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Therapeutic potential of immune privileged Sertoli cells

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Abstract

Sertoli Cells (SCs) in the testes have evolved to possess unique immune privileged properties to protect the developing germ cells from immunological attack. These immune privileged properties are not restricted to the testis, as SCs survive when transplanted across immunological barriers as allo- and xeno-grafts. Here we discuss the therapeutic potential of transplanted SCs in protecting cells, tissues or organs, which could be paramount in the field of transplantation to treat life-threatening diseases. Similar to the testis, transplanted SCs inhibit and/or modulate the immune response locally (at the transplant site) or systemically. Protection of transplanted cells, present in close vicinity of SCs, along with reduction of inflammation at the transplant site support that SC can inhibit and/or modulate the immune response locally. While protection of skin, islets in the contralateral kidney, and organs by SCs support their role in inducing systemic tolerance. Additionally, amelioration of autoimmune diseases, specifically type 1 diabetes mellitus, further supports this notion. Studies exploring SCs role as a vehicle for the cell based gene therapy further widens the horizon of SCs therapeutic potential in transplantation.

Keywords: cell based gene therapy, immune modulation, immune privilege, Sertoli cell, transplantation.

Introduction

Development of the testicular germ cells after the establishment of systemic immune system poses a threat to their survival as they express novel antigens that can be recognized as "foreign" by the host's immune system. Sertoli cells (SCs) play an important role in protecting these auto-immunogenic germ cells from immunological attack by sequestering the majority of the advanced germ cells behind the "Blood-Testis-Barrier/Sertoli cell barrier" and protecting the non-sequestered germ cells by modulating the testicular environment to be immunoregulatory (reviewed in (França *et al*., 2012; Kaur *et al*., 2014a). The ability of the SCs to modulate the immune environment is not restricted to the testis as they survive, when transplanted outside the testis, as allografts (transplantation performed between genetically different individuals of the same species) or xenografts (transplantation performed between different species) without the use of immune suppression (reviewed in Mital *et al*., 2010). Additionally, when SCs are co-transplanted with other immunogenic cells, such as pancreatic islets, they provide immune protection and prolong the survival of the co-grafted cells (reviewed in Mital *et al*., 2010). This demonstrates that their immunoprotective abilities are not restricted to germ cells in the testis.

Sertoli cells and transplantation

Organ or tissue transplantation is the ultimate cure for life-threatening diseases associated with tissue damage or organ failure. Progress made in the transplantation field to augment the survival rate of the transplanted organ/tissue is remarkable. Despite this success, due to the shortage of organ donors, receiving an organ or tissue transplant is still a dream for the majority of patients waiting to receive a transplant. For instance, according to the Organ Procurement and Transplantation Network, more than 122,000 patients are on a transplant waiting list, while in 2013 only 14,258 donors were available resulting in 28,953 total (organ/tissue) transplants (Report, 2012). After receiving a transplant, the next major obstacle faced by the recipient is the life-term dependency on immunosuppressive drugs to avoid graft rejection. The use of immunosuppressive drugs increases the risk of other infections, malignancies and can actually be toxic to the transplanted tissue or organ (Marcen, 2009).

The shortage of organ donors could be resolved by xenotransplantation. For instance, organs (heart, kidney, lung), tissues (islets, skin, cornea) or cells (hepatocytes, neuronal cells) obtained from pigs could be used to cure life-threatening diseases (Ekser *et al*., 2012). However, the immune response generated against xenografts is even more potent than allografts and the concerns involving immunosuppressive drugs still remain (Ekser *et al*., 2012). As mentioned earlier, immune privileged SCs survive transplantation without the use of immunosuppressive drugs. SCs can also protect co-transplanted tissue. Thus, transplanting pig organs/tissues with immune privileged SCs could be utilized in clinical transplantation to provide an unlimited supply of tissue and to avoid the harmful side effects of immunosuppressive drugs. In this review, we will describe the therapeutic potential of SCs in transplantation by providing an overview of their ability

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to protect cells, tissues or organs. Additionally, we will explore the idea of using immune privileged SCs in cell based gene therapy.

Type 1 Diabetes Mellitus

Co-transplantation of Sertoli Cells with pancreatic islets

Insulin dependent (type I) diabetes mellitus (TIDM) results from the autoimmune destruction of the insulin producing pancreatic islets and affects individuals worldwide. It is a serious health problem that can lead to chronic complications such as cardiovascular disease, kidney failure, lower-limb amputations and blindness (National, 2007). Current treatment for this disease is insulin therapy, however, this is costly and in many cases, does not sufficiently control blood glucose levels or deter the serious complications associated with this disease. Transplantation of human islets, using a precise immunosuppressive regimen, has the potential to cure patients with diabetes as it can restore normal blood glucose levels with 80% of these patients remaining insulin independent after 1 year (Shapiro *et al*., 2000; Ryan *et al*., 2002). Unfortunately, within 5 years, 92% of the transplant recipients rejected their transplanted islets and again required exogenous insulin injections (Ryan *et al*., 2005). More recently, preliminary results were presented by four centers indicating insulin independence of over 50% of patients after 5 years (Shapiro, 2011).

The ability of SCs to protect other cells (besides germ cells) came into the limelight in 1993 when Selawry *et al*., co-transplanted allogeneic islets and SCs underneath the kidney capsule of diabetic rats (Selawry and Cameron, 1993) and 64.5% of the co-grafted animals remained normoglycemic (blood glucose levels with the normal range) for over 100 days, while animals that received islets alone never achieved normoglycemia. However, a short course of immune suppression (3 injections of cyclosporine A) was required immediately after transplantation to achieve long-term normoglycemia (Selawry and Cameron, 1993). This study provided the first evidence that SCs can deliver immune protection to co-grafted tissue in an ectopic site (outside the testis) thereby launching the potential of SCs in therapeutic medicine.

