvement of graft tolerance (Ghazizadeh et al. 2012; Walters et al. 2009). To the contrary, it is more practical to construct transgenic animals for use as xenograft donors; therefore, genes and molecules that can ameliorate insults to graft tissues have been studied widely as targets for genetic modification.

Antigen presentation by donor DCs has been well-demonstrated as sufficient to initiate the acute rejection of skin grafts. Thus, the transduction of immunomodulatory genes that can inhibit the function of donor DCs might improve tolerance to skin grafts.

CD200, a membrane glycoprotein that is expressed mainly on the surface of DCs, can transmit an inhibitory signal to certain subsets of T cells that express CD200R. The CD200/CD200R interaction can suppress the phosphorylation of the ERK1/2 mitogen-activated protein kinase in responding T cells and contribute to the production of tolerogenic T cells (Gorczynski 2005). Transgenic overexpression of CD200 significantly attenuated the rejection of allografts through the suppression of inflammation and the upregulation of genes that encode Foxp3, transforming growth factor $\beta$, IL-10, and PD-1/PD-L1/PD-L2 in skin grafts, as well as an increased expression of mRNAs for indoleamine 2,3-dioxygenase (Yu and Gorczynski 2012). However, in this study, both donors and recipients were transfected with CD200; thus, the effect of CD200 overexpression in the graft alone requires further evaluation.

Suppressors of cytokine signalling (SOCS) proteins play pivotal roles in the regulation of macrophage, T cell, and DC activation, development, and differentiation. Silencing of the SOCS1 gene in DCs through RNA interference can contribute to DC maturity and thus increase immune function. Fu et al. (2009) reported that SOCS1 gene-transduced DCs had lower expression levels of CD40 and CD80 and secreted high levels of IL-10. An infusion of these transgenic DCs prolonged the survival of cardiac allografts, which correlated with a substantial increase in the generation of regulatory T cells (Fu et al. 2009). These results suggest that SOCS-1 gene transfer may also improve skin graft survival through the inhibition of donor-derived DC function.

Full T cell activation requires a costimulatory signal in addition to the engagement of the TCR with the antigen-MHC complexes on APCs. The lack of a costimulatory

