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REVIEW

Avenues for immunomodulation and graft protection by gene therapy in transplantation

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Summary

Organ transplantation represents the only definitive therapy for many causes of end-organ failure. However, the universal success of this therapy is limited by chronic allograft rejection, the side effects of chronic immunosuppressive therapy, and a severe shortage of donor organs. Presently, the success of solid-organ transplantation depends on the continuous administration of toxic and nonspecific immunosuppressive agents, therapies that present risks for opportunistic infection, malignancy, and a variety of agent-specific side effects. To promote the use of transplantation with limited risk of long-term sequelae, three dominant research challenges emerge: (i) elimination of the need for exogenous immunosuppression by immunological tolerance induction; (ii) prevention of chronic rejection/graft dysfunction; and (iii) expansion of available organs for transplantation. Gene therapy may provide significant advances and solutions in each of these areas. Rejection of the graft in the immediate post-transplant period has been attacked through the transfer of immunomodulatory molecules in addition to tolerance inducing approaches. Chronic graft rejection may be similarly addressed through permanent tolerance induction or alternatively through the introduction of molecules to resist chronic graft damage. Genetic manipulation of stem cells may ultimately produce transgenic animals to serve as tissue donors to overcome the limited donor organ supply. This review will highlight ongoing developments in the translation of gene therapy approaches to the challenges inherent in transplantation.

Gene therapy in transplantation: overview and approach

While application of gene therapy to human subjects has yet to achieve widespread success, clinical features of transplantation make it uniquely amenable to intervention by this therapeutic modality. As all transplantation requires the isolation and manipulation of donor organs or tissues, there is already an opening in which gene therapy can be both delivered and assessed. Moreover, in most instances only the donor organ would require transgenic modification, a further advantage in that not all cells of the transplant recipient would be exposed to the treatment as is often the case in gene therapy approaches to systemic illness. A final and often unconsidered advantage is that the recipient is likely to be exposed to immunosuppression, at least temporarily. Even if gene therapy approaches can promote immunologic tolerance, the removal of an initial period of immunosuppression from the transplant protocol would certainly be much further in the future. The use and knowledge of immunosuppressive agents in current approaches to transplantation present a unique opportunity for the development and refinement of gene therapy approaches. In many current gene therapy approaches, the success of the technique is limited by immune responses both to the vector and to the gene product that is delivered. The appropriate use of immunosuppression may limit the deleterious effect of the immune system while the vector is established; in fact, the deleterious immune response to the

viral vector and its contents may be partially responsible for negative outcomes in some previous clinical trials [1]. As the gene product in transplantation applications of gene therapy will also be immunomodulatory, transplantation may provide a unique area in which the limitations of current gene therapy protocols may be overcome. The combination of gene therapy targeted at elements of the immune system together with the already extensive knowledge of immunosuppression in the transplant field should assist in bringing candidate interventions to clinical testing.

Gene therapy to prevent acute allograft rejection

The most direct and immediate barrier to the success of allogeneic transplantation is the recipient immune response that necessitates a lifetime of immunosuppressive therapy. Fortunately, aspects of the immune response are highly inviting to intervention by gene therapy approaches. The immune response to an immediately vascularized allogeneic organ is multifaceted and includes both cell- and antibody-mediated effector mechanisms of rejection [2]. Immune activation entails interactions on multiple levels beginning with the stimulation during transplantation of both host and donor antigen presenting cells (APCs). This stimulations is followed by the presentation of allogeneic major hosts compatibility complex (MHC) molecules and peptides by the direct and indirect pathways to recipient T cells, the stimulation of these T cells through the TCR and numerous secondary signals, and finally the infiltration and destruction of grafted tissue by T cells and by other cell types participating in the cytokine-coordinated immune response. The graft may be protected from this complex network through gene-transfer strategies to deliver protein molecules to the graft to modulate host immune responses, a strategy which may limit toxicity when compared with systemic administration. Targets for intervention at this level include selected cytokines that may downregulate or disrupt the nascent immune response in addition to soluble ligands designed to interfere with co-stimulation of graft specific T cells. Qin *et al.* [3] tested this hypothesis by transferring the genes that encode transforming growth factor beta (TGF-α) and interleukin 10 (IL-10) to the mouse myoblast and nonvascularized heart graft. Using a retrovirus- or plasmid-delivery system, grafts transfected with genes that encode IL-10 and TGF-α experienced significantly prolonged survival when compared with the vector alone (12 days with vector compared with 26 days with TGF-α and 39 days with IL-10 expression). The efficacy of transduced IL-10 and TGF-β has been shown in numerous follow-up studies using several vectors for transduction [4–9]. Not only has the effect been demonstrated in cases where the grafted tissue (including heart, islet, and liver) was transduced but there has also been utility in transducing recipient stem cells with these cytokines [10,11]. The latter delivery method is particularly desirable as the mechanism of action likely results in the generation of regulatory (foxp3+) T cells within the recipient and may be facilitated by inoculation of precursor stem cells with regulatory cytokines. This mechanism is supported by the various studies demonstrating reduced cellular proliferation to graft antigen challenge, isolation of CD4+ CD25+ Tregs following therapy, and recent demonstrations of newly generated foxp3+ cells [12,13].

In addition to altering the cytokine balance in the recipient, several studies have described the usefulness of modulating the available co-stimulation via systemic administration of CTLA4Ig in preventing allograft and xenograft rejection [14–19]. CTLA4Ig is a soluble fusion protein that blocks T-cell co-stimulation by binding CD80/86 thereby preventing CD28 signaling; therefore, the local production of CTLA4Ig in the graft might inhibit the immune response directed against the allograft and promote long-term allograft survival. Our center has explored *ex vivo* perfusion of allografts (liver, pancreas or heart) with adenoviral vectors that encode CTLA4Ig cDNA. Olthoff *et al.* [20] reported permanent survival of rat liver allografts transduced with adenoviral CTLA4Ig. The effect of adenoviral-mediated *CTLA4Ig* gene transfer to the pancreas, a highly immunogenic organ, in the prevention of rejection and recurrence of autoimmune diabetes was investigated [18,21,22]. Local expression of the CTLA4Ig-encoding gene permitted long-term graft survival in the rat pancreas transplant model across highly disparate allogeneic barriers. The immunosuppressive effect of the CTLA4Ig protein was essentially limited to the transduced organ, rather than being a systemic phenomenon.

While introducing immunosuppressive molecules directly into the graft appears a promising strategy, gene therapy can also deliver other molecules that may protect the graft from immune surveillance. Lau *et al.* [23,24] have investigated the use of FasL to induce apoptosis in those lymphocytes that survey the area of grafted tissue. They transfected myoblasts of recipient origin *ex vivo* with an expression plasmid containing the cDNA for CTLA4Ig or Fas ligand (FasL) and then transplanted allogeneic islets together with the transfected muscle cells.

