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REVIEW

Skin tolerance: in search of the Holy Grail

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Summary

In 1943, Gibson and Medawar opened the modern era of transplantation research with a paper on the problem of skin allograft rejection. Ten years later Billingham, Brent and Medawar demonstrated that it was possible to induce selective immune acceptance of skin grafts in mice, a state of tolerance. After over six decades, however, the precise mechanism of skin allograft rejection remains still ill-defined. Furthermore, it has not been possible to achieve reliably clinical tolerance allowing the widespread application of skin allotransplantation techniques. The first successful applications of skin allotransplantation have included the hand and face. However, complications from the chronic immunosuppression regimens limit the application of these techniques. Induction of tolerance to skin (and the other tissues in the allograft) would be the most effective way to overcome all these difficulties, but this is yet to be achieved reliably, stimulating some to look for other ways to surmount the current limitations. This paper summarizes alternatives to enlarge the scope of skin allotransplantation techniques, current understanding of mechanisms of skin rejection, and the utility and limitations of animal models used to study skin rejection and tolerance induction. Finally, manipulation strategies to achieve skin tolerance are outlined.

Introduction

In 1943, Thomas Gibson and Peter Medawar opened the modern era of transplantation research with a paper on the problem of skin allograft rejection [1]. Ten years later, taking into account the observations by Owen that naturally occurring chimeric twin calves accepted reciprocal skin grafts [2], Billingham, Brent, and Medawar went on to demonstrate that it was possible to induce selective immune acceptance of skin grafts in mice, a state of tolerance [3]. After over six decades, however, the precise mechanism of skin allograft rejection remains still ill-defined. Furthermore, it has not been possible to achieve reliably clinical tolerance allowing the widespread application of skin allotransplantation techniques.

Autologous skin transplantation is the only available method for adequate reconstruction for many severe defects, but does not always result in satisfactory cosmesis and functional outcomes. Over the last 9 years, the first successful applications of skin allotransplantation have occurred, including 25 hands [4], eight abdominal walls [5], scalp [6], leg, and the three recent partial faces [7]. These have been possible because of the efficacy of modern chronic immunosuppression regimens. The life-enhancing benefits of these allotransplantations have to be weighed against the side-effects from immunosuppression and the risk of chronic rejection. Ultimately, only the patient can assess the balance of benefits and risks; but, improvements in achieving the acceptance of skin allotransplants will promote wider application of skin allotransplantation techniques. Induction of tolerance to skin and the other tissues within the graft would be the most effective way to overcome all these difficulties, but this is yet to be reliably achieved stimulating

some to look for other ways to surmount the current limitations.

This paper summarizes the alternatives to enlarge the scope of skin allotransplantation techniques, the current understanding of mechanisms of skin rejection, and the utility and limitations of animal models used to study skin rejection and tolerance induction. Finally, the manipulation strategies that have been explored to achieve skin tolerance are outlined.

Routes to widespread application of skin allotransplantation techniques

There are three options to overcome the difficulties limiting the expansion of the use of skin allotransplantation: (i) reduction in the toxicity of chronic immunosuppression, (ii) reduction in the dose of immunosuppression by induction of a less alloreactive state, and (iii) obviating the requirement for immunosuppression by tolerance induction.

Reduction of chronic immunosuppression regimen toxicity

The development of novel and less morbid immunosuppressants opened the way for the successes achieved thus far in composite tissue allotransplantation. In the short term, reduction in toxicity of chronic immunosuppression regimens may be most easily achievable with more specific systemic immunosuppressive therapies or the use of site-specific therapies with reduction in, or removal of, systemic immunosuppression.

Future immunosuppressants are likely to offer only modest toxicity reduction on current medications as it is difficult to suppress selectively the graft alloresponse without influencing immune response to other stimuli. Site-specific therapies have been used with some success to treat early rejection episodes in some of the hand transplant recipients [4]. However, it remains unclear whether this is an effective clinical strategy for reducing the maintenance dose of systemic immunosuppression; in small animal models, indefinite skin survival has not been achieved using site-specific therapies [8–10].