Since this initial observation, the ability of SCs to protect co-grafted islets has been extensively investigated and described in other reviews (Mital *et al*., 2010; Kaur *et al*., 2012). Rather than repeat these descriptions, we will highlight a few examples. Another milestone in the therapeutic use of SCs was reached when Korbutt *et al*., modified the method for isolating and culturing SCs prior to transplantation (Korbutt *et al*., 1997). In this study, mild enzymatic treatment was used to isolate SCs and a short recovery period, culturing the isolated SCs for 48 h as aggregates, was added. Interestingly, during the recovery period aggregated SCs formed intercellular tight junctions similar to their morphology in the testis (Fig. 1A). Co-transplantation of these aggregated SCs with allogeneic islets in rats resulted in 100% islet allograft survival for at least 100 days without the requirement of immunosuppression. Another interesting observation reported in this study was that an optimal number of SCs (11 million) was required for prolonged survival of allogeneic islets as a low dose of SCs (5.5 million) was unable to protect the co-transplanted islets (Mean survival time (MST)-11 ± 1 days; Korbutt *et al*., 1997). The importance of the SC dose in protecting co-transplanted islets was further highlighted in the mouse model (Dufour *et al*., 2008a, b). Co-transplantation of allogeneic islets with either a low or high dose of SCs was unable to protect the co-grafted cells, while a dose of 3 or 4 million SCs was optimal as it prolonged the mean islet graft survival to 61.1 ± 6.9 days with 58.8% of the recipients remained nomoglycemic until the graft bearing kidneys were removed (Dufour *et al*., 2008a). The difference in optimal dose between mice and rats was likely due to the size of the islet graft it was protecting (e.g. 2000 islets in rats versus 500 in mice).

The current method of islet transplantation in humans is via portal vein injection. Recently, islets and SCs were isolated from BALB/c mice and co-cultured by hanging drop method prior to transplantation into diabetic C57Bl/6 mice via the portal vein (Takemoto *et al*., 2014; Fig. 1B). Islets and SCs were disassociated to single cells and cultured together for 4 days. Visualization of the fluorescently labeled cells identified aggregates with CellTracker Orange labeled SCs located in the center surrounded by EGFP positive islet cells. Injection of the SC/islet aggregates via the portal vein into the liver resulted in normalization of blood glucose levels and graft survival for over 100 days in 6 of 7 recipients. In contrast, controls receiving islets alone were rejected within approximately 10 days (Takemoto *et al*., 2014). This study established a way to co-localize SCs and islets during portal vein injection allowing the SCs to protect the co-grafted cells without the use of immune suppression.

Similar to allogeneic islets, SCs also protect xenogeneic islets. The initial report demonstrating the ability of SCs to protect xenogeneic islets was published in 1999 (Yang and Wright, 1999). In this study, SCs were co-encapsulated with xenogeneic fish islets and transplanted to diabetic BALB/c mice (Fig. 1C). Animals receiving islets encapsulated alone were used as controls. Co-encapsulation of SCs significantly prolonged the mean graft survival to 46 ± 6.3 days compared to controls (MST-21 ± 6.7 days; Yang and Wright, 1999). Afterwards, three separate studies further validated SCs potential to immunoprotect xenogeneic islets (Luca *et al*., 2001; Dufour *et al*., 2003; Ramji *et al*., 2011). Contrary to allogeneic islets, this protection required the use of either encapsulation

(Yang and Wright, 1999; Luca *et al*., 2001; Fig. 1C) or immunosuppressive drugs (Dufour *et al*., 2003; Ramji *et al*., 2011; Fig. 1D). The ability of SCs to protect xenogeneic pig islets has also been examined in humans (Valdes-Gonzalez *et al*., 2005), also reviewed in Mital *et al*., 2010; Kaur *et al*., 2012). Although promising, this study faced serious criticism due to safety and ethical concerns associated with transplantation of porcine tissue in humans prior to testing in large animal models such as non-human primates.

