Gene Therapy to Improve Pancreatic Islet Transplantation for Type 1 Diabetes Mellitus

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Abstract

Pancreatic islet transplantation is a promising treatment option for Type 1 Diabetics, offering improved glycaemic control through restoration of insulin production and freedom from life-threatening hypoglycaemic episodes. Implementation of the Edmonton protocol in 2000, a glucocorticoid-free immunosuppressive regimen has led to improved islet transplantation success. >50% of islets are lost post-transplantation primarily through cytokine-mediated apoptosis, ischemia and hypoxia. Gene therapy presents a novel strategy to modify islets for improved survival post-transplantation. Current islet gene therapy approaches aim to improve islet function, block apoptosis and inhibit rejection. Gene transfer vectors include adenoviral, adeno-associated virus, herpes simplex virus vectors, retroviral vectors (including lentiviral vectors) and non-viral vectors. Adeno-associated virus is currently the best islet gene therapy vector, due to the vectors minimal immunogenicity and high safety profile. In animal models, using viral vectors to deliver genes conferring local immunoregulation, anti-apoptotic genes or angiogenic genes to islets can significantly improve islet survival in the early post-transplant period and influence long term engraftment. With recent improvements in gene delivery and increased understanding of the mechanisms underlying graft failure, gene therapy for islet transplantation has the potential to move closer to the clinic as a treatment for patients with Type 1 Diabetes.

Introduction

Type 1 diabetes (T1D) is a chronic, life-long autoimmune disease in which the immune system destroys the insulin-producing β-cells within the pancreatic islets. Islet transplantation is a promising treatment approach for T1D, maintaining blood glucose levels to an extent that has not been possible with traditional insulin injections [1, 2]. The potential of islet transplantation as a treatment for T1D was demonstrated in 2000, with the introduction of a steroid-free immunosuppressive protocol, termed the Edmonton Protocol [3]. To date, more than 700 diabetic patients have received an islet transplant [4], and there is considerable clinical evidence to suggest that islet transplantation can improve glycaemic control above that conferred by exogenous insulin treatment. The benefit is particularly significant for diabetic patients that suffer hypoglycaemic unawareness [5].

A major limitation to the success of clinical islet transplantation is the early loss of islet mass 24 – 48 hours post-transplantation. Immediately following transplantation, islets face a number of environmental challenges and stresses, including cytokine assault and inflammation, which lead to significant islet apoptosis. Pancreatic islets are one of the most heavily vascularized structures in the body and disruption of the capillary network during isolation results in islet ischemia and hypoxia. Islets are also highly immunogenic cellular structures, a property which leaves them vulnerable to the effects of alloimmunity and recurrence of anti-islet autoimmunity [6].

The development of highly efficient viral vectors capable of transferring useful genes to human cells has led to the concept of gene therapy, a therapeutic strategy that could be used for a variety of metabolic disorders and autoimmune diseases [7]. Early transplant stresses such as apoptosis and acute rejection may potentially be overcome through the use of *ex vivo* gene therapy strategies to deliver immunomodulatory, anti-apoptotic or angiogenic genes to the islets to improve post-transplant islet viability, engraftment, survival and resistance to rejection.

This review will discuss various viral-mediated gene transfer strategies that have been employed to improve islet survival in preclinical models, including the delivery of anti-apoptotic and angiogenic genes, as well as those conferring local immunoregulation to the islet graft.

Viral-Mediated Gene Transfer to Pancreatic Islets

There are four major classes of viral vectors utilized in gene therapy, adenoviruses (AdV), adeno-associated viruses (AAV), herpes simplex viruses and retroviruses (including lentiviruses) as shown in table **1**. A number of studies have demonstrated the ability of these vectors to infect pancreatic islets of both human and animal origin [8-11].