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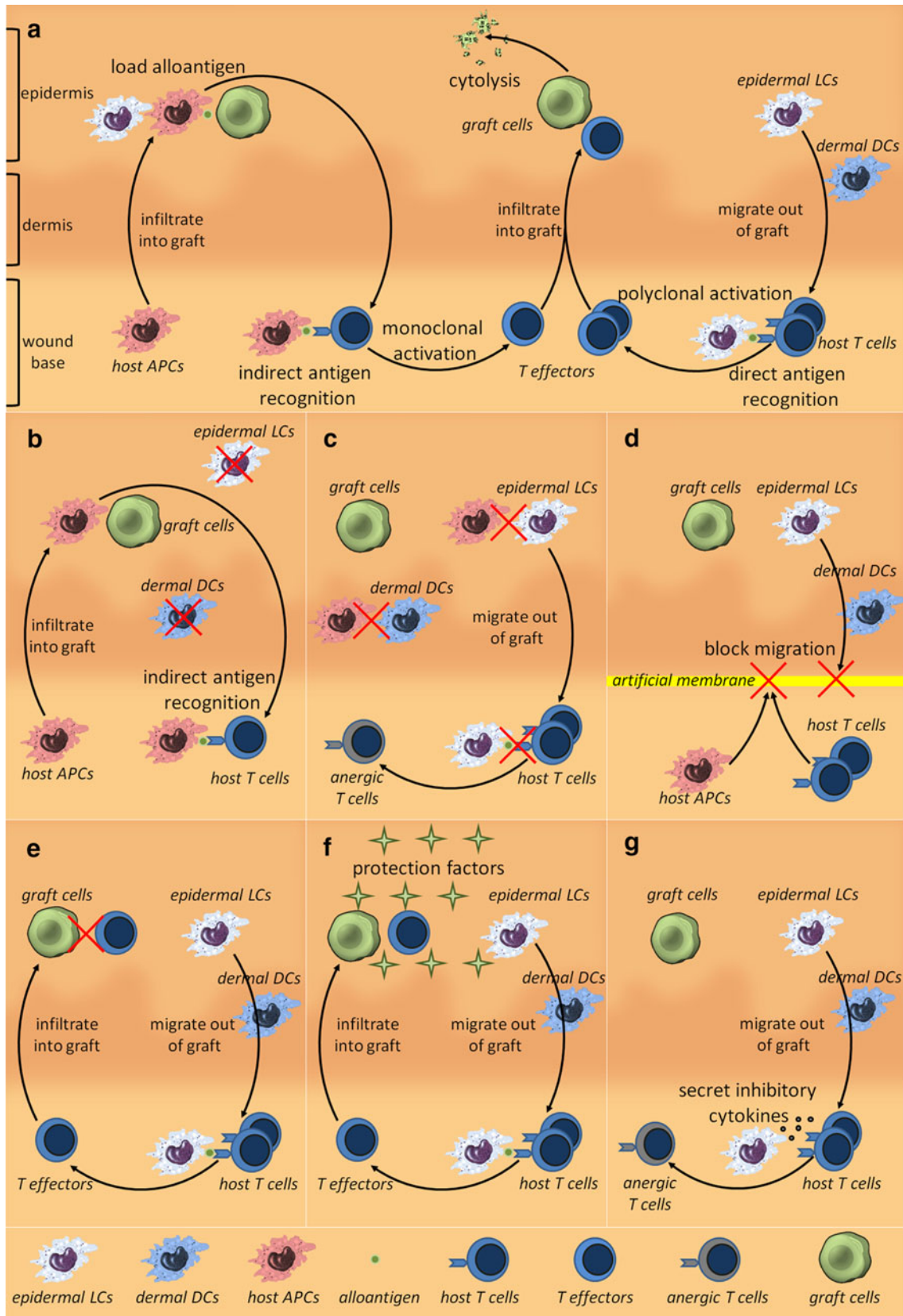
◀ **Fig. 1** Immune responses to skin grafts and putative strategies for tolerance induction. **a** Immune responses to skin grafts. **b** Depletion of donor-derived APCs in the skin graft to block the direct antigen recognition pathway. **c** Inhibition of the antigen-presenting function of donor-derived APCs or modification of the balance of costimulatory signals to intercept the normal activation process in the host's alloreactive T cells. **d** Blocking of the interactions between host immune cells and graft antigens with an artificial matrix membrane. **e** Importation of protective factors into skin grafts through engineering or genetic modifications to reduce damage to graft cells. **f** Inhibition of host T cell function at the effector stage. **g** Upregulation of inhibitory cytokine expression by genetic modification

signal after the engagement of the TCR with cognate antigen results in a partial activation of T cells, which are rendered unresponsive to the encountered antigen, which is a state known as anergy (Durrbach et al. 2010).

Cytotoxic T lymphocyte-associated antigen 4 (CTLA4, CD152) is the most important negative costimulatory signal and acts competitively against CD28. CTLA4-immunoglobulin (CTLA4-Ig) is a soluble chimeric protein that consists of the extracellular domain of CTLA4 at the N-terminus and the constant and hinge regions of IgG at the C-terminus. With the extracellular CTLA4 domain, CTLA4-Ig binds B7 in the same manner as full-length CTLA4 and functions as a competitive inhibitor of the B7 or CD28 costimulatory pathways.