Induction of a less alloreactive state

Some have speculated that the initial hand transplants may coincidentally induce a less alloreactive state because of the donor bone marrow in the graft: hand transplant recipients have required less immunosuppression than was initially expected with stable graft function using dosage regimes comparable with renal allotransplants despite the presumed higher antigenic load caused by the inclusion of skin in the transplant. In addition, cells with a regulatory phenotype (CD4 + CD25 + FoxP3 +) were detected in the allograft dermis of one of the French hand transplant recipients [11], although the functional significance of this remained unclear as the patient was still on immunosuppression.

Interventions to reduce alloreactivity have not been effective for skin allotransplantation. Antithymocyte globulin (ATG) and anti-CD25 monoclonal antibodies were administered in two of the hand transplants [4], anti-CD52 monoclonal antibodies in abdominal wall allograft transplants, and post-transplant bone marrow infusion in the first French face transplant (based on the regimes used in organ transplants [12]) all with no measurable success.

Tolerance

The ultimate goal for skin transplantation is to achieve donor-specific tolerance. This will avoid risks from chronic medication, and possibly the risk of chronic rejection. This goal has been shown to be clinically achievable in renal transplantation [13], with further work required to improve the reliability of the regimen. Skin holds the unenviable title of being the most difficult of all tissues to achieve a state of tolerance when transplanted. However, there are anecdotal reports of skin tolerance in patients [14,15], indicating that clinical skin tolerance is achievable.

Mechanisms of skin rejection

It has long been thought that transplanted skin is more susceptible to rejection than other tissues [16,17]. Four factors that may contribute to skin's particular susceptibility to rejection are its usual mode of transplantation, skin-specific alloantigens, its composition, and allograft size.

Mode of transplantation

The method of skin allograft transferral may influence its immunogenicity: primarily vascularized skin allografts have a small survival advantage over secondarily vascularized skin allografts in some studies [18,19]. Possible mechanisms for the difference in immunogenicity between primarily and secondarily vascularized skin allografts are initial post-transplant ischemic damage and the route of interaction of the allograft with the recipient immune system.

Ischemic damage

In a primarily vascularized allograft, vessels supplying the skin are anastomosed to recipient vessels establishing an

immediate blood supply to the skin and minimizing any ischemic damage. In contrast, in a secondarily vascularized graft, there is a period of relative ischemia for the first 48–72 h until the microvasculature connects to vessels in the wound bed. This causes degeneration and even death of the epidermis [20,21], stimulating an inflammatory response within the graft, which could be a trigger for rejection.

Route of immune interaction

It is likely that the trafficking of immune cells differs radically between primarily and secondarily vascularized skin allografts immediately following transplantation.

There are little data regarding primarily vascularized skin allografts. However, extrapolating from primarily vascularized heart transplant data in mice, it is likely that initial influx and efflux are mainly via the bloodstream involving both recipient and donor dendritic cells [22]. In contrast, initial cell trafficking in secondarily vascularized skin allografts is via lymphatics, as demonstrated by the prolonged survival of secondarily vascularized alymphatic skin allografts [23,24], with no evidence of recipient dendritic cell involvement [25].

The route of sensitization itself may not be a reason for the possible difference in immunogenicity between primarily and secondarily vascularized skin allografts, rather its effect on the maturity, function, and final destination of the dendritic cells [26–28].

Both the route of immune interaction and ischemic damage may contribute to skin's antigenicity. However, the mode of transplantation does not fully explain skin's immunogenicity as primarily vascularized skin is still more easily rejected than other tissues [29].

Skin-specific antigens

The proposal that the susceptibility of skin to rejection is caused by expression of tissue-specific antigens [30,31] was based on the observation that in certain chimeric rodent models, allogeneic donor bone marrow was accepted while skin was rejected. Three skin-specific antigens have been described in mice: Skn-1, Skn-2, and Epa-1.

Skn antigens

Skn antigens (Skn-1 and Skn-2) seem to be truly skin-specific. However, some chimeras accept skin grafts despite making Skn antibodies [32]. The reason for this disparity may be that Skn antigens are not transplantation antigens; acute rejection is T cell mediated, whereas Skn antigens are primarily serologically defined, with incomplete evidence that they can stimulate a T-cell response.