In this setting, local production of CTLA4Ig resulted in prolonged survival (from 11 to 31 days) and expression of the *FasL* gene resulted in an even longer survival time (>90 days) of the transplanted allogeneic islet tissue. However, when subsequent studies have investigated direct transduction of islet cells with FasL, islet cell

destruction either by neutrophilic infiltration or autocrine-induced apoptosis has resulted [25,26]. Overall, these data demonstrate the interesting concept that a molecule may be immunoprotective when delivered on a carrier cell in the vicinity of the graft but may lead to cell death if expressed by the grafted tissue itself. However, this strategy may be worth reconsideration with the recent report of an anti-Fas ribozyme that can reduce Fas expression *in vitro*in beta cells and hence may protect them from Fas-mediated apoptosis following FasL transduction [27]. Other recent studies have attempted to retain the ability of FasL to promote elimination of allogeneic T cells while restricting its intracellular signaling capacity [28,29]. There has been some success with viral delivery of a CTLA4-FasL fusion protein, which may be carried by activated dendritic cells to sites of T-cell activation though further studies will be required to delineate how length of expression correlates with the specificity of tolerance induction [28,29].

Together, the results from initial gene-therapy experiments in the prevention of allograft rejection appear promising, although some of the benefits are limited by an immune response to the viral vector, a complication that may be addressed through adjunct use of immunosuppression. The local production of biologically active proteins from the graft, achieved by gene transfer to the graft *ex vivo*, can inhibit the immune response against the allograft and promote long-term graft survival. Similar studies should be performed in large-animal transplant models, and it is hoped that clinical application of this strategy will follow.

Gene therapy to prevent chronic allograft rejection

While acute rejection remains among the most well-known challenges to transplantation, our current pharmacologic armamentarium is effective in preventing this complication. Despite our successes in the post-transplant period, chronic rejection persists as a distinct problem inadequately addressed by current therapies. It has been postulated that chronic allograft dysfunction is mediated by both alloantigen-dependent (MHC incompatibility, acute rejection) and alloantigen-independent (ischemia and infection) factors [30]. A low-level immune response characterized by perivascular inflammation may induce persistent low-grade damage to the allograft vascular endothelium. In turn, endothelial cells secrete growth factors in response to this damage, stimulating the proliferation of smooth muscle cells and myocyte migration from the media to the intima, forming arteriosclerotic lesions. Ultimately, vascular ischemia and/or interstitial fibrosis develop and characterize chronic allograft rejection.

Ongoing studies have elucidated the mechanisms involved in chronic graft injury and have utilized gene therapy to intervene in candidate pathways. Based on the proposed mechanism, opportunities for therapy exist both for modulation of the immune system and direct inhibition of the graft response. The T cell-mediated graft response involving secretion by T cells of PDGF, VEGF, and Ang1 may contribute to tissue injury and to the stimulation of mitotic activity among intimal cells [31]. While gene therapy approaches are only beginning to address these interactions, there is preliminary evidence that blockade these pathways may facilitate graft survival. Savikko *et al.* [32] recently demonstrated that inhibition of PDGF activity with imatinib was able to decrease chronic allograft dysfunction in a kidney transplant model. The stage is also being set for evaluation of these other mediators as antisense oligonucleotides have been reported to disrupt the secretion of VEGF and TGFα; these strategies may be suitable for future testing in experimental transplant settings.

In addition to limiting T-cell production of factors promoting graft injury, preventing the response of local tissue to these secreted factors may be equally efficacious and may be easily delivered by manipulation of the grafted tissue prior to transplantation. Several studies have used gene therapy approaches to modulate intimal hyperplasia after arterial injury and new therapies are being developed with several strategies aimed at preventing smooth muscle cell migration.

The activation of matrix metalloproteinases in the vascular wall allows smooth muscle cells to digest the surrounding extracellular matrix and migrates from the media into the intima. Expression of the gene that encodes human tissue inhibitor of metalloproteinase (hTIMP) by adenoviral-mediated gene transfer can significantly prevent this smooth muscle cell migration and resultant neointimal formation [33–35]. As the process by which smooth muscle cells migrate has become better understood, the role of the plasminogen activator/urokinase system has also been revealed. Lamfers *et al.* [36] have constructed a novel hybrid protein combining inhibitors of metalloproteinase and urokinase activity and have demonstrated decreased intimal thickening following adenoviral-mediated transfer in saphenous vein culture, a therapy that warrants evaluation in the transplant setting. The potential for application of these novel strategies continues to be developed *in vitro*; construction of a vector containing human TIMP has recently been reported that may permit future applications of this principal to human transplantation [37]. Other gene transfer studies have demonstrated an inhibitory effect on intimal thickening by transfer of a variety of genes that encode anti-apoptotic proteins, antisense oligodeoxynucleotides of

adhesion molecules, nitric oxide synthase or vascular growth factor to endothelial cells [38–41]. Recent evidence suggests the efficacy of increased levels of HO-1 for tissue protection against chronic allograft rejection. Both Chauveau *et al.* and Bouche *et al.* have used adenoviral gene transfer of HO-1 to prevent intimal thickening in aortic graft models [42,43]. The enzyme heme-oxygenase participates in the breakdown of heme and generates CO as one of its products. The increased local CO may down-regulate macrophage activity and protect endothelial cells from apoptosis. Further studies of the CO molecule suggest that this product may be a major mediator of the effect and may act through modulation of nuclear factor kB (NF-kB) pathways [44,45]. Overall, strategies aimed at decreasing chronic inflammatory injury by repressing secreted mediators or changing local tissue metabolism demonstrate significant potential for the prevention and treatment of chronic graft dysfunction.

Gene therapy and xenotransplantation

While new strategies to prevent acute and chronic transplant damage by gene therapy continue to arise, the success of clinical transplantation remains limited by the lack of donor organs. Xenotransplantation represents one means to address the critical organ shortage. However, while the immunologic challenges hindering allotransplantation are significant, the complications are multiplied when species barriers are crossed. In pig-to-human or pig-to-baboon models, the grafted organ is rejected within minutes or hours by antibody-mediated complement activation (hyperacute rejection or HAR) [46]. Because activation of the complement cascade is regulated by various species-specific proteins, overcoming HAR by genetic transfer of human complement regulators has been the major focus in this field. Several groups have successfully introduced genes that encode human complement regulators, such as human decay-accelerating factor (DAF), membrane cofactor protein or CD59, into the pronuclei of fertilized pig oocytes [47–49]. Organs from *DAF*-expressing pigs survived for up to 8 days when transplanted into baboons, in the continued absence of any visible HAR [50].