Commonly used gene delivery viral vectors. Table **1**

Type	Adenovirus	Adeno-associated virus	Herpes Simplex Virus	Retrovirus	Lentivirus
Packaging Capacity	Medium (≤7.5 kilo base pairs)	Low (≤4.5 kilo base pairs)	Large (≥30 kilo base pairs)	Medium (≤7 kilo base pairs)	Medium (≤8 kilo base pairs)
Duration of expression	Transient	Stable, long-term	Stable, long-term	Transient, however stability of expression from newer generation vectors is markedly improved to produce sustained expression	Stable, long-term
Immunogenicity	High	Low	Low	Low	Low
Repeated dosing	Not possible	Possible	Possible	Possible	Possible
Clinical Trials	Yes	Yes	Yes	Yes	Yes
Advantages	Infects both dividing and non-dividing cells, provides transient expression, particularly high short term expression, generates high titer viral stocks	Infects both dividing and non-dividing cells, integrates into host genome, provides long-term expression *in vivo*, elicits minimal immune response, generates high viral titers	Large genome, non-pathogenic, unable to reactivate, broad host range, persists long-term.	Integrates into host genome, provides long-term expression and stable transduction	Infects both dividing and non-dividing cells, genome integration, long-term expression
Disadvantages	Immunogenic, cause mild respiratory disease in humans	Requires helper virus, slow expression onset, inefficient large-scale virus production, small genome limiting packaging capacity	Potentially provoke antiviral responses against HSV-infected cells	Low efficiency *in vivo*, risk of insertional mutagenesis, low titer, host range restricted to dividing cells only	Safety concerns, production inefficient

Adenoviral Vectors

The majority of AdV vectors are based on human serotypes 2 and 5 which are known to cause a mild respiratory disease and are non-oncogenic [12]. More clinical trials utilise AdV-based vectors than any other vector currently available [13] and the applicability and safety of AdV for gene therapy has been studied extensively [14-17].

AdV vectors can trigger the innate immune response through the induction of type I interferons [18, 19]. This reduces the efficacy of gene transfer by eliminating expression of the genes encoded by the construct [20, 21]. In addition, patients treated with AdV constructs may suffer complications or even death [20, 22, 23]. Heavily immunosupressed patients are especially susceptible to severe AdV complications from treatment.

A major advantage of AdV for gene therapy is that they possess a medium size genome of $\leq$7.5 kilo base (kb) pairs, which allows them to accommodate large or multiple transgenes. In addition, AdV generate high titer viral stocks (10^{12}-10^{13} virus particles per ml), and provide high transduction efficiency. AdV infect both dividing and non-dividing cells, an important property when considering the transduction of senescent islet cells. The AdV genome is extra chromosomal, which significantly minimises the risk of insertional mutagenesis resulting from insertion of exogenous DNA into the genome [24]. AdV requires the coxsackie/adenovirus receptor (CAR), heparin sulphate, $\alpha_v\beta_5$ integrin and $\alpha_v\beta_5$ adherin for infection [24, 25]. CAR has been found on murine islets and β-cell lines, which may explain the relatively high efficacy of AdV transduction in islets [26]. Successful AdV infection of rodent pancreatic islets has been described by many research groups [27-30]. Safety studies in human islets demonstrated that AdV infection does not diminish β-cell viability or function *in vitro* [28, 30, 31]. Leibowitz and colleagues [32] compared the three major classes of viral vectors, and found AdV to be the most effective vector for infection of intact islets.

Adeno-Associated Virus Vectors

AAV are small, non-enveloped single-stranded DNA viruses that require a helper-virus for productive infection [33]. AAV possess many properties that make them attractive for use as gene transfer vectors in gene therapy. AAV vectors infect quiescent cells, elicit a minimal immune response and are non-pathogenic [34]. In addition, AAV achieve targeted and stable expression through site-specific integration, and possess low mutagenic and oncogenic potential [35]. AAV may integrate randomly into the host genome at sites of double-stranded DNA breaks [36] but display low frequency of random integration into the genome [37].

A major limitation of AAV vectors is that the maximum size of the gene insert is ≤4.5 kb pairs (smaller than AdV). This limits the potential for inserting multiple genes. AAV gene transfer of the cystic fibrosis transmembrane regulator has progressed to phase II trials in cystic fibrosis patients [34] and other AAV vectors have been evaluated as candidates for Hemophilia A and B treatment with gene therapy in both preclinical and clinical models [38].

The ability of AAV to transduce a given cell type depends on the presence of membrane-associated heparan sulfate proteoglycan (HSPG) receptors [39] and co-receptors including $\alpha_v\beta_5$ integrin heterodimers, fibroblast growth factor receptor type 1 and c-met [40-43]. Kahn and colleagues [44] have identified the presence of HSPG on human islets. Transduction of islets with AAV has been shown by a number of groups [45-49]. Wang and colleagues [45] revealed distinct AAV islet transduction efficiency and gene transfer patterns between different vector serotypes and administration routes. Local intra pancreatic ductal administration of AAV6 showed the best transduction efficiency in β-cells. Intraductal and intraperitoneal administration of AAV8 revealed efficient transduction of both exocrine acinar cells and endocrine β-cells. The transduction efficiency of AAV8 is attributable to the distinct properties of this viral serotype, including its receptor, laminin, which is highly expressed in the pancreas, and facilitates viral entry into the pancreatic cells [46]. Despite a number of AAV serotype studies, the optimal serotype for islet infection is yet to be determined.