Epa-1 antigen

Epa-1 antigen can stimulate T-cell mediated skin rejection, and has a possible homolog in humans; however, it is not skin-specific. Consequently, Epa-1 can trigger rejection of other tissues (e.g. heart) [18]. The other tissues on which Epa-1 is expressed are less immunogenic than skin, suggesting that the cause of skin antigenicity is not just Epa-1.

Skin composition

Skin forms a barrier to the outside world. It is conceptually coherent that for skin to act as an effective first line of defense to any pathogen, it is biased toward a rejection response. The intestine and the lungs perform a similar barrier function and are also particularly susceptible to rejection [33–35]. The cells that make up the skin and dermal structure may both play a role in the particular susceptibility of skin to rejection.

Cells within the skin

The term 'skin immune system' was coined by Bos [36] to indicate that skin is an immunologic organ, with approximately half of its cells having immunologic function. Of the many specialized immune cells within the skin, Langerhans cells are likely to be the most important; the immunogenicity of skin allografts correlates directly with the density of Langerhans cells it contains [37–40]. However, skin allografts from class II knockout mice are acutely rejected at the same rate as wild-type skin grafts [41] demonstrating that direct stimulation by donor class II expressed on these cells is not the sole cause of skin's rejectability.

Dermal structure

The dermis is composed predominantly of collagen and glycosoaminoglycan matrix, which are only weakly immunogenic [42,43]. However, this highly structured environment contains a high concentration of lymphocyte adhesion molecules, thereby making an ideal platform from which effector cells can mount an immune response. Furthermore, the dermis is highly vascular, which allows for rapid immune cell trafficking to the skin.

Graft size

The volume of tissue within the allograft may affect the immune response. Evidence for this comes from both murine models and clinically. In a minor mismatch mouse transplant model, smaller skin and cardiac grafts are rejected acutely whereas larger grafts can avoid acute rejection and are rejected more slowly [44]. In clinic, it has been observed that there is a lower incidence of acute cellular

rejection in recipients of larger volume kidney allografts [45,46]. The correlation between allograft size and avoidance of acute rejection puts transplanted skin at a relative disadvantage as the average skin allograft contains a lot less tissue than the average organ allograft. Additionally, the volume of skin required to avoid acute rejection may be proportionately more than other tissues [47].

The difference in the speed of rejection between large and small grafts appears to be at the effector stage rather than the priming stage [44], and may be because of immunomodulation as well as the influence of graft volume:donor-reactive T-cell ratio. A larger graft may stimulate a stronger regulatory T-cell response than a small graft; these, in turn, may down-modulate the rejection response [48]. Graft size can also influence the speed of rejection by changing the ratio of graft volume to number of donor-reactive T cells. Immediately following transplantation, a threshold number of donor-reactive T cells has to be reached to reject acutely an allograft of a certain size [44,47]. A graft recipient may have enough donor-reactive T cells to reach the threshold required to cause acute rejection of small graft, but this may only be a sub-threshold for rejection of a larger graft.

Graft size may play a role in making the skin more susceptible to acute rejection, particularly in MHC-matched minor-mismatch models. However, the influence of graft size is limited to acute rejection; there is no evidence that larger grafts have a lower incidence of chronic immune damage.

In summary, no single dominant mechanism for skin's antigenicity and susceptibility to rejection has been identified. The mode of transplantation, skin-specific antigens, the composition of skin and allograft volume may all contribute, but more research is required to understand further their specific roles.

Utility of animal models in the development of a strategy for clinical skin tolerance induction

Skin tolerance can be reliably induced across major histocompatibility complex barriers in several small animal models; this has not been possible in large animals or humans. The difficulty in translating between small and large mammals is likely to be because of differences between the models including resilience to toxic induction regimes, endothelial MHC Class II expression, and bystander activation.

Resilience to toxic induction regimes

Rodents are resilient to treatments that cause significant morbidity and mortality in large animal models and the clinic (e.g. lethal irradiation) [49]. This may be partly because of by extrinsic factors such as the highly controlled environment small animals are kept in with minimal exposure to infections, as well as their much shorter lifespan, with death occurring before many complications can develop. However, they are also intrinsically less susceptible to certain complications, such as thrombo-embolism following co-stimulatory blockade [50].