In addition to the inhibition of complement activation, HAR may also be prevented by more direct approaches to avoid the attack of the preformed antibody, so-called xenoreactive natural antibodies (XNAs). As has been described for other specificities in the natural antibody repertoire, the human immune system possesses preformed antibody with affinity for the unique patterns of glycosylation present on xenogeneic tissue because of species-specific expression of unique galactosyltransferases. To address this immunologic obstacle, investigators have attempted to either imbue the recipient immune system with the appropriate transferase or alternatively to eliminate glycosylation from the cells of the donor animal. Bracy *et al.* [51] explored the potential of gene therapy to overcome anti-aGal (Gala1-3Galb1-4GlcNAc-R) antibody-mediated rejection in a murine model and showed that production of anti-aGal XNAs could be inhibited by introducing a gene encoding a-galactosyltransferase in autologous bone marrow. This treatment provides a theoretical basis to circumvent the difficulties associated with engraftment of xenogeneic bone marrow in humans for the purpose of inducing tolerance. While this therapy has provided evidence that tolerance can be induced to species-specific glycosylation, elimination of the alpha-galactosyltransferase within a line of transgenic pigs to circumvent this challenge has been considered as an alternative approach. Tissue from alpha-Gal KO donors was shown to attenuate hyperacute rejection in a renal transplant model and also in a model of lung transplantation [52–54]. Although these methods represent significant advances in addressing principle elements of the hyperacute xenograft response, there remain significant obstacles to xenotransplantation from the humoral immune system. A recent report by Chen *et al.* [55] revealed antibody-mediated rejection of Gal KO pig organs transplanted into baboons suggesting a critical role of antibody against nongal antigens in acute humoral xenograft rejection. Whether the specific targets of these antibodies can be identified and overcome with a similar gene-targeted approach is an area of active new investigation.

However, even if HAR can be avoided, the xenograft is subject to delayed xenograft rejection (DXR) within several days. DXR is a T cell independent but complex process in which many factors, including antibodies, macrophages, NK cells, cytokines, and chemokines are involved [56]. Such DXR is probably initiated by XNAs, many of which are directed against cell surface sugar residues that are expressed on the blood vessel of donor organs, leading to endothelial cell activation – a crucial event in DXR. Endothelial cell activation may contribute further to DXR through activation of proinflammatory genes (such as chemokines, cytokines and adhesion molecules). In addition, activated endothelial cells may themselves serve as antigen presenters as suggested in allotransplant models and may be a more vigorous stimulus in xenogeneic models [57–61]; in addition to antigen presentation, recent studies have implicated expression of ICOSL in endothelial cell-dependent activation of anti-graft CD8 T cells [62,63]. The response of the vascular endothelium to the trauma of transplantation through upregulation of cytokines and costimulatory ligands may both be mediated largely via NF-kB, a transcription factor

that plays a crucial role in controlling many proinflammatory genes [64,65].

While inhibition of NF-kB by IkB was a logical initial intervention, its overexpression acutely sensitized endothelium to apoptosis mediated by TNF [66]. Anrather *et al.* [67] improved on this system with dominant-negative IkB mutants that partially inhibit the myriad functions of NFkB. Overexpression of the gene that encodes p65 RHD in endothelial cells blocked the activation of NF-kB and thus suppressed the upregulation of proinflammatory genes but did not sensitize the endothelial cells to TNF-mediated apoptosis. While the role of the NF-kB pathway has thus far been evaluated primarily in allograft models, its application to xenograft protection where endothelium is targeted by multiple pathways may lead to better engraftment and graft survival. We anticipate renewed interest in these pathways as the initial obstacles in xenotransplantation are managed. As the problems of HAR, and eventually DXR, are overcome, host T-cell responses against the xenograft must finally be addressed. The characterization of T-cell responses to xenografts has been hindered in most discordant models by limited survival beyond the HAR and DXR phases. Nevertheless, experiments with skin and pancreatic islet xenografts, which are not subject to HAR or DXR, demonstrate that T-cell-mediated xenograft rejection is at least vigorous as T-cell-mediated allograft rejection [68,69]. Importantly, conventional immunosuppressive agents may be less effective at prolonging xenograft survival than at prolonging allograft survival [70]. In particular, as our reagents for the inhibition of alloreactivity become more specific, they may further lose the ability to hinder xenorejection. This concept is highlighted in a recent publication by Mirenda *et al.* [69], where variants of the CTLA4 molecule in a xenotransplantation model are selectively able to hinder the direct (presentation by xeno-APCs) and indirect (presentation by allo-APCs) pathways. As it is critical to address both pathways of T-cell activation, we can anticipate that reagents that have been tailored for the allopresentation pathway may be relatively or completely ineffective in preventing presentation by xenogeneic APCs. Therefore, T-cell-mediated rejection of discordant xenografts represents a third hurdle to the success of xenotransplantation. Recently, several groups have investigated the effect of gene transfer on islet xenograft survival. Transfer of the *CTLA4Ig* gene mediated either by adenovirus or a gene gun resulted in a significant prolongation of islet xenograft survival [21,71]. Extension to other organs where direct presentation may play a greater role may be facilitated by targeting both allogeneic and xenogeneic CD28 molecules as described above. In addition, we showed that transfer of the genes that encode IL-10 and TGF-β resulted in prolonged islet xenograft survival in a highly discordant dog-to-rat islet transplant model, but the same strategy actually accelerated islet xenograft rejection in a rat-to-mouse islet transplant model, probably by enhancing the humoral immune response [72]. These data point out the importance of better understanding cytokine regulation and interaction in the xenoimmune response to design better gene therapy approaches to this challenging problem.

Gene therapy approaches to successful islet transplantation

While direct control of acute and chronic graft-reactivity is critical to the success of allo and xeno-transplantation, our investigation into islet transplantation has also highlighted additional areas in which gene therapy may benefit transplantation. The implementation of islet transplantation is largely limited by the need for an average of two donor pancreata for the treatment of each recipient, a stringent challenge given the organ shortage. In addition, the continued requirement for long-term immunosuppression challenges our ability to balance the benefits and risks for all patients with diabetes. To optimize the application of this therapy, it is necessary to limit the need for immunosuppression and improve the number of recipients that can be cured from a single donor. Gene therapy again offers a number of therapeutic opportunities based on our knowledge of the immune process leading to beta cell destruction as well as our appreciation of basic beta cell physiology.

While T cells are the final effectors of islet cell death, the mechanism by which islet cells are destroyed remains under active investigation. Although cell–cell contact between CD4 or CD8 T cells and islets may be a factor in this progression, many studies have suggested that such intimate contact is unnecessary [73,74]. Rather, secreted cytokines may mediate direct toxic effects on islet tissue and activate islet cell apoptosis resulting in beta cell destruction and diabetes [75,76]. Within the cytokine pathway, secretion of IFNa, Il-1, and TNF-á has been most reliably associated with progression to diabetes [77–81]. In fact, alterations in the availability of these factors or in their signal transduction apparatus have been associated with the prevention of autoimmunity in diabetes-prone non-obese diabetic (NOD) mice. Islet cells themselves possess the ability to receive signals through these mediators and contact with these ligands involves activation of multiple islet factors including STAT1, AP-1, and NF-kB [75,82]. Ultimately, transduction via these stress-related pathways leads to production of pro-apoptotic genes such as Bax and islet cell death. These pathways have been amenable to intervention through a variety of gene therapy approaches. Interference in the IL-1-mediated apoptotic

pathway has been most reliably obtained by transduction of islet cells with a construct expressing IRAP, the interleukin-1 receptor antagonist protein. Tellez *et al.* recently showed improved islet cell survival and replication following adenoviral transduction with this cytokine, an extension of the prior findings of Giannoukakis [83,84]. Protection from cytokine-mediated apoptosis has also been achieved following viral induction of islet cell expression of TNFRIg, baculovirus p35 protein, and insulin-like growth factor IGF-1 [85–87]. In addition to preventing the initial activation of the apoptotic pathway, strategies to overexpress antiapoptotic proteins such as the TNF-a inducible transcription factor A20 or bcl-2 have also been effective in extending islet survival by preventing islet loss [38,88,89].