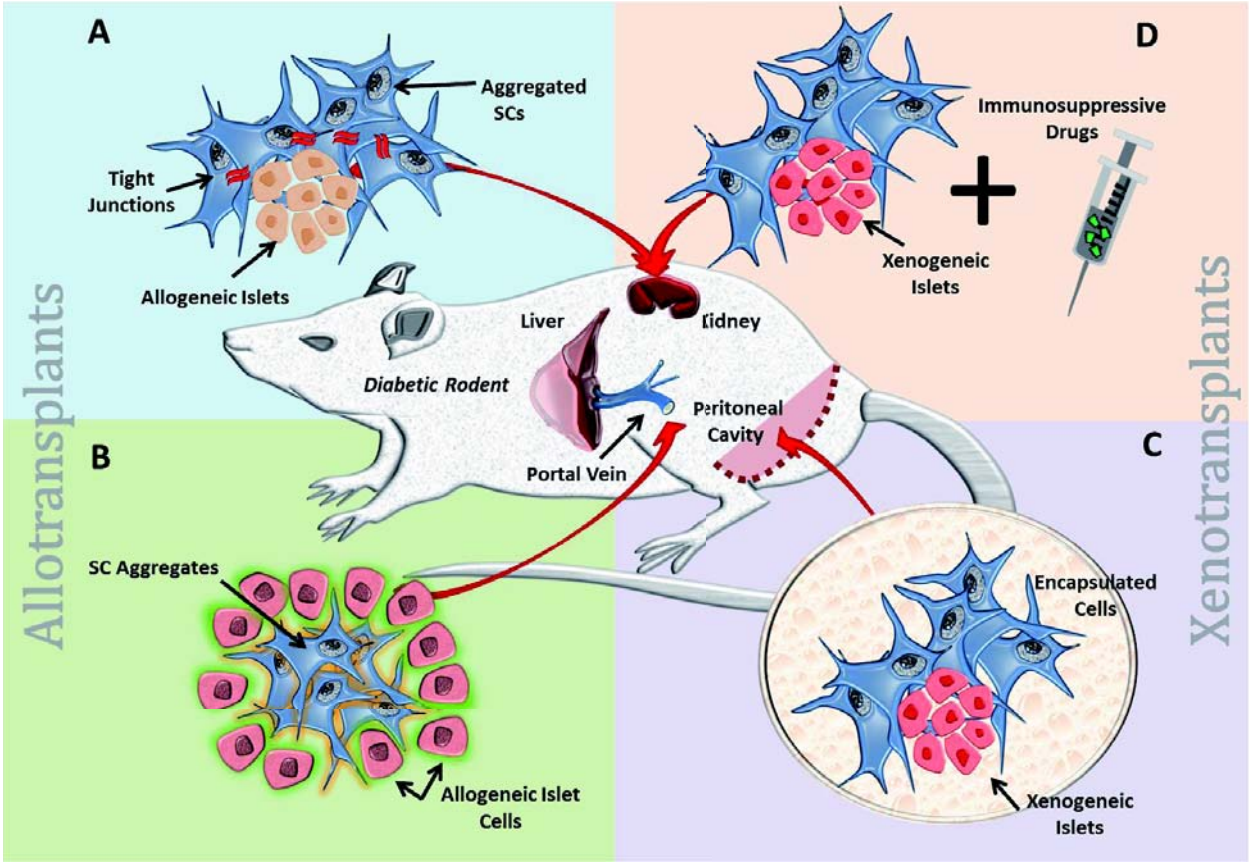

Figure 1. Sertoli cells co-transplanted with pancreatic islets. Allogeneic transplantation: Allogeneic islets were either combined with SCs (A) or co-cultured with SCs for 4 days (B) prior to transplantation. SC-islets were either transplanted underneath the renal subcapsular space (A) or injected intrahepatically via the portal vein (B) into the diabetic rodents without the use of any immune suppressive drugs. Xenogeneic transplantation: Xenogeneic islets were either co-encapsulated with SCs (C) or combined with SCs (D) prior to transplantation into diabetic rodents. Protection of naked (without encapsulation) xenogeneic islets by SCs required immune suppressive therapy (D).

Regeneration of pancreatic islets after Sertoli cell transplantation

As mentioned, T1DM is an autoimmune disease. A common school of thought was that most autoimmune diseases result from an emergence of a cytotoxic environment surrounding the targeted cells. This is most likely the case, however recent evidence indicates that the hostile environment also results from the loss of the immune tolerant or immune regulatory environment (Putnam *et al*., 2005; Carbone *et al*., 2014). Several studies provide evidence that SCs express and/or secrete factors, which can either inhibit and/or alter the immune response from destructive to protective type (Fig. 2; Wyatt *et al*., 1988; Selawry *et al*., 1991; De Cesaris *et al*., 1992; Campese *et al*., 2014). One particular study demonstrated that SC may induce systemic tolerance (Suarez-Pinzon *et al*., 2000). In this study, islets were transplanted underneath the left kidney capsule while SCs were transplanted underneath the right kidney capsule of diabetic nonobese diabetic (NOD) mice. NOD mice were used as a model of type 1 autoimmune diabetes. Sixty-four percent of the mice that received islets and SCs remained normoglycemice for over 60 days despite transplantation of the islets and SCs in contralateral kidneys. In contrast, 0% of mice that received islets only were normoglycemic. The resulting protection was associated with an anti-inflammatory/protective cytokine profile in the normoglycemic mice (Suarez-Pinzon *et al*., 2000).

An additional study indicated that transplanted SCs may even be capable of regenerating pancreatic islets in NOD mice by modifying the immune response.

Encapsulated neonatal porcine SCs or empty capsules (controls) were implanted in the intraperitoneal cavity of severely diabetic NOD mice (Fallarino *et al*., 2009). The animals that received empty capsules had a survival time of 28-62 days (and none were normoglycemic), while 81% of the SC transplanted animals survived for >120 days with 10 of these becoming normoglycemic. Additionally, SC transplantation into prediabetic NOD mice was even more beneficial as 88% of these mice remained disease free. Although, the mean number of islets in the SC treated versus control mice was not significantly different, SC therapy increased the mean area of individual islets and the mRNA expression (30-60 fold) of the islet progenitor cell genes. SC transplantation also created a regulatory environment inferred by analysis of immune cells from the spleen and pancreatic lymph node. A significant increase in expression of regulatory factors with a decrease in pro-inflammatory factors was detected compared to controls (Fallarino *et al*., 2009). Interestingly, in these mice, the onset of thyroiditis (another autoimmune disease) was also suppressed suggesting SCs therapeutic effect in autoimmune diseases is not limited to T1DM. Collectively, SC transplantation leading to diabetes prevention and reversion was associated with restoration of systemic tolerance along with regeneration of pancreatic islets. These studies denote the therapeutic effects of SCs on their surroundings, and specifically how they may provide a lasting treatment for people in the early onset stages of T1DM before the majority of pancreatic islet cells have been destroyed. However, once the majority of the islet cells have been destroyed, other methods to replace islet cell function, such as islet transplantation or gene therapy, must be explored.