Vascular endothelium immune function

Vascular endothelium is a likely principle target for the host–antigraft response. There are significant differences in the expression of molecules involved in the immune response on rodent versus human and large animal endothelial cells [51]. For example, large animals constitutively express MHC Class II on their endothelium, where as in rodents it is only inducible [52,53]. This difference in Class II expression may not actually lead to a difference in a transplant scenario, as MHC Class II expression may be induced on rodent endothelium by the act of transplantation. It is also possible that endothelial class II MHC has different functions in small animals compared to large animals. In mice, endothelial class II MHC does not activate direct alloreactive CD4 + cells [54], and may even induce the generation of CD4 + 25 + FoxP3 + regulatory cells [55]; this has not been examined in large animals.

Bystander activation

Large animals and humans are exposed to a variety of antigenic stimuli to which they mount an immune response with the consequent formation of memory cells. One or more clones of these memory cells may also be activated by the allograft because of antigenic similarity between the original stimulus and the graft (heterologous immunologic memory) [56,57]. In contrast, small animals are often bred in controlled environments and therefore are less likely to have previously formed memory cells that can be activated by the allograft.

In summary, differences between large and small animals mean that it is possible to induce skin tolerance in many small animal models, but rarely in large animals or humans. Therefore, although small animals are useful for outlining new approaches and for mechanistic studies, large animals, with their greater physiologic and immunologic similarity to humans, possibly better simulate the human condition and the development of clinically translatable protocols.

Strategies for skin tolerance induction

A tolerance induction strategy involves selection of the stage of immune development at which to induce tolerance

Table 1. Methods of immune manipulation to achieve tolerance.

Non-hematopoietic stem cell transfer (HCT)	Immunosuppression T-cell depletion Costimulatory blockade
HCT	Nil Immunosuppression T-cell/lymphocyte depletion Costimulatory blockade Dendritic cells

and the method of immune manipulation used to induce one or more tolerance mechanisms (see Table 1). Each of these elements will be considered in turn.

Stage of immune development

Tolerance can be induced *in utero*, or during neonatal or adult life. The immature immune system of *in utero* models often requires less manipulation to induce donor-specific tolerance. Adult tolerance induction models often require more aggressive manipulation; however, they have a much wider scope of application as they can be used to treat acquired disorders not present *in utero* and avoid risk of triggering abortion by *in utero* manipulation. Neonatal models theoretically combine advantages of both *in utero* and adult models, with minimal manipulation required of the still developing immune system to achieve tolerance without risk of abortion. Initial work in small animal neonatal models was successful in achieving the donor tolerance across a major MHC barrier to a delayed musculoskeletal allograft with the infusion of bone marrow cells [58]. However, similar strategies to induce skin tolerance with neonatal injection of bone marrow into the thymus [59] or the simple intra-peritoneal injection of bone marrow with or without epithelial cells [60] only resulted in modest prolongation of skin graft survival. There has been no improvement in induction of skin tolerance neonatally since Boyse and Old's successful neonatal skin tolerance radiation mouse model [61], which was no less toxic than successful regimes used in adult models. The theoretical advantage of the neonatal model does not seem to be born out in practice for skin tolerance induction.

Method of immune manipulation

Manipulations that have been used for skin tolerance induction attempts can be divided in to those that involve donor hematopoietic stem cell transfer (HCT) and those that do not (non-HCT) (see Table 1).

Hematopoietic stem cell transfer approaches may have a fundamentally different mechanism than non-HCT approaches due to the transfer of hematopoietic stem cells (HSCs). HSCs have the ability to replicate indefinitely as well as differentiate in to cells of all lympho-hematologic lineages. If donor HSCs stably engraft in the recipient they will provide donor antigen to the thymus allowing life-long negative selection of newly arising donor-reactive thymocyes (central deletion) [62] and creation of naturally occurring regulatory cells. It is likely that with near complete replacement of recipient by donor HSCs central deletional mechanisms are dominant. However, at lower levels of HSC chimerism, the mechanism of tolerance induction may not be very different from non-HCT approaches with regulatory cells having a greater role [63–65]. Regulatory cells can be 'naturally occurring' thymic derived or be 'inducible' in the periphery [66]. Inducible regulatory T cells can stimulate mature T cells to change to a regulatory phenotype (infectious tolerance [67]).