While protecting islet cells from cytokine-mediated injury may both prolong their survival and decrease the number of cells needed for curative transplantation, we have also investigated gene therapy approaches to induce islet cell proliferation or to augment directly the function of the transplanted tissue to promote disease cure with fewer donor islets. We have demonstrated effective islet cell proliferation following lentiviral-mediated cellular transduction with a chimeric construct in which the erythropoietin receptor signaling apparatus is fused to FK binding protein (FKBP)-binding domains, which can be cross-linked by exposure to a chemical inducer of dimerization [90]. In this study, there was successful proliferation demonstrated both *in vitro* and *in vivo*, retention of appropriate glucose responsiveness, and stability of the differentiated phenotype of the transduced cells. The induction of islet cell proliferation with decreased minimal islet mass for cure has also been found following islet transduction with hepatocyte growth factor (HGF) [91,92]. The physiologic role of this pathway has been supported in more recent studies demonstrating decreased islet function in the absence of the HGF receptor [93]. In addition to the induction of islet proliferation, we have investigated the augmentation of individual islet cell function such that one islet may subserve the function of many, a 'superislet' approach. As the major function of the transplanted islet tissue is the secretion of insulin in response to glucose and other nutritional stimulation, we have hypothesized that transduction of islet beta cells with the insulin gene would increase the ability of individual beta cells to produce insulin in response to hyperglycemic challenge [94]. We have demonstrated that the required islet mass for cure of diabetes in mice is significantly reduced following this modification with only 25–50% of the previously needed islet mass now rendered curative. Further improvement in these approaches may enable living-related islet cell donation in the future, which would greatly expand the potential for transplantation for autoimmune diabetes.

Gene therapy and transplantation tolerance induction

While solutions may be tailored via gene therapy to address individually, the myriad challenges of organ transplantation, the future combination of gene therapy, and transplantation will seek the ultimate remedy – the induction of permanent donor-specific tolerance. Despite the enormous success achieved in rodent models, induction of permanent graft tolerance in large-animal models and humans has not been achieved routinely. Fortunately, the application of gene therapy may provide numerous approaches to achieve or enhance induction of tolerance in phylogenetically advanced mammals. While acute rejection is effectively managed by pharmacologic means, the induction of long-term tolerance can be sought by usurping the function of the normal tolerance maintaining machinery including both central (thymically mediated) and peripheral tolerance mechanisms. There has been considerable effort in introducing donor-specific proteins into the recipient thereby manufacturing an expansion of the recipient definition of 'self' with associated protection from immune destruction. This manipulation has been achieved temporarily through donor-specific transfusion and is provided on a more permanent basis through the generation of microchimerism in which the recipient bone marrow is altered to express donor proteins. Numerous groups have repeatedly demonstrated long-term tolerance induction following gene transfer of MHC class I or class II to create matching to donor organs in murine models of transplantation [95–101]. These models have demonstrated efficacy even with single haplotype matching, a finding that may significantly simplify the vectors needed for clinical transplantation, where the degree of MHC mismatching is often large and unpredictable. While much insight has been gained into the utility of gene therapy to fabricate immunologic matches, the success of these early therapies also highlights the challenges inherent within the system. Present approaches focus on the transduction of autologous bone marrow and aim at minimizing the preparative and myeloablative regimens necessary to inoculate critically ill recipients with reconstituting bone marrow. While other gene delivery approaches have been presented, most notably the direct inoculation of the recipient thymus with MHC-encoding cDNAs, this technique has not been fully developed for clinical application and if developed would face the challenge of thymic involution that characterizes the aged population most likely to be recipients [102]. Nonetheless, the introduction of donor MHC into recipient bone marrow or thymus with simple conditioning and treatment regimens remains a plausible therapy for the induction of permanent allograft tolerance.

Although there has been much success with and much enthusiasm for experimental approaches to generate microchimerism for the purpose of tolerance induction, the need in most systems for reinfusion of recipient bone marrow may limit the applicability of this strategy as many patients in need of transplantation are critically ill and may not tolerate this procedure. Yet, the core concept – manipulation of the specificities recognized by the recipient immune system – can be modified based on current research. The new millennium has seen a renewed interest in regulatory T cells, CD4+ CD25+ Foxp3+ cells that can control the responsiveness of other T lymphocytes. Several recent reports suggest that these cells can prolong allograft survival or promote indefinite tolerance [103–106]. Initial reports of this phenomenon focused on polyclonal regulatory cells without known specificity. However, we and others have recently reported the ability of antigen-specific regulatory cells to mediate prolongation for grafted tissue expressing their cognate antigen [107–109]. Of critical importance from the gene therapy perspective, these cells can be generated from CD4+ CD25− negative precursors by transduction with the *foxp3* gene, the master mediator of regulatory cell development [110]. These artificially generated regulatory cells can prevent graft versus host disease (GVHD) and promote skin allograft survival. While other attempts have been made to imbue graft reactive T cells with graft protective cytokines such as Il-10 or TGFα, the induction of the full regulatory cell pattern via the master transcriptional regulator may represent the most effective means to generate tolerance through Tregs. There would be a number of benefits if this process could be similarly applied to human research including the use of autologous cells for gene-modification thereby avoiding potential GVHD. The efficacy of this strategy would be enhanced if transferred tolerance to defined antigens is demonstrated to expand to other epitopes; this 'linked' tolerance has been a reported characteristic of tolerant systems in which regulatory cell function is the predominant mechanism. So-called 'infectious tolerance' suggests that regulatory cells specific for one epitope may cause other naïve cells recognizing distinct antigens shared by the graft to deviate along a regulatory path of development [111,112]. In this way, tolerance may become widespread against multiple graft antigens with minimum requirements for transduction efficiency and may also be self-perpetuating.

Moreover, the development of antigen specific immune regulation would be a substantial improvement over previous approaches. This specificity could be produced either through genetic strategies such as coupling foxp3 transduction with co-inoculation of engineered T-cell receptors [113,114] or by classic immunologic methods such as expanding donor reactive cells via alloantigen exposure prior to or coincident with foxp3 expression. Specific targeting of the desired proteins against which to provide regulation by taking advantage of the specificity inherent in the TCR-MHC interaction may limit global immunosuppression, minimize side effects, and offer better prospects for long-term safety.