Herpes Simplex Viral Vectors

Herpes Simplex Virus (HSV) is a double-stranded linear DNA virus, 152 kb in size with a virion structure consisting of an envelope, tegument, capsid and core. HSV-1 forms part of the larger *Herpesviridae* family, and is the most frequently used herpes virus for gene transfer. HSV-1 infection is common in the general population, manifesting itself as cold-sores however in rare cases it can cause encephalitis [50].

HSV-1 possesses a broad host range with the ability to infect many cell types. In particular, HSV-1 vectors provide efficient transduction and gene expression within the nervous system [53-55]. Therefore, most of the research utilizing HSV-1 vectors has focused on therapies to target neurological diseases. The large genome of HSV-1 (≥30 kb pairs) allows the vector to accommodate large or multiple transgenes or regions including regulatory elements or promoters [51]. In addition, the HSV-1 vector remains as an extrachromosomal episome, which decreases the likelihood of insertional mutagenesis within the host's genome [52]. These properties combined with the ability of HSV-1 to infect non-dividing cells makes it a suitable vector system for use in islet gene therapy [51].

To date, there have been a limited number of studies demonstrating the ability of HSV-1 to infect pancreatic islets [62, 63]. Liu and colleagues [63] have shown that murine islets and a β-cell line were efficiently transduced by a HSV vector, and that cytokine-mediated β-cell apoptosis was blocked by transduction with an anti-apoptotic Bcl-2 expressing HSV-1 vector.

In order for HSV to become a viable option for clinical islet gene therapy two major disadvantages of this vector system must be overcome. Firstly, HSV vehicles can be toxic and have the ability to provoke potent antiviral responses against HSV-infected cells [64]. Secondly, HSV provide only short transgene expression. New generation, completely defective HSV vectors are currently being developed that could allow these vector obstacles to be overcome [65].

Retroviral vectors

The first human gene therapy clinical trial was based on a retroviral vector, for correction of adenosine deaminase deficiency. In this study, both integrated retroviral vector and adenosine deaminase gene expression persisted for several years [66]. Retroviral vectors possess many advantages over other viral vectors for long-term treatment or correction of gene defects. Retroviral vectors allow for an insert size of up to 7 kb pairs and they integrate into the target cell genome, resulting in sustained gene expression.

There are over 250 currently approved retroviral gene therapy clinical trials, accounting for nearly 30% of the total clinical trials approved worldwide [67]. However, retroviral vectors are unable to infect non-dividing cells, such as islets, severely limiting their potential for use in islet gene therapy. Lentiviruses however are a subset of retroviruses with the ability to infect non-dividing cells and are therefore a logical retroviral candidate for use in islet gene therapy [68-70]. The first successful transduction of adult human pancreatic β-cells was performed using lentivirus, by Ju and colleagues in 1998 [11]. One study comparing infection of intact islets found that while no infection was achieved using retroviral vectors, up to 25% of the β-cells could be infected with lentivirus [32].

Lentiviral vectors have been shown to efficiently transduce dispersed islets [11], monolayer cultures of islets [32] and intact islets [10, 71-74] from different species including human. Giannoukakis and colleagues [10] demonstrated the ability of lentiviral vectors to transduce islets at a comparable efficiency to AdV without the drawbacks of immunogenicity. Furthermore, lentiviral vectors can efficiently transduce whole islets [9] and lentivirus transduced rat islets display no changes in islet morphology or function [75], further supporting their use in islet gene therapy.

Non-Viral-Mediated Gene Transfer to Pancreatic Islets

Various non-viral islet transduction strategies such as bacterial plasmids, cationic lipid- and polymer-based carriers, gene gun technology and calcium phosphate precipitation have been considered [76-84], with low transduction efficiency being the major obstacle reported to date.

A number of research groups [84-86] have investigated the use of protein transduction technology in islet gene therapy. This is a novel technique which allows delivery of specific proteins or peptides fused to small cell-penetrating peptides known as protein transduction domains to cells or tissues [87]. Delivery of a JNK inhibitory peptide via this system prevented islet apoptosis following isolation and

improved islet graft function. Furthermore, an NF-κB inhibitor infused into the mouse pancreatic duct prior to isolation yielded islets with enhanced viability [88].

Non-viral vectors offer several advantages over viral vectors including high clinical safety, no immunogenicity and ease of production. Despite this, non-viral vectors provide low islet transduction efficiency, owing to both the large size of the islets [83] and the diffusive barrier created by the islet nuclear membrane [78]. In addition, non-viral vectors offer only transient gene expression and require high doses [77, 81, 82] when compared to viral vectors. At present, despite extensive investigation of non-viral approaches for use in islet gene therapy, no studies have progressed to clinical trials.