To attain tolerance, it has been reported that regulatory T cells may only be required to a small number of antigens in an allograft; cells within the allograft expressing other antigens attaining protection by 'linked' or 'bystander' suppression [68,69]. However, it is unlikely that this mechanism will be relevant to attaining skin tolerance within a composite tissue allograft because bystander suppression appears to require both regulatory cells and bystander cells to be in the same tissue and not just adjacent to each other [68]. This is supported by the observation of 'split tolerance' in composite tissue allotransplantation models with tolerance to the musculoskeletal element but eventual rejection of the skin element of the allograft [70].

It is possible in some non-HCT approaches for transfer of donor cells to occur with the achievement of microchimerism (i.e. detectible only by PCR). Some have suggested that a microchimeric state can lead to tolerance [71], and there is evidence of central deletion with microchimerism [72]. However, microchimerism and tolerance do not always correlate [73–75]. The apparent disparity may be because the term 'microchimerism' is often used without specifying the donor cell type or their location (e.g. peripheral blood, bone marrow, and thymus), meaning there are differing immunologic processes occurring in different models all demonstrating 'microchimerism'.

Hematopoietic stem cell transfer approaches are of particular interest in composite tissue allotransplantation because many allografts contain vascularized bone marrow. Donor marrow cells within composite tissue allotransplants may contribute to tolerance induction [76]. However, it remains unclear what role they have in the maintenance of tolerance: Siemionow found that recipient donor marrow cells are substituted by donor cells over time in a rat model [77]; however, Mathes found, in a

pig model, that the presence of donor cells within the allograft diminished over time with no evidence for donor substitution in recipient marrow [78]. In addition, vascularized bone marrow may have limited application clinically: the bones contained within a hand transplant have minimal hematopoietic activity in adult life, and face transplants will contain little, or no, bone marrow. To counteract the possible effect of lack of bone marrow within the transplant, donor bone marrow infusions were given to the first facial allotransplant recipient [7].

Non-HCT Approaches

Short course of immunosuppression

There are clinical reports of skin tolerance following just a short course of immunousuppression [15,79]. However, these were not formally studied to confirm pretransplant alloreactivity or their immune status post-transplant.

A short course of FK506 in the MGH miniature swine induced tolerance to kidney allografts across full double haplotype MHC barriers [80]. However, subsequently applied donor skin grafts were rejected, without rejection of the organ allograft.

T-cell depletion

Depletion of alloreactive T cells reduces the initial alloreactive response allowing development of peripheral tolerance mechanisms. This is often combined with a short course of immunosuppression to give further bias towards a tolerogenic versus an alloreactive state. This has been successful in small animals. Siemionow demonstrated prolonged survival of vascularized skin allografts in rats treated with αβTCR Ab and a short course of cyclosporine or FK506 [81]. Strom attained skin graft tolerance across MHC barriers using rapamycin with an IL2-IL15 fusion protein that depleted cytopathic T cells while sparing regulatory T cells [82]. In murine models, CD4 and CD8 antibody blockade without T-cell depletion can achieve tolerance to class 1 MHC mismatch as well as minor mismatched skin allografts [83] indicating that T-cell depletion is not essential to achieve skin tolerance via peripheral mechanisms in small animals.

T-cell depletion has been less successful in large animals with only prolonged skin allograft survival (from 9.25 to 22–26 days) achieved in nonhuman primates by the administration of ATG [84].

Costimulatory blockade

Costimulatory blockade is usually considered to act by preventing activation of alloreactive T cells. However, there is evidence that anti-CD154 may heighten the suppressive activity of regulatory cells as well [85]. Tolerance to skin allografts has been achieved using costimulatory blockade in mice [86]. However, repeated intravenous injection of anti-CD154 achieved only a modest increase in skin allograft survival (7.3–13.3 days) across MHC barriers in primates. Survival was significantly prolonged with the addition of both rapamycin and donor specific transfusion (DST) (mean: 142.7 days) [87]. Also, repeated anti-CD154 antibody treatment given both intravenously and into the graft bed achieved markedly prolonged skin allograft survival to greater than >202 days [88] with only a marginal increase in survival (>236 days) with the addition of DST [89].