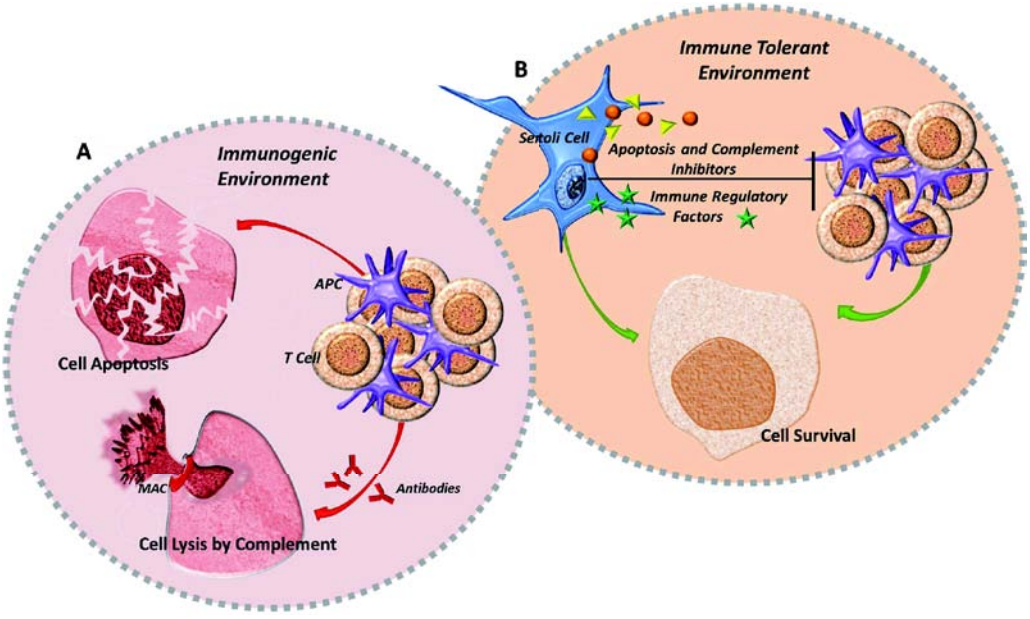

Figure 2. Inhibition and/or modulation of the immune response by Sertoli cells. Immunogenic Environment (A): Recognition of foreign antigens on the transplanted allo- or xeno-geneic grafts (cells/tissues/organs) activates antigen-presenting cells (APCs, dendritic cells or macrophages) which in turn activates the cell-mediated immune response (e.g. T cells). Activated immune cells destroy the transplanted graft by apoptosis. Additionally, the humoral immune response (involves antibodies) can also destroy the transplanted grafts by activating the complement cascade, which results in the formation of pores in the cell membrane and eventually cell lysis. Immune Tolerant Environment (B): SCs express or secrete complement and apoptosis inhibitors and thereby can protect cells, transplanted in close vicinity of SCs, from immunological attack. Additionally, SCs also secrete immunomodulatory factors and convert the cytotoxic immune response to regulatory type thereby inducing systemic tolerance and protecting tissues or organs, which are not transplanted in close vicinity of SCs.

Sertoli cell based gene therapy to deliver insulin

Although SCs can protect co-transplanted islets, the ability of SCs to survive transplantation is better than their ability to protect other cells. For instance, when allogeneic SCs were co-transplanted with islets, allogeneic islet graft survival was prolonged only 60% of the time, while over 90% of the grafts contained large number of surviving SCs (Dufour *et al*., 2008a). This improved survival suggests SCs can be used as a vehicle for cell based gene therapy. Specifically, SCs could be genetically engineered to express therapeutic proteins and these engineered SCs could then be transplanted across immunological barriers to ameliorate various diseases without the use of immunosuppressive drugs. In the future, transgenic pigs, with SCs expressing therapeutic proteins, could be created to provide an unlimited supply of transplantable tissue.

The ability of genetically modified SCs to secrete biologically active insulin at levels to restore normoglycemia was tested when neonatal porcine and pre-pubertal BALB/c mouse SCs were transduced with an adenovirus, carrying furin-modified human proinsulin cDNA (Halley *et al*., 2010; Fig. 4F). These SCs expressed and secreted insulin and when transplanted underneath the kidney capsule of streptozotocin (STZ) induced diabetic SCID mice, they were able to transiently restore normoglycemia. More recently, a mouse SC line (MSC-1 cells) was transduced with a lentivirus carrying furin-modified human proinsulin cDNA to achieve long-term, stable expression of insulin (Kaur *et al*., 2014b). In this study, *in vitro* stable expression of insulin was achieved for >2 years through several freeze thaw cycles. *In vivo*, when transplanted underneath the kidney capsule of STZ induced diabetic BALB/c mice, these transduced MSC-1 cells not only survived in the immune competent mice, they continued to express insulin for up to 50 days. However, as is often the case with lentiviral compared to adenoviral transduction, secretion levels of insulin were significantly lower and normoglycemia was not achieved (Kaur *et al*., 2014b). Further studies to increase insulin secretion are indicated if genetically modified SCs expressing insulin are to be therapeutic in the treatment of T1DM.

Neurodegenerative diseases

Co-transplantation of dopamine producing tissue with Sertoli cells

Neurodegenerative diseases are caused by the gradual and progressive loss of neuronal cells (Brown *et al*., 2005). An ideal treatment is either preventing the death of neurons or replacement of the lost neurons. Although, the brain is considered an immune privileged site, similar to the testis, it is immunologically responsive and can reject intracerebral grafts. For instance, animal studies have demostrated that tissue (allo- or xeno-grafts) transplanted into the brain is rejected and the use of immune suppression or other means to reduce the immunogenicity of the transplanted tissue is mandatory (Larsson *et al*., 2000, 2001; Armstrong *et al*., 2001).

The idea to use SC co-transplantation to treat a neurodegenerative disease such as Parkinson's was first tested by Sanberg *et al*., in 1996 (Sanberg *et al*., 1996). SCs were co-transplanted with xenogeneic bovine adrenal chromaffin cells (non-neuronal dopamine producing cells) into the striatum of the rat brain (Fig. 3A). Xenogeneic adrenal chromaffin cells transplanted to the contralateral side of the brain served as controls. Tissue collected at 2 month post-transplantation demonstrated that SCs were closely associated with viable chromaffin cells as evident by characteristic SC nuclei and the presence of SC-SC junctional complexes, while chromaffin cells transplanted alone were rejected. Co-transplantation of SCs also resulted in a 7-fold decrease in the microglial response, which was statistically significant compared to chromaffin cells transplanted alone (Sanberg *et al*., 1996). These data suggest that by decreasing microglial activation, SCs prolong the survival of co-transplanted xenogeneic chromaffin cells without the use of immunosuppressive drugs.