Challenges and prospects

Gene therapy strategies offer the potential to prevent allograft and xenograft rejection by several approaches. To date, the results of experiments in animal models have been encouraging and have demonstrated proof of principle. However, major challenges in the application of gene therapy in transplantation remain. Such problems include: [1] a low level of gene expression using currently available gene-delivery systems; [2] immune responses to gene-delivery vectors, such as adenoviral vectors, or risks associated with these viral vectors; [3] an incomplete understanding of the mechanisms of both rejection and tolerance, such as the details of regulatory cytokine networks, MHC-antigen interactions during the rejection process, and a complete understanding of costimulatory factors and their functions.

It is expected that, as gene therapy technology improves, more clinically acceptable and efficient means of gene transfer will develop. The mechanisms of tolerance and rejection and the role of MHC class I and class II molecules will be better understood, such that strategies that favor the development of transplantation tolerance will emerge. Similarly, as the details of cytokine regulation of the immune response become clear, more effective manipulation of the immune response through the control of local or systemic levels of specific cytokines will be achieved. We look forward to the extension of findings in allogeneic murine systems, where tolerance is readily achieved to the more complex setting of cross-species immunity in xenotransplantation. We anticipate greater understanding of the xenorejection process and, with new successes, greater knowledge of our opportunities for intervention. By combining genetic modification of donor tissue or whole donor animals with complementary changes to the recipient immune systems, long-term graft survival without ongoing immunosuppression is an achievable goal.

References

1. Somia N, Verma IM. Gene therapy: trials and tribulations. *Nat Rev Genet* 2000; **1**: 91.
2. Hayry P Molecular pathology of acute and chronic rejection. *Transplant Proc* 1994; **26**: 3280.
3. Qin L, Chavin KD, Ding Y, *et al.* Retrovirus-mediated transfer of viral IL-10 gene prolongs murine cardiac allograft survival. *J Immunol* 1996; **156**: 2316.

4. Zhang YC, Pileggi A, Agarwal A, *et al*. Adeno-associated virus-mediated IL-10 gene therapy inhibits diabetes recurrence in syngeneic islet cell transplantation of NOD mice. *Diabetes* 2003; **52**: 708.
5. Ozden H, Kabay B, Guven G, Acikalin MF, Erbis H, Alatas O. Interleukin-10 gene transfection of donor pancreas grafts protects against rejection after heterotopic pancreas transplantation in a rat model. *Eur Surg Res* 2005; **37**: 220.
6. Vassalli G, Fleury S, Li J, Goy JJ, Kappenberger L, von Segesser LK. Gene transfer of cytoprotective and immunomodulatory molecules for prevention of cardiac allograft rejection. *Eur J Cardiothorac Surg* 2003; **24**: 794.
7. Fujisawa K, Saito S, Okada Y, *et al*. Suppression of allogeneic response by viral IL-10 gene transfer. *Cell Transplant* 2003; **12**: 379.
8. Cheng J, Xia SS, Xie S, Tang LG, Shi XF. Transforming growth factor beta1-modified donor cell transfusion induced allo-heart tolerance. *Transplant Proc* 2005; **37**: 2360.
9. Luo X, Yang H, Kim IS, *et al*. Systemic transforming growth factor-beta1 gene therapy induces Foxp3+ regulatory cells, restores self-tolerance, and facilitates regeneration of beta cell function in overtly diabetic nonobese diabetic mice. *Transplantation* 2005; **79**: 1091.
10. Salgar SK, Yang D, Ruiz P, Miller J, Tzakis AG. Viral interleukin-10 gene therapy to induce tolerance to solid organ transplants in mice. *Transplant Proc* 2004; **36**: 397.
11. Salgar SK, Yang D, Ruiz P, Miller J, Tzakis AG. Viral interleukin-10- engineered autologous hematopoietic stem cell therapy: a novel gene therapy approach to prevent graft rejection. *Hum Gene Ther* 2004; **15**: 131.
12. Csencsits K, Wood SC, Lu G, Bishop DK. Transforming growth factorbeta1 gene transfer is associated with the development of regulatory cells. *Am J Transplant* 2005; **5**: 2378.
13. Fu S, Zhang N, Yopp AC, *et al*. TGF-beta induces Foxp3+ T-regulatory cells from CD4+ CD25-precursors. *Am J Transplant* 2004; **4**: 1614.
14. Jiang GP, Hu ZH, Zheng SS, Jia CK, Zhang AB, Wang WL. Adenovirus mediated CTLA4Ig gene inhibits infiltration of immune cells and cell apoptosis in rats after liver transplantation. *World J Gastroenterol* 2005; **11**: 1065.
15. Kanaya K, Tsuchida Y, Inobe M, *et al*. Combined gene therapy with adenovirus vectors containing CTLA4Ig and CD40Ig prolongs survival of composite tissue allografts in rat model. *Transplantation* 2003; **75**: 275.
16. Lin H, Bolling SF, Linsley PS, *et al*. Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. *J Exp Med* 1993; **178**: 1801.
17. Sayegh MH, Akalin E, Hancock WW, *et al*. CD28-B7 blockade after alloantigenic challenge in vivo inhibits Th1 cytokines but spares Th2. *J Exp Med* 1995; **181**: 1869.
18. Uchikoshi F, Yang ZD, Rostami S, *et al*. Prevention of autoimmune recurrence and rejection by adenovirus-mediated CTLA4Ig gene transfer to the pancreatic graft in BB rat. *Diabetes* 1999; **48**: 652.
19. Lenschow DJ, Zeng Y, Thistlethwaite JR, *et al*. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4lg. *Science* 1992; **257**: 789.
20. Olthoff KM, Judge TA, Gelman AE, *et al*. Adenovirus-mediated gene transfer into cold-preserved liver allografts: survival pattern and unresponsiveness following transduction with CTLA4Ig. *Nat Med* 1998; **4**: 194.
21. Liu C, Deng S, Yang Z, *et al*. Local production of CTLA4-Ig by adenoviral-mediated gene transfer to the pancreas induces permanent allograft survival and donor-specific tolerance. *Transplant Proc* 1999; **31**: 625.
22. Liu C, Jiang K, Deng S, Gelman A, Shaked A, Brayman KL. Long-term localized transgene expression in the pancreas achieved by intra-arterial adenoassociated virus-mediated gene transfer. *Transplant Proc* 2001; **33**: 603.
23. Lau HT, Yu M, Fontana A, Stoeckert Jr CJ. Prevention of islet allograft rejection with engineered myoblasts expressing FasL in mice. *Science* 1996; **273**: 109.
24. Kang SM, Hoffmann A, Le D, Springer ML, Stock PG, Blau HM. Immune response and myoblasts that express Fas ligand. *Science* 1997; **278**: 1322.
25. Kang SM, Schneider DB, Lin Z, *et al*. Fas ligand expression in islets of Langerhans does not confer immune privilege and instead targets them for rapid destruction. *Nat Med* 1997; **3**: 738.
26. Allison J, Georgiou HM, Strasser A, Vaux DL. Transgenic expression of CD95 ligand on islet beta cells induces a granulocytic infiltration but does not confer immune privilege upon islet allografts. *Proc Natl Acad Sci USA* 1997; **94**: 3943.
27. Klein D, Ricordi C, Pugliese A, Pastori RL. Inhibition of Fas-mediated apoptosis in mouse insulinoma betaTC-3 cells via an anti-Fas ribozyme. *Hum Gene Ther* 2000; **11**: 1033.
28. Feng YG, Jin YZ, Zhang QY, Hao J, Wang GM, Xie SS. CTLA4- Fas ligand gene transfer mediated by adenovirus induce long-time survival of murine cardiac allografts. *Transplant Proc* 2005; **37**: 2379.
29. Jin Y, Qu A, Wang GM, Hao J, Gao X, Xie S. Simultaneous stimulation of Fas-mediated apoptosis and blockade of costimulation prevent autoimmune diabetes in mice induced by multiple low-dose streptozotocin. *Gene Ther* 2004; **11**: 982.
30. Sayegh MH, Carpenter CB. Tolerance and chronic rejection. *Kidney Int* 1997; **58** (Suppl.): S11.
31. Lemstrom KB, Nykanen AI, Tikkanen JM, *et al*. Role of angiogenic growth factors in transplant coronary artery disease. *Ann Med* 2004; **36**: 184.
32. Savikko J, Taskinen E, Von Willebrand E. Chronic allograft nephropathy is prevented by inhibition of platelet-derived growth factor receptor: tyrosine kinase