Other co-stimulatory molecules including CD28 [86], CD134 [90] and OX40 [91] have all been shown to prolong skin allograft survival in murine MHC mismatch models. The utility of these in large animal models has not yet been reported on.

HCT approaches

HCT alone

The permissive immunologic environment of the fetus *in utero* allows for HCT and engraftment without additional therapy. Tolerance is attained by central deletion, with peripheral mechanisms to control alloreactive T cells that escape thymic processing [92]. *In utero* induction of skin tolerance in mice was first demonstrated by Medawar [3]. However, this was in part due to a fortuitous strain combination with only an MHC class 1 mismatch (CBA → A). *In utero* induction of skin tolerance has subsequently been attained, in the small animal, across MHC class 1 and 2 barriers [93]. Skin grafts showed only prolonged acceptance (27 days vs. 7–9 days for controls) in swine with stable low-level multilineage chimerism [94]. Interestingly, these animals did not demonstrate a second set reaction, or develop antibodies upon regrafting from the same donor; it is possible that this may have been due to regulatory tolerance mechanisms that limited accelerated rejection following regrafting, but were not strong enough to prevent rejection completely (evidence for a regulatory mechanism was the finding of noninflammatory graft infiltrating lymphocytes (GILs) [95]).

Marginally prolonged secondarily vascularized skin allograft survival was demonstrated in primate models following donor leukocyte [96] and antigen [97] infusions. The mechanism of prolongation may be similar to following donor-specific transfusion with stimulation of a regulatory response [98].

HCT and a short course of immunosuppression

In the MGH miniature swine model, administration of a 12-day course of cyclosporine induced tolerance to MHC-matched, minor mismatched musculoskeletal allografts [99]. Biopsies demonstrated noninflammatory GILs

indicating a possible regulatory mechanism [95]. However, subsequent skin grafts (nonvascularized) from the donors were rejected, without breaking of tolerance to the musculoskeletal graft [100] (a state of 'split tolerance') demonstrating the rejectability of skin.

In further development of this approach, a vascularized hind limb allograft, which included a skin paddle, was transplanted across an MHC-matched minor-mismatched barrier in six animals [101]. The musculoskeletal element was accepted in all animals. In addition, one animal accepted the skin element of its vascularized graft with the others demonstrating split tolerance. This acceptor animal received a cryopreserved donor skin graft 120 days later. The skin graft was rejected by 60 days with simultaneous rejection of the epidermal element of the hind limb graft. This finding suggests three things. Firstly, skin tolerance can be achieved across a minor mismatch barrier using this approach. The variability in success may have been due to a more close matching of minor antigens (although the skin graft rejection demonstrated that they were not completely matched), or may have been due to the recipient having a tolerant phenotype [102]. Secondly, the mode of transplantation may affect the outcome of skin transplantation, with acceptance of immediately vascularized skin while rejecting the skin graft. Thirdly, tolerance to the epidermis in this model can be broken more easily than to the dermal and musculoskeletal elements.

HCT and T-cell/lymphocyte depletion

Transplanted donor HSCs (i.e. not transferred in the bone marrow contained within the graft) will not engraft in an adult recipient without manipulation of the immunologic environment. Some regimes have used high-dose irradiation to deplete alloreactive T cells and create 'immunologic space' to allow the donor HSCs to engraft in the recipient bone marrow. This has led to skin graft tolerance across MHC barriers in rodent models [103], and across a minor histocompatibility barrier in dog models [104,105]. Other regimes have achieved HSC engraftment with lower doses of irradiation by the addition of T-cell depleting antibodies. This approach has achieved skin graft tolerance across MHC barriers in the mouse [106]. In the MGH miniature swine, tolerance to skin grafts between MHC-matched, minor-mismatched animals was achieved in two out of six cases [107]. The others rejected their skin grafts despite showing prior tolerance to a cardiac allograft (a state of split tolerance). Tolerance to the cardiac graft was not broken by rejection of the skin.