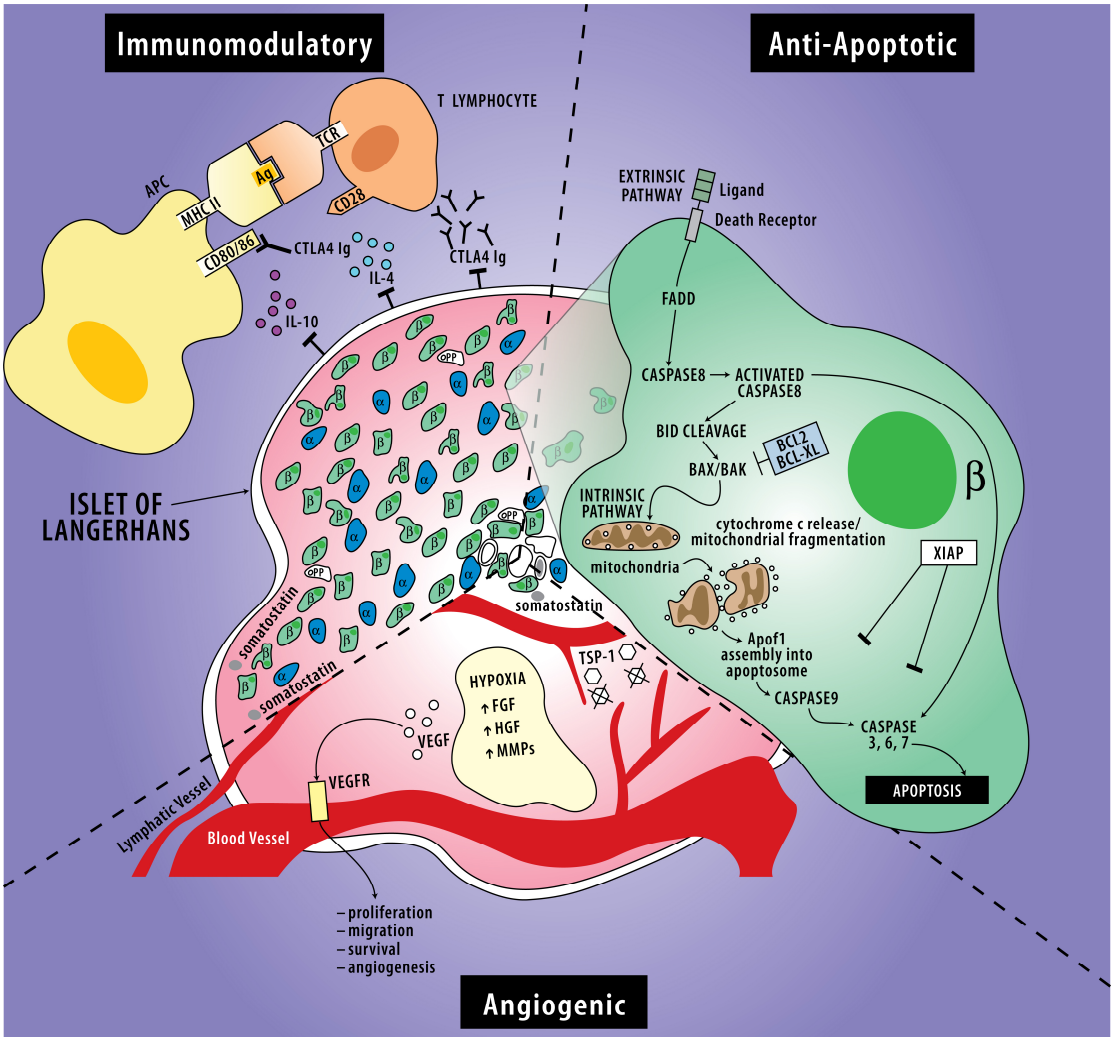
Summary

The four groups of viral gene therapy vectors described have specific strengths and weaknesses regarding their usefulness for islet transplantation. AAV have no known disease association and provide site-specific integration of DNA, whereas other viruses such as lentivirus insert randomly into the host genome and exhibit preference for integration at active transcription sites, generating concern regarding risk of insertional mutagenesis [89]. New-generation integration-deficient lentiviral vectors (IDLVs) have been developed that exhibit a reduced risk of causing insertional mutagenesis [90] and therefore may be a useful vector to pursue in future gene therapy studies.