inhibitors as a potential therapy. *Transplantation* 2003; **75**: 1147.
33. Cheng L, Mantile G, Pauly R, *et al.* Adenovirus mediated gene transfer of the human tissue inhibitor of metalloproteinase-2 blocks vascular smooth muscle cell invasiveness in vitro and modulates neointimal development in vivo. *Circulation* 1998; **98**: 2195.
34. George SJ, Baker AH, Angelini GD, Newby AC. Gene transfer of tissue inhibitor of metalloproteinase-2 inhibits metalloproteinase activity and neointima formation in human saphenous veins. *Gene Ther* 1998; **5**: 1552.
35. George SJ, Johnson JL, Angelini GD, Newby AC, Baker AH. Adenovirus-mediated gene transfer of the human TIMP-1 gene inhibits smooth muscle cell migration and neointimal formation in human saphenous vein. *Hum Gene Ther* 1998; **9**: 867.
36. Lamfers ML, Grimbergen JM, Aalders MC, *et al.* Gene transfer of the urokinase-type plasminogen activator receptor-targeted matrix metalloproteinase inhibitor TIMP-1.ATF suppresses neointima formation more efficiently than tissue inhibitor of metalloproteinase-1. *Circ Res* 2002; **91**: 945.
37. Xia D, Yan LN, Tong Y, Wang XP, Zhang MM, Zhao LY. Construction of recombinant adenoviral vector carrying human tissue inhibitor of metalloproteinase-1 gene and its expression in vitro. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 259.
38. Hancock WW, Buelow R, Sayegh MH, Turka LA. Antibody-induced transplant arteriosclerosis is prevented by graft expression of anti-oxidant and anti-apoptotic genes. *Nat Med* 1998; **4**: 1392.
39. Poston RS, Ennen M, Pollard J, Hoyt EG, Billingham ME, Robbins RC. Ex vivo gene therapy prevents chronic graft vascular disease in cardiac allografts. *J Thorac Cardiovasc Surg* 1998; **116**: 386.
40. der Leyen HE, Gibbons GH, Morishita R, *et al.* Gene therapy inhibiting neointimal vascular lesion: in vivo transfer of endothelial cell nitric oxide synthase gene. *Proc Natl Acad Sci USA* 1995; **92**: 1137.
41. Muhs A, Heublein B, Schletter J, *et al.* Preclinical evaluation of inducible nitric oxide synthase lipoplex gene therapy for inhibition of stent-induced vascular neointimal lesion formation. *Hum Gene Ther* 2003; **14**: 375.
42. Chauveau C, Bouchet D, Roussel JC, *et al.* Gene transfer of heme oxygenase-1 and carbon monoxide delivery inhibit chronic rejection. *Am J Transplant* 2002; **2**: 581.
43. Bouche D, Chauveau C, Roussel JC, *et al.* Inhibition of graft arteriosclerosis development in rat aortas following heme oxygenase-1 gene transfer. *Transpl Immunol* 2002; **9**: 235.
44. Martins PN, Reuzel-Selke A, Jurisch A, *et al.* Induction of carbon monoxide in the donor reduces graft immunogenicity and chronic graft deterioration. *Transplant Proc* 2005; **37**: 379.
45. Sato K, Balla J, Otterbein L, *et al.* Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants. *J Immunol* 2001; **166**: 4185.
46. Dorling A, Riesbeck K, Warrens A, Lechler R. Clinical xenotransplantation of solid organs. *Lancet* 1997; **349**: 867.
47. Fecke W, Long J, Richards A, Harrison R. Protection of hDAF-transgenic porcine endothelial cells against activation by human complement: role of the membrane attack complex. *Xenotransplantation* 2002; **9**: 97.
48. Zhou CY, McInnes E, Copeman L, *et al.* Transgenic pigs expressing human CD59, in combination with human membrane cofactor protein and human decay-accelerating factor. *Xenotransplantation* 2005; **12**: 142.
49. Fodor WL, Williams BL, Matis LA, *et al.* Expression of a functional human complement inhibitor in a transgenic pig as a model for the prevention of xenogeneic hyperacute organ rejection. *Proc Natl Acad Sci USA* 1994; **91**: 11153.
50. McCurry KR, Kooyman DL, Alvarado CG, *et al.* Human complement regulatory proteins protect swine-to-primate cardiac xenografts from humoral injury. *Nat Med* 1995; **1**: 423.
51. Bracy JL, Sachs DH, Iacomini J. Inhibition of xenoreactive natural antibody production by retroviral gene therapy. *Science* 1998; **281**: 1845.
52. Yamada K, Yazawa K, Shimizu A, *et al.* Marked prolongation of porcine renal xenograft survival in baboons through the use of alpha1,3-galactosyltransferase gene-knockout donors and the cotransplantation of vascularized thymic tissue. *Nat Med* 2005; **11**: 32. Epub 2004 Dec 2026.
53. Kuwaki K, Tseng YL, Dor FJ, *et al.* Heart transplantation in baboons using alpha1,3-galactosyltransferase gene-knockout pigs as donors: initial experience. *Nat Med* 2005; **11**: 29 Epub 2004 Dec 2026.
54. Schroeder C, Allan JS, Nguyen BN, *et al.* Hyperacute rejection is attenuated in GalT knockout swine lungs perfused ex vivo with human blood. *Transplant Proc* 2005; **37**: 512.
55. Chen G, Qian H, Starzl T, *et al.* Acute rejection is associated with antibodies to non-Gal antigens in baboons using Gal-knockout pig kidneys. *Nat Med* 2005; **11**: 1295. Epub 2005 Nov 1227.
56. Lin Y, Goebels J, Xia G, Ji P, Vandeputte M, Waer M. Induction of specific transplantation tolerance across xenogeneic barriers in the T-independent immune compartment. *Nat Med* 1998; **4**: 173.
57. Kreisel D, Krupnick AS, Balsara KR, *et al.* Mouse vascular endothelium activates CD8+ T lymphocytes in a B7-dependent fashion. *J Immunol* 2002; **169**: 6154.
58. Rothermel AL, Wang Y, Schechner J, *et al.* Endothelial cells present antigens in vivo. *BMC Immunol* 2004; **5**: 5.

