Gene therapy and solid-organ transplantation

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Gene therapy and solid-organ transplantation. Recent developments in transplantation medicine improved the short- and long-term survival of solid-organ transplantation. However, chronic allograft rejection, the side effects of the long-term immunosuppressive treatment, and organ shortage are still the major obstacles to achieving long-term survival. Gene therapy has the potential to meet these challenges and has unique advantages in transplantation. In this review we summarize the studies using gene therapy in solid-organ transplantation.

The introduction of new immunosuppressive medications significantly increased the short-term and long-term survival of kidney grafts from both living and cadaver donors. The projected half-life of cadaver transplantation was increased to 13.8 years, and living-related transplantation was increased to 21.6 years in 1995. Despite the reduction of the rate of acute rejection, it is still one of the major obstacles to achieving long-term survival. The projected half-life of cadaver transplants with an episode of acute rejection was only increased to 8.8 years from 7.0 years [1]. Chronic rejection remains the most important cause of graft loss in long-term studies, and acute rejection is the major predictor of chronic rejection. Death with graft function is increasingly becoming an important cause of graft loss, occurring in 10 to 40% of transplanted patients, due to long-term immunosuppressive treatment in particular the increased risk of infection, malignancy, and cardiovascular disease [2]. Delayed graft function is another independent risk factor for long-term survival [3]. Another hurdle in transplantation is the increasing number of patients on a waiting list because of organ shortage, limiting the opportunities for transplantation. To overcome these obstacles, three main research areas in the field of transplantation have increased interest: (1) the prevention of acute and chronic rejection with new immunotherapies with less systemic immunosuppression and preferably that induce transplantation tolerance, as defined by graft acceptance in the absence of immunosuppressive treatment, (2) the prevention of nonimmunologic causes of graft loss, such as ischemia-reperfusion injury and arteriosclerosis, and (3) xenotransplantation as a potential solution for organ shortage.

Gene therapy has the potential to meet these challenges and has unique advantages in transplantation. Allotransplantation requires the removal of tissues from one individual and offers the possibility of treating cells or organs ex vivo with gene transfer vectors prior to implantation, thus avoiding many of the hurdles encountered with in vivo gene transfer. This gives the advantage of local production of immunosuppressive molecules, without exposing the recipient to the systemic side-effects of the drugs and may potentially achieve antigen specific immunosuppression. Higher concentrations of vector can be administered to organs ex vivo or cells in vitro, and excess vector washed away, decreasing chances for toxicity, immunogenecity, and systemic transduction or expression of the vector. One of the major limitations to the gene therapy is that gene expression and protein production are transient, which significantly limits the ability of gene transfer techniques for the treatment of inborn errors of metabolism, genetic deficiencies, or chronic disease. However, in the setting of transplantation, transient gene expression may be sufficient for immune modulation or even desirable because the potential toxicities and adverse effects of prolonged vector persistence and expression or immunosuppressive molecule production could be avoided [4].

Gene therapy can be used for the transfer of gene that induces secreted proteins [such as interleukin-10 (IL-10), transforming growth factor-β (TGF-β)], overexpression of membrane-bound molecules (such as Fas ligand) or intracellular proteins (such as antiapoptotic molecules). In this review, we summarize the studies using gene therapy in solid-organ transplantation.

IMMUNOMODULATION

The alloimmune response is a T-cell-dependent process that involves the orchestration of different amplification and effector mechanisms, including natural killer
cells, B cells, macrophages, cytokines, and chemokines. T cell recognizes the antigenic peptides in the groove of major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells (APCs). This so-called signal 1 delivers intracellular signals and confers antigen specificity to the immune response but alone is insufficient for full T-cell activation. The second signal, or “costimulatory signal,” is provided by interactions between specific receptors on the T cells, CD28, CTLA4 (CD152), CD40 ligand, and their respective ligands on APCs, B7-1 (CD80), B7-2 (CD86), and CD40. Blocking the costimulatory signals with the fusion protein CTLA4Ig and/or anti-CD40 ligand antibodies results in long-term allograft acceptance [5].

Although allo-MHC is the main target for alloimmune response, exposure of these donor MHC antigens through the thymus or hematopoietic stem cells could serve as a strategy for inducing antigen-specific tolerance [6]. The involution of the adult thymus makes the thymic approach not feasible for human gene therapy. However, DNA-mediated gene transfer of MHC classes I and II molecules to recipient bone marrow cells prolonged survival of murine cardiac allografts [7]. By using a similar approach, it was shown that pretransplant administration of a single donor class I MHC molecule was sufficient for the indefinite survival of cardiac allografts [8]. Retroviral transduction of bone marrow with the donor MHC gene significantly prolonged murine skin allograft survival and miniature swine kidney allograft survival [9, 10]. A recent study, using liposomes to transfet cultured recipient hepatocytes with plasmid DNA encoding the soluble donor MHC class I Ag, abrogated HAR and prolonged allograft survival in presensitized rat cardiac allograft model [11]. These studies demonstrate that the application of gene therapy of donor MHC molecules through bone marrow cells provides a new strategy for inducing transplantation tolerance.