Vectors such as AdV that carry large inserts may be favoured over others for some applications, however the safety concerns surrounding AdV, herpes simplex virus and lentiviral vectors limit their clinical potential. Non-viral vectors offer high clinical safety over their viral vector counterparts, but they have the disadvantage of very low islet transduction compared to AdV vectors which provide very high islet transduction efficiency [10]. Of the currently available viral vectors, AAV provides the best gene therapy tool available for islet transplantation due to its minimal immunogenicity, safety profile and its ability to produce high viral titers relevant for clinical use. However, expanded understanding of AAV biology and differences between vector serotypes is required to lead to improved and varied clinical applications for AAV gene therapies in the future.

Gene Transfer Strategies to Improve Islet Transplantation

There are a number of cellular processes that may be targeted by gene therapy to improve the outcomes of islet transplantation. Once the appropriate gene therapy vector has been selected, this must be paired with the optimal gene to be delivered. Effective therapy may result from the overexpression of an active protein, or the inhibition of a deleterious gene. Genes that are likely to be useful for islet transplantation fall into three main categories: immunomodulatory, anti-apoptotic and angiogenic (summarized in Fig. **(1)**). Numerous studies have investigated the potential of gene therapy for islet transplantation in animal models (Table **2**).



Gene transfer strategies to improve islet transplantation. Figure **1**

Three areas of islet cell biology that are currently being targeted by gene therapy are depicted. Immunomodulatory strategies (upper left panel) include co-stimulation blockade with CTLA4Ig and production of immunomodulatory interleukin-10 (IL-10) and interleukin-4 (IL-4). Anti-apoptotic approaches (upper right panel) target extrinsic (via stimulation of death receptors) and intrinsic (release of apoptotic factors by mitochondria) apoptosis pathways in the β-cell. Bcl-XL and Bcl2 (blue box) block pro-apoptotic proteins while XIAP (white box) directly inhibits caspases 3 and 9. Angiogenic (lower panel) factors VEGF, HGF, FGF and matrix metalloproteinases are induced during hypoxia and can be therapeutically overexpressed to enhance revascularization. Antisense blockade of angiostatic TSP-1 (white hexagons) improves the potency of proangiogenic factors. Figure represents a whole islet, with constituent α- (blue; glucagon-producing), β- (green; insulin-producing) and PP-(white; pancreatic polypeptide-producing) cells depicted in the upper left panel. Enlarged representations of infiltrating immune cells are shown (APC = antigen presenting cell; Ag = islet-specific antigen).

Genes delivered to pancreatic islets using viral vectors. Table **2**

Pathway	Transgene	Vector	Comment
Immunomodulation	CTLA4Ig	Adenovirus	Expression of CTLA4Ig in islets can prolong islet graft survival [93-96]
		Lentivirus	Islets transduced with CTLA4Ig prolong graft survival in a rat to mouse transplantation model [74]
	Interleukin-10	Adeno-associated virus	IL-4 transduced islets resulted in impaired metabolic function in recipient mice and normoglycaemia in only 1/7 mice [102]. Viral IL-10 introduced systemically sustained suppression of autoimmune responses and prolonged islet allograft survival [101]
	Interleukin-4	Adeno-associated virus	AAV-8 mediated IL-4 gene transfer to islets prevented the onset of diabetes in NOD mice [103]
	CTLA4Ig/CD40Ig	Adenovirus	Results in simultaneous blockade of co-stimulation pathways [97]
Anti-Apoptotic	Bcl-2	Adenovirus	Over expression of Bcl-2 in islet cells failed to prevent cytokine induced toxicity [110] and reduce inflammation in porcine islets [112]
		Lentivirus	Bcl-2 transduction of an pancreatic β-cell line provided protection against apoptosis induced by various stimuli including hypoxia and pro-inflammatory cytokines and corrected hyperglycaemia for several months when transplanted under the kidney capsule of diabetic C3H mice [111]
		Herpes Simplex Virus-1	Cytokine-mediated beta-cell apoptosis was blocked by transduction with an Bcl-2 expressing HSV-1 vector [63]
	Bcl-XL	Adenovirus	Bcl-XL transduction of a rat insulinoma cell line blocked cytokine induced apoptosis [114]
	XIAP	Adenovirus	Adenoviral-XIAP transduced βTC-Tet cells and human islets are highly resistant to hypoxia and cytokine induced apoptosis *in vitro* and βTC-Tet cells transplanted into SCID mice successfully reverse diabetes in 3 days compared to 21 for control cells [121-123]
Angiogenic	VEGF	Adenovirus	Rat islet grafts with elevated VEGF production exhibited significantly increased microvasculature, insulin content and reversed hyperglycaemia in diabetic mice [136]
	HGF	Adenovirus	Co-expression of hHGF and hIL-1Ra led to significant decrease in caspase-3 induced in human islets by cytokine challenge *in vitro*. Transduction of human islets improved the outcome of islet transplantation [137]. Pre-transplant islet gene therapy with HGF markedly improved islet transplant outcomes even in the setting of immunosuppressant-induced insulin resistance and β-cell toxicity [140]