59. Bagai R, Valujskikh A, Canaday DH, *et al.* Mouse endothelial cells cross-present lymphocyte-derived antigen on class I MHC via a TAP1-and proteasome-dependent pathway. *J Immunol* 2005; **174**: 7711.
60. Brossay A, Harang S, Herault O, Bardos P, Watier H. The active role played by xenogeneic endothelial cells in the indirect presentation pathway is not lymphocyte trans-co-stimulation. *Transpl Int* 2005; **17**: 787. Epub 2005 Feb 2015.
61. Coleman TS, Pittman HK, Purser SM, Haisch CE, Verbanac KM. Human T-cell-porcine endothelial cell interactions induce human Th1 cytokines and porcine activation markers. *J Surg Res* 2001; **97**: 184.
62. Khayyamian S, Hutloff A, Buchner K, *et al.* ICOS-ligand, expressed on human endothelial cells, costimulates Th1 and Th2 cytokine secretion by memory CD4+ T cells. *Proc Natl Acad Sci USA* 2002; **99**: 6198.
63. Klingenberg R, Autschbach F, Gleissner C, *et al.* Endothelial inducible costimulator ligand expression is increased during human cardiac allograft rejection and regulates endothelial celldependent allo-activation of CD8+ T cells in vitro. *Eur J Immunol* 2005; **35**: 1712.
64. Kempe S, Kestler H, Lasar A, Wirth T. NF-kappaB controls the global proinflammatory response in endothelial cells: evidence for the regulation of a pro-atherogenic program. *Nucleic Acids Res* 2005; **33**: 5308. Print 2005.
65. Zhou LF, Yin KS, Zhu ZL, *et al.* Adenovirus-mediated overexpression of novel mutated IkappaBalpha inhibits nuclear factor kappaB activation in endothelial cells. *Chin Med J (Engl)* 2005; **118**: 1422.
66. Bach FH Genetic engineering as an approach to xenotransplantation. *World J Surg* 1997; **21**: 913.
67. Anrather J, Csizmadia V, Brostjan C, Soares MP, Bach FH, Winkler H. Inhibition of bovine endothelial cell activation in vitro by regulated expression of a transdominant inhibitor of NF-kappa. *B J Clin Invest* 1997; **99**: 763.
68. Smyth MJ, Kershaw MH, Trapani JA. Xenospecific cytotoxic T lymphocytes: potent lysis in vitro and in vivo. *Transplantation* 1997; **63**: 1171.
69. Mirenda V, Golshayan D, Read J, *et al.* Achieving permanent survival of islet xenografts by independent manipulation of direct and indirect T-cell responses. *Diabetes* 2005; **54**: 1048.
70. Deng S, Buhler L, Lou J, *et al.* Study of concordant xenografted islets of Langerhans rejection: humoral or cellular mechanism? *Transplant Proc* 1994; **26**: 1184.
71. Guo Z, Mital D, Mo YY, *et al.* Effect of gene gun-mediated CTLA4IG and Fas ligand gene transfection on concordant xenogeneic islet graft rejection. *Transplant Proc* 1998; **30**: 589.
72. Deng S, Ketchum RJ, Yang ZD, *et al.* IL-10 and TGF-beta gene transfer to rodent islets: effect on xenogeneic islet graft survival in naive and B-cell-deficient mice. *Transplant Proc* 1997; **29**: 2207.
73. Horwitz MS, Bradley LM, Harbertson J, Krahl T, Lee J, Sarvetnick N. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. *Nat Med* 1998; **4**: 781.
74. Sarukhan A, Lechner O, von Boehmer H. Autoimmune insulitis and diabetes in the absence of antigen-specific contact between T cells and islet beta-cells. *Eur J Immunol* 1999; **29**: 3410.
75. Lee MS, Chang I, Kim S. Death effectors of beta-cell apoptosis in type 1 diabetes. *Mol Genet Metab* 2004; **83**: 82.
76. Papaccio G, Graziano A, D'Aquino R, Valiante S, Naro F. A biphasic role of nuclear transcription factor (NF)-kappaB in the islet beta-cell apoptosis induced by interleukin (IL)-1beta. *J Cell Physiol* 2005; **204**: 124.
77. Suk K, Kim S, Kim YH, *et al.* IFN-gamma/TNF-alpha synergism as the final effector in autoimmune diabetes: a key role for STAT1/IFN regulatory factor-1 pathway in pancreatic beta cell death. *J Immunol* 2001; **166**: 4481.
78. Nicoletti F, Zaccone P, Di Marco R, *et al.* Prevention of spontaneous autoimmune diabetes in diabetes-prone BB rats by prophylactic treatment with antirat interferon-gamma antibody. *Endocrinology* 1997; **138**: 281.
79. Dinarello CA. Reduction of inflammation by decreasing production of interleukin-1 or by specific receptor antagonism. *Int J Tissue React* 1992; **14**: 65.
80. Yang XD, Tisch R, Singer SM, *et al.* Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I The early development of autoimmunity and the diabetogenic process. *J Exp Med* 1994; **180**: 995.
81. El-Sheikh A, Suarez-Pinzon WL, Power RF, Rabinovitch A. Both CD4(+)and CD8(+)T cells are required for IFN-gamma gene expression in pancreatic islets and autoimmune diabetes development in biobreeding rats. *J Autoimmun* 1999; **12**: 109.
82. Eizirik DL, Mandrup-Poulsen T. A choice of death – the signal-transduction of immune-mediated beta-cell apoptosis. *Diabetologia* 2001; **44**: 2115.
83. Giannoukakis N, Rudert WA, Ghivizzani SC, *et al.* Adenoviral gene transfer of the interleukin-1 receptor antagonist protein to human islets prevents IL-1beta-induced beta-cell impairment and activation of islet cell apoptosis in vitro. *Diabetes* 1999; **48**: 1730.
84. Tellez N, Montolio M, Biarnes M, Castano E, Soler J, Montanya E. Adenoviral overexpression of interleukin-1 receptor antagonist protein increases beta-cell replication in rat pancreatic islets. *Gene Ther* 2005; **12**: 120.
85. Hollander K, Bar-Chen M, Efrat S. Baculovirus p35 increases pancreatic beta-cell resistance to apoptosis. *Biochem Biophys Res Commun* 2005; **332**: 550.
86. Giannoukakis N, Mi Z, Rudert WA, Gambotto A, Trucco M, Robbins P. Prevention of beta cell dysfunction and apoptosis activation in human islets by adenoviral gene transfer of the insulin-like growth factor I. *Gene Ther* 2000; **7**: 2015.