Systemic administration of CTLA4Ig and anti-CD40 ligand has been tested in numerous allograft and xenograft models, and it has been shown in some studies that they significantly prolong survival and induce antigen-specific tolerance [5]. The effect of local expression of CTLA4Ig has been studied by using replication-defective adenoviral vector in cold-preserved murine liver allograft model [12]. Ex vivo perfusion of the donor hearts with recombinant adenovirus encoding CTLA4Ig cDNA induced indefinite graft survival in rat cardiac allotransplantation [13]. Transplantation of allogeneic mouse islets expressing the human CTLA4Ig cDNA following gene gun delivery also prolonged allograft survival significantly [14]. Gene transfer of anti-CD40 ligand has not yet been studied.

The role of cytokines in the induction of allograft acceptance and transplantation tolerance has been largely studied in the context of the Th1/Th2 paradigm. This paradigm proposes that Th1-derived cytokines (IL-2, interferon γ) promote allograft rejection by mediating delayed-type hypersensitivity reactions, cytotoxic T-lymphocyte generation, and macrophage activation and that Th2-derived cytokines (IL-4 and IL-10) protect rejection by down-regulating immune responses. Previous studies in rat transplantation models showed that the induction of tolerance in some studies is associated with the inhibition of Th1 cytokines and the sparing Th2 cytokines [15]. TGF-β can also suppress T-cell, B-cell, neutrophil, and inflammatory cytokine functions. Many studies have investigated the role of these immunomodulatory cytokines by using gene therapy. IL-10 gene therapy by using different vectors (such as, naked plasmid DNA, retroviral, herpes simplex viral and adenoviral vector or lipid mediated) significantly prolonged murine and rabbit cardiac allograft survival [16–19]. In addition, TGF-β gene therapy was shown to prolong the murine cardiac and liver allograft survival [16, 20, 21]. Along with the immunosuppressive properties of TGF-β, it induces fibrosis and may play a role in chronic rejection. This issue has not been addressed in these studies. One recent study investigated the effects of IL-4 gene therapy by using recombinant adenovirus vector in rat heart transplantation model. Although IL-10 gene therapy significantly prolonged the cardiac allograft survival, IL-4 gene therapy had no effect [22].

Chemokines are members of a family of small, inducible, and secreted proteins that are produced in inflammation and regulate leukocyte and lymphocyte recruitment. Increasing number studies have shown that chemokines play an important role in allograft rejection [23]. vMIP-II and MC148 are two recently identified chemokine homologues encoded by human herpes virus 8 and molluscum contagiosum that have antagonistic bone marrow cells provides a new strategy for inducing transplantation tolerance.

This approach resulted in a marked decrease of donor-specific cytotoxic T lymphocytes infiltrating the grafts and inhibited alloantibody production. Targeting the chemokine functions by gene therapy can be another novel approach for inhibition of alloimmune response.

Following the activation of effector mechanisms, alloimmune responses need to be down-regulated. This is carried out by several mechanisms that inhibit T cells at the later stages of the alloimmune response. CTLA4 molecules expressed on activated T cells interact with B7 molecules on APCs and deliver inhibitory signals to T cells. The Fas/Fas ligand (FasL) system plays an important role in the induction of lymphoid apoptosis and can turn off immune responses. Two immune-privileged sites, the eye and the testis, constitutively express...
FasL and protect cells from immune destruction. Gene therapy, using FasL, has been studied by several groups in different transplantation models, but the results are controversial. Gene transfer of murine FasL by replication-defective adenovirus significantly prolonged rat renal allograft survival [25]. Similar results were obtained in the rat liver transplantation model by using rat pFasL conjugated to liposome vesicles (hemagglutinating virus of Japan liposome) [26]. However, heart allografts from FasL transgenic mice were more rapidly rejected than nontransgenic control allografts [27].

A recent interesting study demonstrated the importance of secondary lymphoid tissue in alloimmune response. In this study, cardiac allografts were accepted indefinitely in recipient mice that lack secondary lymphoid tissue, indicating that the alloimmune response to a vascularized organ cannot be initiated in the graft itself and requires the presence of secondary lymphoid tissue [28]. This study may challenge the idea of using gene therapy methods locally at the transplanted organ and may suggest that secondary lymphoid organs could be potential targets. Dendritic cells are highly specialized APCs that initiate and modulate immune responses and play an essential role in T-cell activation and central and peripheral tolerance. A recent study used dendritic cells for adenoviral delivery of CTLA4Ig and induced alloantigen-specific T-cell hyporesponsiveness [29].