Immunomodulatory Genes

A variety of immunomodulatory gene transduction strategies have been employed to confer islet transplant survival, by controlling or limiting local immune-mediated destruction.

CTLA4Ig

CTLA4Ig is an immunomodulatory molecule that is composed of the binding domain of human cytotoxic T lymphocyte associated antigen-4 attached to the Fc portion of human IgG1. CTLA4Ig binds to CD80/86 and blocks costimulatory signaling through CD28 thus preventing T cell activation - a process important for maintenance of the inflammatory response. As an immunosuppressive strategy, CTLA4Ig has been approved for the treatment of rheumatoid arthritis with the therapeutic drug Abatacept [91]. A second generation drug, Belatacept, is in phase III clinical trial for renal transplantation [92].

Feng and colleagues [93] demonstrated prolonged islet graft survival when AdV-CTLA4Ig transduced rat islets were transplanted into streptozotocin treated mice. A number of studies have shown that systemic administration of CTLA4Ig is more effective than local delivery in both allograft and xenograft models of islet transplantation [94] [95] This is probably reflective of the action of CTLA4Ig which blocks antigen presentation during T cell activation, a process likely to occur at a distant site such as the regional lymph nodes.

Another costimulatory pathway, CD40/CD40L has also been targeted using gene therapy to prolong graft survival. Local blockade of the CD40/CD40L pathway provided little to marginal improvement in survival of islet xenografts [96]. Combination gene therapy using CTLA4Ig and anti-CD40Ig has also been applied to xenogeneic islet transplant models. AdV expression of both CD40Ig and CTLA4Ig results in simultaneous blockade of co-stimulation pathways, meaning this may be an acceptable method to induce immune tolerance [97].

Whilst most of the studies of co-stimulatory molecule gene transduction have been performed with AdV, Fernandes and colleagues [74] compared AdV with lentiviral gene delivery using combined CTLA4Ig and TGF-β, and concluded that lentivirus gave no significant advantage over AdV in terms of immune protection.

Interleukin-4 and Interleukin-10

Both auto- and allo-immunity are major contributing factors to islet loss post-transplantation. Skewing of the alloimmune response away from a TH1 and towards either a TH2 or regulatory T cell phenotype may improve graft survival. Transduction of pancreatic islets with two key TH2 cytokines (Interleukin (IL)-10 and IL-4) is a promising strategy to subvert these immune responses.

IL-10 is a pleiotropic cytokine affecting a wide range of immune cells including dendritic cells, down-regulating the expression of co-stimulation molecules CD80/86 and inhibiting production of the TH1 promoting cytokine IL-12 [98]. Pure cellular IL-10 stimulates NK cells and cytotoxic T cells, whereas the Epstein Barr viral IL-10 homologue has immunosuppressive properties without potential immunostimulation. Both systemic and local administration of IL-10 has been investigated in the setting of islet transplantation. Islets from transgenic mice which over express IL-10 show no survival advantage when compared to wild type murine islets [99]. In fact, under some circumstances, expression of IL-10 within the pancreatic islet has been associated with early and rapid development of diabetes in autoimmune-prone NOD mouse [100]. However, when viral IL-10 was introduced systemically using AAV in NOD mice, there was sustained suppression of autoimmune responses and prolongation of islet allograft survival was observed [101].

IL-4 transduced murine islets displayed impaired metabolic function in a syngeneic model [102]. However other studies by Rehman and colleagues [103] found that local expression of murine IL-4 in islets prevented islet destruction and blocked autoimmunity, partly through regulation of T cell function. In a combinatorial approach, human and murine islets have been successfully transduced with IL-4 and IL-10 [49].

Summary

These studies demonstrate that the introduction of immunomodulatory genes to islets by gene therapy has generally not been as effective in blocking xenogeneic, allo- and auto-immune responses as the systemic expression of these genes. While the delivery of immunomodulatory cytokines (such as IL-4) that alter the local inflammatory milieu may prevent immune injury to some extent, other approaches that target initial immune recognition (such as CTLA4Ig) may require systemic expression to be effective. Therefore, islet transplantation outcomes may ultimately be enhanced by the local expression of a carefully selected immunomodulatory gene in conjunction with a transgene directed at another target.