87. Machen J, Bertera S, Chang Y, *et al*. Prolongation of islet allograft survival following ex vivo transduction with adenovirus encoding a soluble type 1 TNF receptor-Ig fusion decoy. *Gene Ther* 2004; **11**: 1506.
88. Rabinovitch A, Suarez-Pinzon W, Strynadka K, *et al*. Transfection of human pancreatic islets with an antiapoptotic gene (bcl-2) protects beta-cells from cytokine-induced destruction. *Diabetes* 1999; **48**: 1223.
89. Bach FH, Ferran C, Hechenleitner P, *et al*. Accommodation of vascularized xenografts: expression of ''protective genes'' by donor endothelial cells in a host Th2 cytokine environment. *Nat Med* 1997; **3**: 196.
90. Kobinger GP, Deng S, Louboutin JP, *et al*. Pharmacologically regulated regeneration of functional human pancreatic islets. *Mol Ther* 2005; **11**: 105.
91. Lopez-Talavera JC, Garcia-Ocana A, Sipula I, Takane KK, Cozar-Castellano I, Stewart AF. Hepatocyte growth factor gene therapy for pancreatic islets in diabetes: reducing the minimal islet transplant mass required in a glucocorticoid-free rat model of allogeneic portal vein islet transplantation. *Endocrinology* 2004; **145**: 467. Epub 2003 Oct 2009.
92. Rao P, Cozar-Castellano I, Roccisana J, Vasavada RC, Garcia-Ocana A. Hepatocyte growth factor gene therapy for islet transplantation. *Expert Opin Biol Ther* 2004; **4**: 507.
93. Roccisana J, Reddy V, Vasavada RC, Gonzalez-Pertusa JA, Magnuson MA, Garcia-Ocana A. Targeted inactivation of hepatocyte growth factor receptor c-met in betacells leads to defective insulin secretion and GLUT-2 downregulation without alteration of betacell mass. *Diabetes* 2005; **54**: 2090.
94. Deng S, Vatamaniuk M, Lian MM, *et al*. Insulin gene transfer enhances the function of human islet grafts. *Diabetologia* 2003; **46**: 386. Epub 2003 Mar 2007.
95. Ildstad ST, Wren SM, Bluestone JA, Barbieri SA, Sachs DH. Characterization of mixed allogeneic chimeras. Immunocompetence, in vitro reactivity, and genetic specificity of tolerance. *J Exp Med* 1985; **162**: 231.
96. Forman D, Tian C, Iacomini J. Induction of donor-specific tolerance in sublethally irradiated recipients by gene therapy. *Mol Ther* 2005; **12**: 353.
97. Emmanouilidis N, Larsen CP. Induction of chimerism and tolerance using freshly purified or cultured hematopoietic stem cells in nonmyeloablated mice. *Methods Mol Med* 2005; **109**: 459.
98. Colson YL, Xu H, Huang Y, Ildstad ST. Mixed xenogeneic chimerism induces donor-specific humoral and cellular immune tolerance for cardiac xenografts. *J Immunol* 2004; **173**: 5827.
99. Starzl TE Chimerism and tolerance in transplantation. *Proc Natl Acad Sci USA* 2004; **101**: 14607. Epub 12004 Aug 14619.
100. Hayashi H, Mayfield RS, Sawada T, *et al*. Role of the thymus in donor specific hyporesponsiveness induced by retroviral transduction of bone marrow using an MHC class I gene. *Transplant Proc* 1997; **29**: 1133.
101. Bagley J, Tian C, Sachs DH, Iacomini J. Induction of T-cell tolerance to an MHC class I alloantigen by gene therapy. *Blood* 2002; **99**: 4394.
102. Knechtle SJ, Wang J, Graeb C, *et al*. Direct MHC class I complementary DNA transfer to thymus induces donor-specific unresponsiveness, which involves multiple immunologic mechanisms. *J Immunol* 1997; **159**: 152.
103. Cohen JL, Salomon BL. Therapeutic potential of CD4+ CD25+ regulatory T cells in allogeneic transplantation. *Cytotherapy* 2005; **7**: 166.
104. Akl A, Luo S, Wood KJ. Induction of transplantation tolerance-the potential of regulatory T cells. *Transpl Immunol* 2005; **14**: 225. Epub 2005 Apr 2026.
105. Koksoy S, Elpek KG, Yolcu ES, Shirwan H. Tolerance to rat heart grafts induced by intrathymic immunomodulation is mediated by indirect recognition primed CD4+ CD25+ Treg cells. *Transplantation* 2005; **79**: 1492.
106. Schenk S, Kish DD, He C, *et al*. Alloreactive T cell responses and acute rejection of single class II MHC-disparate heart allografts are under strict regulation by CD4+ CD25+ T cells. *J Immunol* 2005; **174**: 3741.
107. Tamura S, Beck Y, Ando Y, Tahara H. Intrathymic inoculation of donor HLA class I-derived peptide generates donor-specific CD4+ CD25+ regulatory T cells. *Transplant Proc* 2005; **37**: 40.
108. Karim M, Feng G, Wood KJ, Bushell AR. CD25+ CD4+ regulatory T cells generated by exposure to a model protein antigen prevent allograft rejection: antigenspecific reactivation in vivo is critical for bystander regulation. *Blood* 2005; **105**: 4871. Epub 2005 Feb 4815.
109. Lee MKT, Moore DJ, Jarrett BP, *et al*. Promotion of allograft survival by CD4+ CD25+ regulatory T cells: evidence for in vivo inhibition of effector cell proliferation. *J Immunol* 2004; **172**: 6539.
110. Chai JG, Xue SA, Coe D, *et al*. Regulatory T cells, derived from naive CD4+ CD25− T cells by in vitro Foxp3 gene transfer, can induce transplantation tolerance. *Transplantation* 2005; **79**: 1310.
111. Cobbold S, Waldmann H. Infectious tolerance. *Curr Opin Immunol* 1998; **10**: 518.
112. Wise MP, Bemelman F, Cobbold SP, Waldmann H. Linked suppression of skin graft rejection can operate through indirect recognition. *J Immunol* 1998; **161**: 5813.
113. Engels B, Noessner E, Frankenberger B, Blankenstein T, Schendel DJ, Uckert W. Redirecting human T lymphocytes toward renal cell carcinoma specificity by retroviral transfer of T cell receptor genes. *Hum Gene Ther* 2005; **16**: 799.
114. Hughes MS, Yu YY, Dudley ME, *et al*. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005; **16**: 457.