This same group has also co-transplanted allogeneic SCs with dopaminergic neurons obtained from fetal rat ventral mesencephalon into the 6-hydroxy-dopamine (6-OHDA) lesioned rat striatum (model of Parkinson's disease; Willing *et al*., 1999a; Fig. 3B). Contrary to the xenogeneic chromaffin transplants, in this study a short course of immune suppression was administered (cyclosporine was given on day 0 and +1). Immunostaining the brain tissue for tyrosine hydroxylase (TH, a marker for dopamine neurons) at 1 and 7 days post-transplantation revealed that 85% of the SC-dopamine neuron co-grafts survived compared to only 60% graft survival when dopamine neurons were transplanted alone. Additionally, a significant increase in the number of TH-positive neurons was detected in SC co-grafts (59% more at day 1 and 72% more at day 7 post-transplantation) compared to controls (Willing *et al*., 1999a). These data suggest that besides protecting co-transplanted neurons, SCs also increased their proliferation by secreting growth factors. Additionally, SCs ability to protect a xenogeneic immortalized cell line of human neuron-like (hNT) cells, in 6-OHDA lesioned rat striatum was also tested with promising results (Willing *et al*., 1999b; Fig. 3B). Although, the above-mentioned studies provide ample evidence that SCs protect co-transplanted neuronal or non-neuronal dopamine producing cells by decreasing the microglial response, amelioration of motor defects in the 6-OHDA transplanted rat model of Parkinson's disease was not clearly stated. In order to strengthen and expand the therapeutic horizon of SCs in neurodegenerative diseases, studies demonstrating improvements in motor defects need to be carried out.

Protection of degenerating neurons with Sertoli cell transplantation

Besides protecting co-transplanted neuronal cells, there is also evidence that SCs protect vulnerable neurons, such as motor neurons that are targeted in amyotrophic lateral sclerosis (ALS). In ALS the loss of motor neurons results in weak and spastic muscles as well as muscle atrophy that often results in paralysis. Oxidative stress has been identified as one culprit in the destruction of neurons in neurodegenerative diseases (Adams *et al*., 1989; Adams and Odunze, 1991; Multhaup *et al*., 1996). Many biological antioxidants have evolved to protect cells from oxidative stress, including those in the testes where many free radicals

are produced during spermatogenesis (Banfi *et al*., 2001; Kumagai *et al*., 2002). One specific antioxidant enzyme identified in the testes is superoxide dismutase (SOD). Specifically, extracellular SOD has been shown to be highly expressed by SCs (Aravindan *et al*., 1996, 1997). In a study by Hemendinger *et al*., they injected SCs into the parenchyma of the spinal cord of SOD1 transgenic mice (Fig. 3C; Hemendinger *et al*., 2005). SOD1 transgenic mice express a mutant form of the human SOD1 gene and serve as a model for ALS. A significant increase in motor neuron density was observed proximal to the injection site compared to non-injected SOD1 mice. However, the injected SCs were unable to delay the onset of neurodegeneration symptoms or prolong the survival of SOD1 mice compared to controls (Hemendinger *et al*., 2005). This could be attributed to the low dose (10,000) of SCs along with localized intraspinal injection that might not be therapeutically sufficient. Although, in this study, SC transplantation was unable to ameliorate the disease, it suggests that SCs have the potential to protect the motor neurons from oxidative stress.

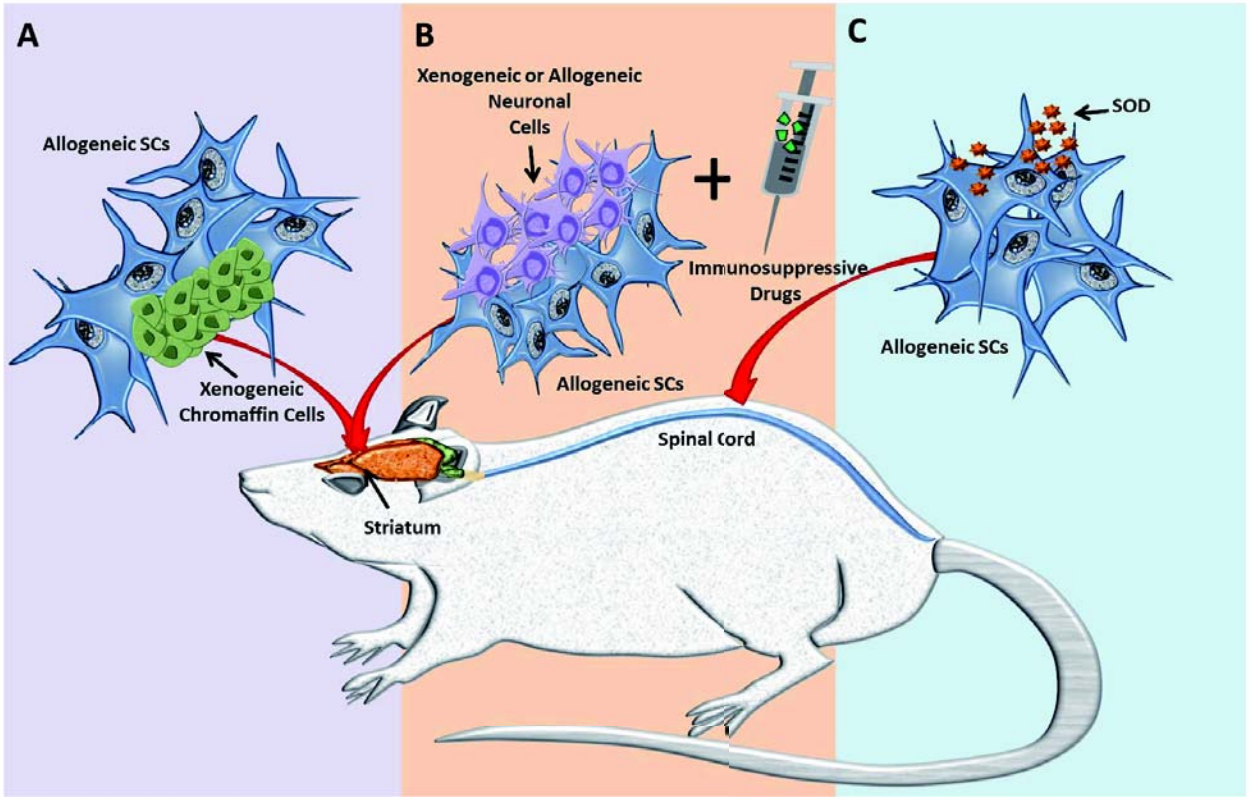

Figure 3. Sertoli cells co-transplanted with neuronal or non-neuronal tissue. Xenogeneic bovine adrenal chromaffin cells were co-transplanted with SCs into the striatum of normal rats without the use of any immunosuppressive drugs (A). Allogeneic dopaminergic neurons or xenogeneic human neuron-like cells were co-transplanted with SCs into the striatum of 6-OHDA-rat brain (a model for Parkinson's disease). Survival of allo- or xeno-geneic neuronal cells, in 6-OHDA-rats, required immune suppressive therapy along with SCs (B). A single injection of allogeneic SCs was injected into the parenchyma of the spinal cord of SOD1 transgenic mice (a model for ALS) without any immunesuppressants (C).