The soluble CTLA4-Ig Belatacept (trade name *Nulojix*) has proved to be an effective immunosuppression agent and has been approved for maintenance therapy after renal transplantation. However, the systemic administration of CTLA4-Ig might lead to an increased risk of viral infections or lymphoproliferative disorders (Larsen et al. 2010). Our group has successfully induced a skin-specific overexpression of human CTLA4-Ig in transgenic murine and porcine models (Luo et al. 2012; Wang et al. 2006). The transgenically expressed CTLA4-Ig protein suppressed lymphocyte proliferation in vitro. The survival of transgenic mouse skin grafts on rat wounds was prolonged remarkably compared to wild-type skin grafts from the same mouse strain ($13.7 \pm 4.8$ vs. $7.2 \pm 1.8$ days; $p < 0.01$). Transient levels of circulating CTLA4-Ig protein were detected in recipient sera; however, no extensive immunosuppression was observed (Wang et al. 2008).

When the CTLA4-Ig transgenic porcine skin grafts were applied to patients with partial-thickness burn wounds, the transgenic skin grafts also showed prolonged survival times of approximately 5 days compared with grafts transfected with an empty vector. In a multi-centre, randomised parallel control clinical trial with 200 patients, we observed no detectable systemic side effects that were associated with CTLA4-Ig-gene transfected grafts (Luo et al. 2012).

OX40 (CD134) is a tumor necrosis factor (TNF) receptor related costimulatory molecule that strongly regulates the division and survival of conventional CD4$^+$ and CD8$^+$ T cells and the cross-talk of these cells with APCs (Croft 2010). In a recent study, the ex vivo transfection of allogeneic skin flaps with an OX40-immunoglobulin (OX40-Ig) expressing lentiviral vector led to a significantly increased survival time that was correlated with the diminished secretion of IL-2 and interferon (IFN)-γ. When OX40-Ig was transfected in combination with CTLA4-Ig, the protective effects on the allografts were further enhanced (Zhang et al. 2012).

Cytokines are other important factors that affect transplantation immune responses. The balance among the immunoregulatory cytokines can determine the outcome of the T cell response. The overexpression of cytokines with negative immunoregulatory functions is assumed to improve graft survival. The local overexpression of IL-10 in donor hearts by an ex vivo intracoronary infusion of a liposome-mediated vector significantly decreased both CD4$^+$ and CD8$^+$ T cell responses and expression levels of Th1 cytokine genes such as IL-2, IFN-γ, and TNF-α, which led to the prolonged survival of allogeneic hearts in animals (Furukawa et al. 2005; Hong et al. 2002).

Although IL-10 transduction significantly improved the survival of some solid organ grafts and studies showed that IL-10-transduced foetal liver cells could also prolong the survival of mouse skin grafts (Hase et al. 2005), the effects of local IL-10 overexpression on skin grafts, which are more difficult immune rejection models, are still not confirmed.

## Conclusions and Future Directions

In summary, the transplantation of allogeneic and xenogeneic skin grafts is associated with potent immune responses that involve the migration of donor-derived DCs, antigen recognition, activation of host T cells, and ultimately, the elimination of donor cells and the destruction of grafts. Nevertheless, the immune system is a complicated network. In this process, immune cells, cytokines and signal pathways interact with one another and together lead to graft rejection. The long-term survival of a skin graft requires the delicate control of immune homeostasis to direct the outcome from rejection to donor-specific tolerance.

However, owing to the limited conditions and techniques, current studies normally focus on one factor at a time, and as a consequence, the interactions between these elements have not been thoroughly elucidated. Thus, the results that were obtained in accurately controlled experimental conditions could not always be replicated in other situations or applied in clinical settings. An insight into the interactions between the immune cells that are involved in

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graft rejection and the balance of activating and inhibitory signals in the immune response could help us to find new targets for therapies or effective methods for tolerance induction.

According to current research results, graft pre-treatments or modifications may be promising methods with which to decrease immune responses and improve the survival of skin grafts without general immune suppression. However, in most studies, graft pre-treatments and modifications have exhibited only modest effects. Thus, engineering or genetic modifications may enhance the availability of skin grafts as temporary wound dressings but are not enough to induce long-term donor-specific tolerance. Graft pre-treatments might be combined possibly with sub-therapeutic immunosuppressive drug treatments to augment the effects of pre-treatment and avoid side effects from systemic immunosuppressive therapy.

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