MODULATION OF NONIMMUNOLOGIC CAUSES OF GRAFT LOSS

Ischemia-reperfusion injury is an important cause of early nonimmune loss of the allograft. It induces the formation of reactive oxygen species by endothelial damage, which may cause cell death within the graft or may induce acute or chronic rejection by activating cytokines and adhesion molecules and recruiting leukocytes. This subject has also been of interest in gene therapy. Mitochondrial superoxide dismutase gene was transferred to the mouse liver prior to the lobar ischemia-reperfusion injury. It was shown to reduce acute liver damage significantly by inhibiting the redox-mediated activation of nuclear factor-κB and activator protein-1, which are the immediate early transcription factors that represent common pathways by which cells respond to environmental stress [30].

Apoptosis plays an important role in organ preservation and rejection. Overexpression of antiapoptotic genes in the allograft may prevent ischemia/reperfusion-induced apoptosis. Bcl-2 gene transfer to the mouse liver by recombinant adenovirus before procurement significantly decreased hepatocyte injury and the number of apoptotic cells [31]. It was also demonstrated that the expression of genes that encode heme oxygenase-1, bcl-xl, and A20 prevent antibody-induced transplant arteriosclerosis in mouse cardiac allografts [32].

Endothelium-derived nitric oxide is an endogenous inhibitor of vascular lesion formation. Gene transfer of endothelial cell nitric oxide synthase by the Sendai virus/liposome in vivo gene transfer technique to the endothelium of rat carotid arteries significantly decreased the balloon injury-induced damage and neointima formation [33]. Adenoviral-mediated inducible nitric oxide synthase gene transfer completely suppressed the development of arteriosclerosis in both untreated or cyclosporine A-treated rat aortic allografts [34].

Intercellular adhesion molecule-1 (ICAM-1) is an important mediator of cell adhesion and T-cell costimulation. The use of ex vivo hyperbaric transfection of antisense oligodeoxynucleotides that blocked ICAM-1 resulted in the reduction of chronic graft vascular disease and reperfusion injury in rat cardiac allografts [35, 36].

Cyclin-dependent kinase 2 is an important enzyme in the transition from the G1-S phase of the cell cycle and mediates smooth muscle cell proliferation. The use of gene transfer of antisense phosphorothioate oligodeoxynucleotide against this enzyme inhibited intimal hyperplasia and expression of vascular cell adhesion molecule-1 [37].

XENOTRANSPLANTATION

Organ shortage is one of the major limitations in transplantation, and this has stimulated a great deal of research into the possibility of using nonhuman donors for transplantation. The preferred donor animal species for xenotransplantation is the pig. The initial barrier to xenotransplantation is the pig. The initial barrier to xenotransplantation is hyperacute rejection (HAR), which results in the loss of graft within a few minutes to hours because of antibody-mediated complement activation of the graft endothelium. This xenoreactive natural antibody predominantly recognizes the unique carbohydrate structure, Galα1-3Gal, which is found as the terminal sugar on glycolipids and glycoproteins present on the donor endothelium. Humans do not express this antigen and have antibody to this antigen, in a similar fashion to blood group antigens. This epitope is synthesized by an enzyme called α-galactosyltransferase. It was shown that introducing a functioning gene that encodes this enzyme by retroviral gene transfer into autologous bone marrow cells overcame the HAR in a mouse model [38]. The effector mechanism of HAR is the activation of complement cascade. There are complement-regulatory proteins (CRPs) present on the donor endothelium that regulate the activation of complement. These proteins include decay-accelerating factor (DAF:CD55), membrane cofactor protein (MCP:CD46), and CD59. Xenograft endothelium is severely damaged because of a lack of these CRPs. Transgenic pigs that express human CRPs have been developed to limit complement-induced dam-
age. Heart and kidneys derived from transgenic pigs that express DAF did not develop HAR if transplanted into nonhuman primates [39–42]. Furthermore, the use of transgenic pigs that express DAF with CD46 or CD59 as donors for kidney or heart transplantation into baboons show to prolong graft survival without immunopathological findings of HAR [43].

If HAR can be avoided, the xenograft is subject to acute vascular rejection (delayed xenograft rejection) within a few days to weeks post-transplantation, which is probably T-cell independent and is caused by antibodies, macrophages, cytokines, and chemokines. If these problems are overcome, which is very difficult, a third hurdle to the final success of the xenotransplantation is T-cell-mediated rejection. Similar approaches as in allotransplantation (using CTLA4Ig, IL-10, TGF-β gene transfer) can be used in xenotransplantation to prevent T-cell-mediated rejection.

CONCLUSION

Gene therapy, which is in the early stages of development, holds great promise for allotransplantation and xenotransplantation. Improvements in transfer vectors and delivery protocols to obtain efficient transfection with minimal toxicity, along with a better understanding of alloimmune responses to identify the most efficient gene or gene combinations, would make gene therapy an important field of transplantation in this century.

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REFERENCES


