Gene Therapy

Allograft Transplantation

Rapid progress in understanding some of the molecular mechanisms involved in the pathogenesis of rejection and tolerance, coupled with the development of gene delivery technology, has stimulated novel approaches for therapy in transplantation. Conventional management for organ allograft transplantation requires the life-long administration of systemic immunosuppressive agents to promote graft acceptance. This therapy is associated with considerable morbidity and mortality, and it is not yet effective in preventing chronic rejection. Gene therapy has compelling potential to develop unique methods for achieving long-term allograft survival and immune tolerance.

Rejection of an allograft is primarily a cellular immune response. Five elements of the response to allografts have been modified by gene therapy in experimental models reported so far. These are (1) increasing the levels of the immunomodulatory cytokines IL-10 and TGF-β; (2) blunting the effects of IL-12, a proinflammatory cytokine; (3) inducing expression of the Fas ligand and recipient MHC molecules in donor cells to enhance peripheral tolerance; (4) blocking the interaction of cytotoxic T lymphocytes with antigen-presenting cells; and (5) preventing transport of MHC class I gene products out of the endoplasmic reticulum and decreasing the expression of adhesion molecules.

Viral IL-10 inhibits monocyte/macrophage activation and subsequent cytokine synthesis and also suppresses alloreactivity of peripheral blood lymphocytes. Hepatic and cardiac allografts transduced with different gene therapy vectors to express viral IL-10 without additional immunosuppression survive for a significantly longer period of time. Similarly, decreased alloreactivity to pancreatic islets has been reported after genetic modification of the islet to induce secretion of viral IL-10 with replication-defective adenoviral vectors. Preliminary studies show that transfection of islet grafts with IL-10 abrogates primary non-function and prolongs graft survival.

When the gene for TGF-β1, a potent immunoregulatory polypeptide, was transduced into syngeneic and allogeneic cardiac grafts, no toxic effects or fibrosis were evident and survival of the grafts increased without systemic immunosuppression. After orthotopic transplantation of liver allografts expressing TGF-β1, enhanced TGF-β1 production was associated with decreased levels of the pro-inflammatory cytokines TNF-α and IFN-γ when compared with nontransfected controls.

Another approach has been to modify proinflammatory cytokine production induced after transplantation. IL-12 induces IFN-γ production by activated T cells and natural killer cells and augments cytotoxic T lymphocyte generation. The IL-12 p40 subunit is responsible for receptor binding and serves as an antagonist. Accordingly, transducing the IL-12 p40 gene into allogeneic myoblasts can induce potent local immunosuppression and has potential as a novel therapy for preventing graft rejection.

Interactions of Fas with its ligand, FasL, are thought to play a major role in the maintenance of immunological homeostasis and peripheral tolerance. A high percentage of human corneal transplants are accepted without tissue matching or immunosuppressive therapy, a unique condition that has been attributed to expression of FasL on the cornea. FasL expression in the anterior chamber of the eye and in the testes has also been postulated to be responsible for the immunologically privileged status of these sites.

Extending this paradigm, Henry Lau and co-workers at the University of Pennsylvania showed that pancreas islet allograft rejection could be prevented by co-transplantation of syngeneic myoblasts transfected with the FasL gene. These studies await confirmation but suggest that local expression of FasL may be able to provide immune protection of islet allografts. Other studies to examine the role of FasL gene therapy to induce immunological privilege in allografts have been controversial, suggesting that additional regulatory molecules may be required.

An alternative strategy for promoting acceptance of incompatible grafts is to reduce the antigenicity of the graft through gene transfection and expression of recipient major histocompatibility complex class I and II antigens. Increased graft survival has been achieved as well by infusing autologous bone marrow cells transduced with retrovirus to express allogeneic MHC genes. This approach has the potential of avoiding the usual risk of graft-vs.-host disease. At this time, it is possible with new retroviral vectors to transfer either one class I or one class II gene into bone marrow cells.