Anti-Apoptotic Genes

Following transplantation, islets undergo extensive apoptosis induced by various intrinsic and extrinsic stimuli (see Fig. **(1)**). One approach to preserve β-cell mass in the early post transplant period has been to directly inhibit the apoptotic cascade. Some of the earliest efforts in prevention of β-cell death following transplantation involved inhibition of cytokine and Fas-mediated apoptosis [104-108].

Bcl-2

Bcl-2 is an anti-apoptotic protein, located in the membranes of the endoplasmic reticulum, nuclear envelope and outer membranes of the mitochondria. The over-expression of Bcl-2 has been reported in various β-cell lines [109-111] and has been shown to prevent both cytokine- and hypoxia-induced cell death *in vitro*. Although this effect has not always been replicated in the whole islet [110] possibly due

to the requirement of the gene to be expressed in every protected cell. While some authors have reported a cytoprotective effect of Bcl-2 expression *in vivo* [112] it is thought that Bcl-2 alone is not sufficient to increase preservation of islet mass following transplantation [113].

Bcl-XL

Bcl-XL is an anti-apoptotic member of the Bcl-2 family of proteins and is an important regulator of cell death. Transduction of a rat insulinoma cell line with Bcl-XL blocked both inducible nitric oxide synthesis (iNOS), cytokine-mediated mitochondrial changes, and subsequent apoptosis [114]. In islets, Bcl-XL is important for survival and deletion of Bcl-XL in pancreatic β-cells renders them abnormally sensitive to apoptotic stimuli *in vitro* and *in vivo* [115].

X-linked Inhibitor of Apoptosis Protein

X-linked Inhibitor of Apoptosis Protein (XIAP) is another potent inhibitor of apoptosis, which works by blocking the activation of multiple downstream caspases [116-120]. AdV-XIAP transduction of human and mouse β-cell lines and whole islets confers resistance to apoptosis following exposure to hypoxia and cytokines *in vitro* [121-123]. AdV-XIAP over expression preserved both β-cell viability and glucose responsiveness, the latter being a critical function that ordinarily disappears very early on during hypoxic stress. Furthermore, an AdV-XIAP transduced β-cell line transplanted into SCID mice, showed successful reversal of diabetes in 3 days, compared to 21 days for control cells.

In allogeneic islet transplantation, a recent study has shown that 90% of streptozotocin-induced diabetic animals receiving XIAP transduced islets survived up to 72 days, compared with a mean allograft survival of 17 days in control grafts [123]. The *in vitro* allogeneic response of splenocytes isolated from recipients of XIAP-expressing grafts 8 weeks post-transplant was similar to that seen in non-primed allogeneic mice, suggesting that XIAP over expression may lead to the acceptance of islet allografts in diabetic recipients. The mechanism behind this remains to be determined, but may involve T-cell anergy or the production of alternative regulatory cytokines.

Summary

Anti-apoptotic genes, such as Bcl-2, Bcl-XL and XIAP, have the potential to prevent islet cell death following transplantation. However, the difficulty lies in the requirement for all or most cells to express the gene in order to gain protection. Despite this, XIAP appears to be an effective target, as it can prevent apoptosis triggered by a number of stimuli *in vitro* and has resulted in significant *in vivo* islet protection in some models.

Angiogenic Genes

Pancreatic islets are a heavily vascularized tissue, utilizing around 10% of pancreatic blood flow despite only making up 1% of the tissue mass. During isolation, islets are removed from the blood supply and become rapidly hypoxic, exacerbating early cell death and β-cell loss [124, 125]. Following transplantation, islets slowly revascularize, but the ultimate vascular density and function is reduced compared to that of native pancreatic microvasculature.

During development and throughout life, islets produce a wide number of pro-angiogenic and angiostatic factors that maintain the dense, specialized vasculature while avoiding aberrant vessel production. Gene therapy, involving the delivery of pro-angiogenic genes or the inhibition of angiostatic factors, may be an ideal approach to improve the efficiency of engraftment and enhance the function of transplanted pancreatic islets.

Vascular Endothelial Growth Factor

Vascular Endothelial Growth Factor (VEGF) is an angiogenic factor for pancreatic islets. It is highly expressed during development [126] and is largely responsible for the resultant dense intra-islet capillary network and the maintenance of fenestrations on the endothelial cells in adulthood [127]. Genetic studies have suggested that VEGF may play a protective role in the development of Type I diabetes [128] and exogenous VEGF prevents islet death induced by serum starvation in human islets *in vitro* [129].