Some regimes have used T-cell depleting antibodies without irradiation. Siemionow demonstrated tolerance to a hind-limb allograft (containing both vascularized bone marrow and skin) across an MHC barrier in rats conditioned with either antilymphocyte serum [108] or αβTCR Ab [109–111] followed by a short course of cyclosporine. The mechanism of tolerance induction was thymus dependant [112], indicating a role for either central deletion and/or naturally occurring regulatory cells. In contrast, Waldmann achieved skin tolerance in murine MHC class 1 mismatch models with bone marrow transplantation following CD4 and CD8 antibody blockade instead of T-cell depletion. A peripheral tolerance mechanism is likely in this model as the mature T cells are not removed [113].

In the MGH miniature swine, tolerance was achieved to the musculoskeletal elements of a limb transplanted immediately following T-cell depletion with a porcine CD3 immunotoxin, pCD3-CRM9 [114] under the cover of a short course of cyclosporine across a full MHC mismatch barrier. However, the skin only showed prolonged acceptance of between 42 and 70 days (immunosuppression was stopped on day 30) [70]. In two of the five long-term survivors, just the epidermis was rejected, with full-thickness skin rejection in the other three cases. Peripheral mechanisms are likely to be involved in tolerance induction in this model because the induction regime does not completely T-cell deplete the recipient. The involvement of the dermis in skin rejection may, in some cases, be because of selective epidermal alloresponse with the secondary destruction of dermal bystander cells in an antigen nonspecific manner [115].

HCT and costimulatory blockade

Costimulatory blocking agents to the CD40/CD154 or CD28/B7.1/B7.2 pathways, and an increased HCT dose can achieve skin graft tolerance across MHC barriers without the need for irradiation or T-cell depletion, in mouse models [116–119]. In these models, anergy, suppression and peripheral deletion are important in the induction of tolerance with central deletion being the dominant mechanism in the long-term maintenance of tolerance [63,120].

HCT and dendritic cells

Both recipient and donor dendritic cell infusions have been used in protocols attempting to achieve skin tolerance. Unactivated recipient dendritic cells loaded with donor antigen and injected prior to transplantation of a hind limb allograft across a major MHC barrier in rats achieved only a small increase in survival (8 vs. 5 days) [121]. This may have been via a thymic dependent mechanism [122]. Beriou achieved tolerance to skin transplanted across a major allogeneic barrier in mice that were already tolerant to a cardiac transplant following infusion of immature bone marrow-derived recipient dendritic cells with a short course of a deoxyspergualin analog (LF 15-0195) [123].

The use of donor dendritic cells has only achieved prolonged skin graft survival. Markees showed rapid rejection of major mismatched allogeneic skin grafted on to mice treated with Flt3-ligand induced donor dendritic cells, and only prolonged survival with the addition of anti-CD154 (61 vs. 7 days) [124].

Conclusions

The widespread use of skin allotransplantation techniques would transform the field of reconstructive surgery. The risk-benefit ratio of immunosuppression is still an issue. A tolerogenic process would overcome these difficulties.

The methods used thus far to induce tolerance have not achieved clinical tolerance against skin. The mode of skin transplantation, skin-specific antigens and skin's composition may all contribute to the susceptibility of skin to rejection. Although there has been success in small animal models in achieving indefinite skin survival across MHC barriers, tolerance in the large animal model has only been attained across minor antigen barriers with prolonged survival between MHC mismatched animals.

However, it may not be necessary to reach the ultimate goal of true tolerance to achieve a favorable risk-benefit ratio required for widespread application of CTA techniques. Adequate reduction in systemic immunosuppressive toxicity may be possible with novel immunosuppressive therapies, site-specific adjuvant treatments, or by the induction of a less alloreactive state.

Fifty-four years after Medawar first demonstrated that it was possible to induce tolerance to skin in a murine model, the Holy Grail of clinical skin tolerance is yet to be unearthed. The barrier of transferring techniques that are effective in the small animal to large animal models is largely unbreached. However, with methods to reduce the toxicity of chronic immunosuppression regimens and progress towards induction of a less alloreactive state, we may have alternatives that go some way towards achieving the true goal of transplantation with minimal risk.

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