Allografts can also be modified to block the interaction between T cells and antigen-presenting cells. Investigators at the University of Pennsylvania have demonstrated

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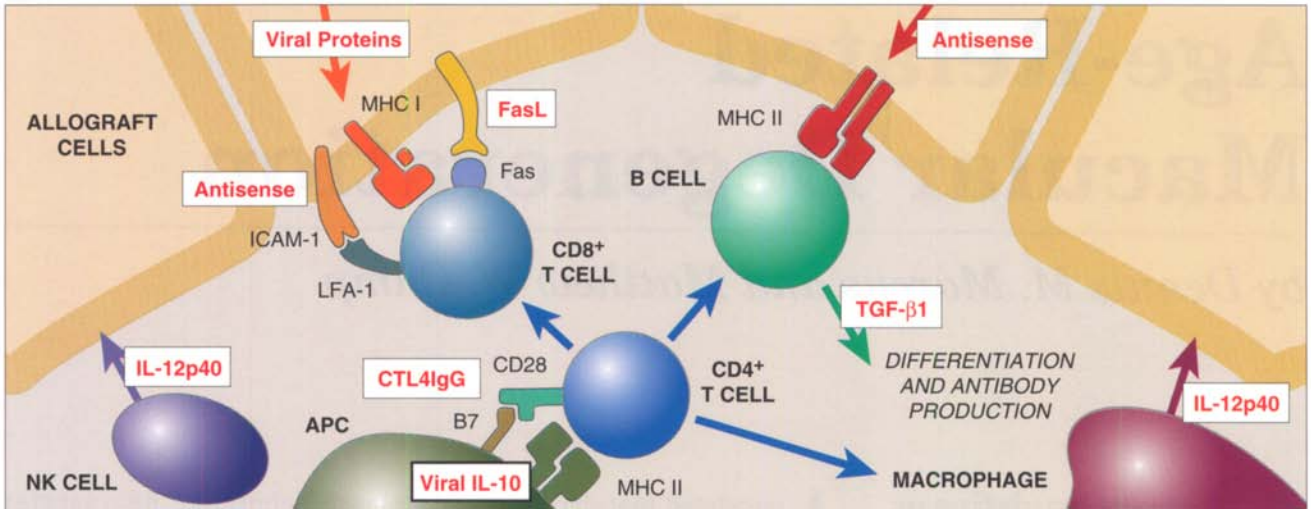

that local production of CTL4IgG, an inhibitor of the CD28/B7 costimulatory pathway in T cell-APC interaction, can significantly inhibit the alloimmune response to liver grafts. Transfection of this gene may be useful in preventing chronic rejection and also has potential for tolerance induction.

Introduction of viral genes that have evolved to regulate virus-host interactions and to evade the immune system represents another strategy to increase allograft survival. Protein products of genes of early transcription region 3 (E3) of adenovirus inhibit several different pathways of the host immune response. One of these proteins has been shown to bind to the heavy chain of MHC class I gene products and prevent their transport out of the endoplasmic reticulum. Prolonged survival of pancreatic islet allografts has been shown after insertion of adenovirus immunoregulatory genes.

Synthetic oligonucleotides can inhibit the expression of a gene in a sequence-specific manner at the transcriptional or translational level. Antisense-mediated inhibition of gene expression is a potentially valuable tool in transplantation. Blocking expression of intracellular adhesion molecule 1 (ICAM-1) and leukocyte antigen 1 prolongs allograft survival. This technology has been examined in rat heart and kidney and in monkey kidney transplant models with promising results. Preliminary results with antisense oligonucleotides also showed the feasibility of reducing expression of MHC class II molecules. This technology has potential applications for brief down-regulation of critical molecules involved in early phases of rejection.

The thymus plays a pivotal role in T cell development via positive and negative selection. It is known that tolerance to self-MHC antigens develops via the presentation of these antigens to the thymus (so-called central tolerance). By this means, lymphocytes programmed to react against autoantigens are eliminated. When donor-type MHC class I cDNA was injected directly into the thymus prior to liver transplantation in rats, 70% of the animals showed prolonged survival with donor-specific unresponsiveness.

Infusion of bone marrow cells has long been known to promote acceptance of allografts in a natural or induced state of T cell deficiency without chronic immunosuppression. It has been suggested that infused bone marrow cells persist in recipient tissues, resulting in a chimeric state that is required for induction and maintenance of tolerance. Graft-derived chimerism has been demonstrated clinically in long-surviving recipients of human solid organ transplants.

Further insight into the role of bone marrow cells in the induction and maintenance of tolerance has been obtained by monitoring chimerism derived from retrovirally transduced bone marrow cells carrying a neomycin resistance gene marker. The bone marrow cells remained transduced for at least six months, as assessed by molecular and immunohistochemical techniques. Transducing donor bone marrow cells with gene markers may provide an effective means to monitor the kinetics and localization of bone marrow cells and provide novel opportunities to investigate the role of chimerism in organ transplantation.

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