SC based gene therapy to deliver neurotrophin

Not only can SCs provide a protective environment for neurons, they can also be modified to deliver neurotrophins to aid in the regeneration of neurons. Transplantation of genetically modified "non-immuneprivileged" cells to deliver neurotrophin-3 (NT-3) after the spinal cord injury (SCI) resulted in axonal regrowth and led to partial functional recovery. However, this therapy either required syngeneic cells to avoid immune response against the transplant or when allogeneic cells were used it required immune suppressants (Ruitenberg *et al*., 2003; Tobias *et al*., 2003; Tuszynski *et al*., 2003). More recently, Trivedi *et al*., allotransplanted Lewis rat SCs modified to express NT-3 by adenovirus into the injured spinal cord of Sprague-Dawley rats which were not treated with immune suppressants (Trivedi *et al*., 2006; Fig. 4F). The transduced, allogeneic SCs survived for a significant period of time (42 days); however, NT-3 expression was lost by day 3 due to the transient nature of the adenoviral vector. Nevertheless, *in vitro* the NT-3

expressed by the SCs resulted in significantly higher neurite plasticity in cortical neurons cultured with conditioned medium from the transduced cells compared to neurons cultured with medium from non-transduced SCs. Contrary to diabetes, where stable expression is necessary to treat the disease, transient expression of neurotrophins can be beneficial and allow for the onset of neuron regrowth.

Not only did the aforementioned study provide data to support the use of SCs as delivery vehicles for neurotrophins in the treatment of SCI without the use of immune suppressants, it also further confirmed that SCs may have beneficial effects in inflammatory disease. Macrophage infiltration is indicative of an inflammatory response and is usually observed with SCI. This infiltration is also involved in early secondary pathogenesis following SCI (Mabon *et al*., 2000). Both transduced and non-transduced SCs, not only survived this hostile environment, but also significantly reduced the macrophage infiltration at the injury site, indicating again that SCs have a profound immunomodulatory effect on their surrounding environment.

Liver disease

Co-transplantation of Sertoli cells with hepatocytes

Acute liver failure also known as fulminant hepatic failure can be caused by a variety of agents resulting in liver cell dysfunction, which can rapidly progress to hepatic encephalopathy (mental disorientation) and eventually death from multi-organ failure (Lee, 2003; Riordan and Williams, 2003; van Paassen *et al*., 2012). Although, liver transplantation is the gold standard to treat hepatic diseases, a shortage of organ donors hampers this therapy. Instead, hepatocyte transplantation can be used as an alternative as it can sustain normal liver function allowing time for the host's liver to regenerate or until a donor organ becomes available (Rust and Gores, 2000; Dhawan *et al*., 2006; Akhter *et al*., 2009). So far, more than 60 clinical trails, transplanting either freshly isolated or cryopreserved hepatocytes, have been conducted. (Akhter *et al*., 2009) Although promising, variable success rates were reported (Dhawan *et al*., 2006; Akhter *et al*., 2009). This variability was mainly attributed to the health of the transplanted hepatocytes since most high quality donor livers were utilized for organ transplantation and therefore, the human hepatocytes available for clinical trails were isolated from livers considered unfit for whole organ transplantation (Rust and Gores, 2000; Dhawan *et al*., 2006; Akhter *et al*., 2009). The use of immunosuppressive drugs was an additional issue (Rust and Gores, 2000; Dhawan *et al*., 2006; Akhter *et al*., 2009).

To date, only one study has investigated the therapeutic effect of SCs on protecting co-transplanted hepatocytes (Rahman *et al*., 2005). Syngeneic rat SCs were co-encapsulated with xenogeneic HepG2 cells (a human hepatocyte-derived cell-line) and transplanted into the peritoneal cavity of normal rats or animals with acute hepatic failure without any immunosuppressive drugs (Fig. 4A). HepG2 cells encapsulated alone or empty capsules were used as controls. In normal rats, the HepG2 capsules, recovered at 1 week post-transplantation, contained viable and healthy cells. However, at 4 weeks post-transplantation, capsules were often fragmented and contained very few viable HepG2 cells. An intense immune cell response, surrounding or even infiltrating the capsules was observed. This immune reaction was HepG2 cell specific as empty capsules recovered at 1 month post-transplantation did not induced any immune reaction. In contrast, the HepG2-SC capsules contained viable and healthy HepG2 cells and SCs with very few immune cells surrounding the capsules. In rats with acute hepatic failure, 50% of the animals transplanted with co-encapsulated HepG2-SCs survived for 1 month compared to 20% transplanted with capsules containing Hep2 cells only and 0% transplanted with empty capsules. The capsules recovered at 1 month post-transplantation revealed that capsules containing HepG2 cells alone were heavily fibrosed, infiltrated with leukocytes and contained no viable cells, while the HepG2-SCs capsules contained viable cells (Rahman *et al*., 2005). Collectively, these data demonstrate that transplantation of co-encapsulated xenogeneic hepatocytes with SCs protected the hepatocytes from immune response and prolonged the survival of the animals with acute hepatic failure.