VEGF is also essential for the revascularization of transplanted pancreatic islets [130]. Upon isolation, VEGF expression in islets increases, but is significantly reduced within 2–3 days post-transplant [131]. This impairment is further pronounced in the presence of prevailing hyperglycemia, which coincides with delayed and insufficient islet revascularization in diabetic mice [131-134].

A number of studies have investigated VEGF in islet transplantation, either delivered as a protein or via gene therapy [135, 136]. Olsson and colleagues [137] found that while the culture of islets with VEGF improved graft revascularization one month post-transplantation, VEGF treated islets had similar or worse vascular engraftment compared to control islets. Zhang and colleagues [136] transferred human VEGF to murine islets, followed by transplantation into diabetic mice. Islet grafts with elevated VEGF production exhibited significantly increased microvasculature and insulin content, contributing to the reversal of hyperglycemia in diabetic recipient mice. Chae and colleagues [135] have demonstrated effective glycemic control achieved by transplanting non-viral liposome-mediated VEGF transfected islets in streptozotocin-induced diabetic mice using a non-viral cationic lipid reagent as a VEGF gene carrier.

Additional angiogenic factors

While VEGF appears to be one of the most important angiogenic factors, other factors with angiogenic activity such as hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and matrix metalloproteinases are also expressed naturally by islets, particularly during hypoxia [138]. The potential use of gene therapy to deliver these molecules to islets prior to transplantation has been shown in a number of studies [139, 140].

Inhibition of angiostatic factors

Pancreatic islets express a number of angiostatic factors that prevent aberrant vessel formation in adulthood [138]. One of the most important factors in islet transplantation is thrombospondin-1 (TSP-1) which, unlike other factors, is not downregulated by hypoxia and may contribute largely to the poor revascularization of islets following transplantation. At least one study has shown that the inhibition of TSP-1 in murine islets by siRNA transfection improves the revascularization and function of the islets transplanted into nude mice [141].

Summary

Experimental models provide proof-of-principle that local over-expression of angiogenic molecules in islet grafts can stimulate graft angiogenesis and enhance islet revascularization, thereby improving the outcome of marginal islet transplantation with better glycemic control in diabetic mice. Angiogenic factors are naturally produced by islets in response to hypoxia, but their potency may be improved by concurrently inhibiting endogenous angiostatic factors. One concern with an angiogenic approach to improve transplant engraftment, is that it may hasten allorecognition and immune attack. However, in the clinic, islet transplants are administered under the cover of systemic immunosupression. Thus, improvement of revascularization may be the more important consideration.

State of The Art: Islet Gene Therapy

Islet transplantation is limited by a myriad of early post-transplantation factors that conspire to limit islet function and survival. In order to improve the outcome of islet transplantation, measures that promote islet engraftment and revascularization or prevent rapid apoptosis following transplantation need to be improved. To this end, isolated islets are ideal candidates for local gene therapy, where the tissue is treated *ex vivo* prior to transplantation. The field must focus on several areas that present potential intervention targets, such as immunosuppressive β-cell toxicity, apoptosis, autoimmunity recurrence and angiogenesis. Furthermore, the field of islet gene therapy should focus future efforts on identifying the optimal combination of transgenes to be delivered to isolated islets in culture, whilst simultaneously identifying the most effective vector that provides the least immunogenicity. Pro-

survival Insulin Like Growth Factor, angiogenic thioredoxin-1 and immunomodulatory IL-4 may represent a valid combination of targets for islet transplantation and could be investigated further. With the current state of gene vector technology, including the development of mutants with improved transduction and safety profiles, AAV vectors are likely to be the best choice for providing safe delivery of transgenes to pancreatic islets at lower vector doses.

Conclusion

Gene therapy is a powerful and novel therapeutic technique, allowing modulation of the processes that are occurring in the local islet environment, thus avoiding potential systemic side effects. While AdV-based vectors have been the most commonly used vectors used in preclinical studies, the improved safety profile of AAV vectors makes them the candidate of choice for islet transplantation. The successes presented by the field to date have provided important insights into the delivery of immunomodulatory, anti-apoptotic and angiogenic genes to islets. The advantage of secreted proteins, such as anti-inflammatory cytokines and angiogenic factors, is that they do not need to be expressed in every cell. While all three gene classes hold potential for preventing significant islet loss following islet transplantation, it is likely that a combinatorial approach will be required in future studies aiming to promote early islet survival following transplantation.

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