Skin wounds or burns

Autologous skin grafting is a widely acceptable method to treat large full thickness skin defects resulting from burns, wounds or skin necrosis (Bahar *et al*., 2012; Biedermann *et al*., 2013). The immune response to autologous skin grafts is negligible as the skin is transferred from one site to another on the same patient. However, autologous skin grafting is unrealistic in patients with third degree burns who have limited uninjured tissue (Bahar *et al*., 2012). Additionally, in patients who experience nonhealing complications (diabetic and elderly), creating a new wound by removing skin for autologous transplantation increases the risk of further complications (Bahar *et al*., 2012). In these cases, commercially available skin substitutes or allogeneic cadaver skin are routinely used (Hermans, 2011; Biedermann *et al*., 2013). Skin is a highly immunogenic tissue and survival of allogeneic skin after transplantation requires continous use of immunosuppressive drugs. Thereby making the patient vulnerable to opportunistic life-threatning infections.

Co-transplantation of an allo- or xeno-geneic skin grafts with immune privileged SCs has potential to prolong skin survival without the harmful effects of

immunosuppressive drugs. Shamekh *et al*., provided the first report demonstrating the beneficial effect of SCs on xenogeneic skin grafts (Shamekh *et al*., 2006). One month after the intravenous injection of rat SCs, the mice received a xenogeneic rat skin graft (Fig. 4B). Mice receiving a skin graft without a SC injection were used as controls. The control mice rejected the xenogeneic skin by 9.5 ± 0.5 days while rejection of skin grafts in SC transplanted mice was significantly delayed to 18.8 ± 1.79 days (Shamekh *et al*., 2006). Similar to the NOD mouse study mentioned earlier, where SCs protected islet grafts in the contralateral kidney, this study further suggested that the SCs can induce systemic tolerance by suppressing pro-inflammatory cytokines. Recently, the beneficial effect of SC transplantation on allogeneic skin graft survival was reported (Bistoni *et al*., 2012). In this study, encapsulated SCs were injected intra-peritoneally one week prior to transplantation of allogeneic skin grafts (Fig. 4B). Transplanted SCs prolonged the survival of allogeneic skin (MST-16 ± 2 days) compared to controls (MST-6 ± 2 days), which received empty capsules only. Graft survival was attributed to an absence of immune cell infiltration at the graft site and a significant increase in regulatory T cells in the peripheral lymph nodes. Interestingly, removal of the encapsulated SC transplants did not disrupt the tolerance generated toward skin grafts (Bistoni *et al*., 2012).

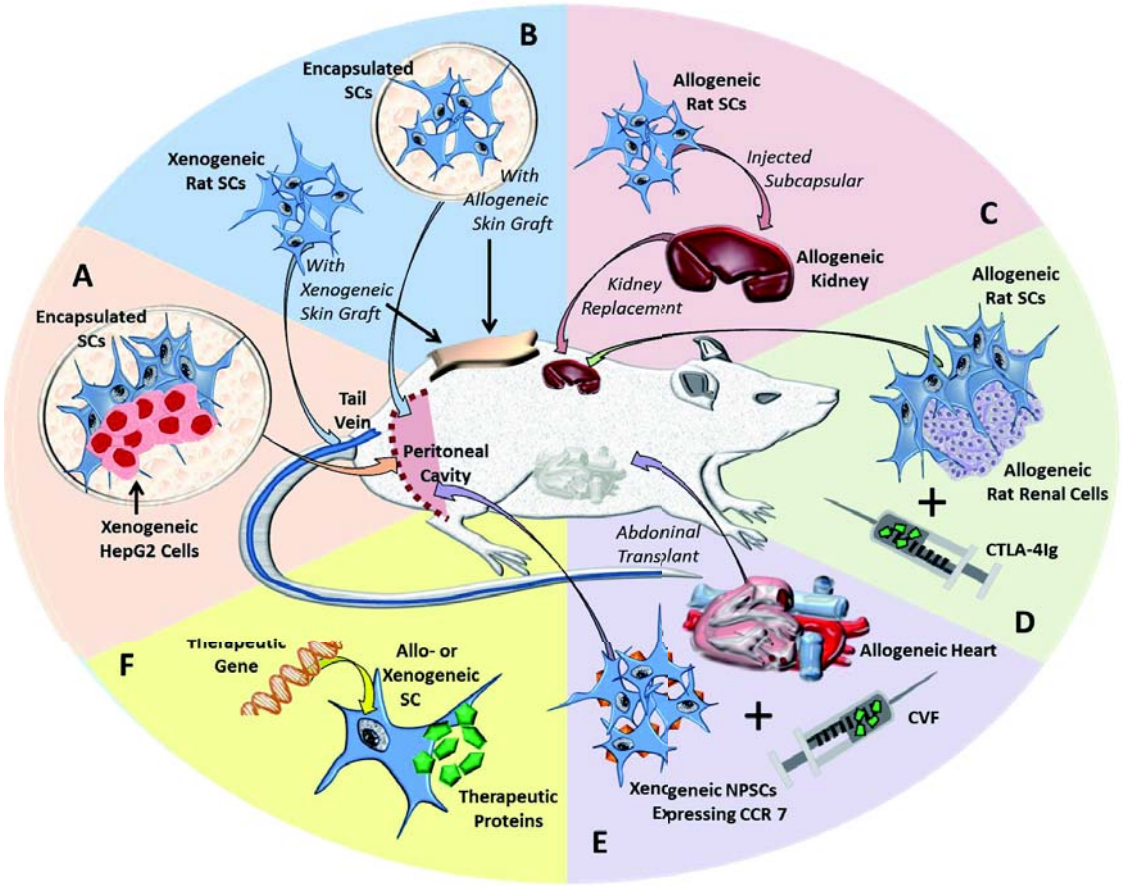

Figure 4. Other Sertoli cell transplantation studies. Liver Disease (A): Syngeneic rat SCs and HepG2 cells (a human hepatocyte-derived cell-line) were co-encapsulated and transplanted into the peritoneal cavity of normal rats or animals with acute hepatic failure without any immunosuppressive drugs. Skin Grafts (B): Xenogeneic rat SCs were injected intravenously in the tail vein of C57/BL6 mice one month prior to transplanting xenogeneic rat skin grafts. Additionally, encapsulated xenogeneic porcine SCs were injected in the peritoneal cavity of Wistar rats one week prior to transplanting allogeneic Long Evans rat skin grafts. Kidney Disease - Kidney Transplant (C): Allogeneic kidney grafts with SCs injected into the subcapsular space were transplanted in rats. Renal/SC Co-transplantation (D): Allogeneic renal cells and SCs were co transplanted underneath the kidney capsule of rats. Transplanted animals also received immunosuppressive (CTLA-4Ig) drug. Heart Transplant (E): Neonatal porcine SCs (NPSCs) expressing CCR7 were injected into the peritoneal cavity of the animal prior to allogeneic heart transplant. Immune suppression (CVF) was required along with SCs to prolong heart survival. SC Gene Therapy (F): SCs have been genetically engineered to express therapeutic proteins such as insulin and neurotrophins and transplanted as allo- and xeno-grafts.

Protection provided to transplanted organs by Sertoli cells

Co-transplantation of SCs with other cells or tissue has been well described. However, in some situations organ transplantation is mandatory. For instance, in the case of end stage heart failure or renal disease, the patient's life can only be saved by heart or kidney transplantation, respectively. SC protection of transplanted organs has only recently been reported with two separate studies investigating the therapeutic effect of SCs on transplantation of heart and kidney (Lim *et al*., 2009; Mai *et al*., 2012). The study investigating the beneficial effects of SCs on allogeneic heart transplantation used a neonatal pig SC line transfected with chemokine receptor (CCR) 7 (Lim *et al*., 2009; Fig. 4E). The authors reasoned that CCR7 expression on SCs would result in their migration to secondary lymphoid organs, where antigen presentation occurs, thereby resulting in systemic tolerance. The intra-peritoneal injection of SCs expressing CCR7 was unable to prolong the survival of transplanted heart compared (MST- 8.2 ± 0.84 days) to controls (a heart transplanted alone, MST-8.0 ± 2.92 days). However, when animals injected with SCs were given cobra venom factor (inhibit complement mediated cell lysis), from day 0 to day 5 post-transplantation, it significantly prolonged the survival of transplanted hearts (MST-25.5 ± 7.1 days) compared to control heart grafts receiving cobra venom factor alone (MST-9.5 ± 0.6 days; Lim *et al*., 2009).

The therapeutic potential of SCs in renal disease was also evaluated. Initially, it was demonstrated that SCs in combination with immune suppression (CTLA-4Ig) protected co-transplanted allogeneic renal cells (Zhao *et al*., 2002; Fig. 4D). Nevertheless, only survival, and not function of the transplanted renal cells was investigated in this study. More recently, the ability of SCs to protect allogeneic kidney grafts was investigated (Fig. 4C). Rats that received kidney grafts containing SCs injected within the kidney subcapsular space survived a mean duration of 19.5 ± 4.3 days, which was statistically different from the control group that received kidney grafts without SCs (MST-14.5 ± 3.1 days; Mai *et al*., 2012). Although, the transplanted kidneys failed to restore serum creatine levels to the normal range, a significant decrease in serum creatine levels was observed in the animals that received kidney-SC grafts compared to controls (received kidney grafts only) at 10 days post-transplantation. Additionally, at day 10 post-transplantation, kidney grafts in the control group exhibited a typical acute rejection while the severity of rejection was less in the kidney-SC group (Mai *et al*., 2012). While further work is needed to improve graft survival, these studies demonstrate that SC transplantation can extend organ graft survival in rodent models. Besides having a potential benefit for whole organ transplantation, these studies are also interesting since they provide further evidence that SCs may induce systemic tolerance.

Other therapies utilizing immune privileged Sertoli cells

Besides protecting co-transplanted tissue or organs, the use of SCs to deliver therapeutic drugs to the lungs has also been investigated (Kumar *et al*., 2011). In this study, allogeneic SCs phagocytosed FITC-labeled or curcumin (anti-inflammatory compound)-coupled nanoparticles (Kumar *et al*., 2011). These preloaded SCs were then injected through the lateral tail vein (i.v.) into normal or ovalbumin (OVA) challenged mice (animal model of pulmonary inflammation). At 15 min post-injection, SCs were detected in the deep lungs. At the same time, 92% of the FITC-labeled nanoparticle load was delivered specifically to the lungs. At 1 h post-injection, SCs were lysed and cleared from the lungs and 65% of the nanoparticle load remained. The OVA-challenged mice, injected with SCs containing curcumin-coupled nanoparticles, showed no evidence of pulmonary inflammation and contained ~90% of the curcmin-coupled nanoparticles in the lungs. While the untreated OVA-challenged control lungs showed extensive perivascular inflammation as indicated by the presence of inflammatory cells (Kumar *et al*., 2011). These data clearly demonstrates the therapeutic potential of SCs in drug delivery.

As discussed previously, the intra-peritoneal injection of encapsulated SCs was beneficial to the regrowth of pancreatic islet cells in the treatment of early T1DM. This same therapy was tested for the treatment of Laron Syndrome. Laron Syndrome is a genetic disease where growth is severally and irreversibly retarded due to a defect in growth hormone receptor. The current therapy requires multiple daily injection of recombinant human insulin growth factor -1 (IGF-1). This therapy is only 13% effective in females and 19% effective in males (Fintini *et al*., 2009). Interestingly, SCs express high amounts of IGF-1 (Chatelain *et al*., 1987) which has been identified as an important regulator in the development of the male reproductive system and in spermatogenesis. When encapsulated SCs were injected into the intra-peritoneal cavity of a Laron mouse model, significant proportional growth was observed compared to the empty capsule control, although serum IGF-1 levels remained low (Luca *et al*., 2013).

Summary

The immune system is an intricate and complicated network that has evolved over millions of years. The fact that SCs can modulate an immune response to allow for their own survival and that of other cells within their jurisdiction is extraordinary. The

studies we discussed in this review highlight three potential therapeutic venues for SCs; 1) SC protection of other cells vital for the treatment of disease and injury, 2) inhibition and/or alteration of the local and/or systemic environment by SCs, creating a refuge for damaged cells and tissues to recover, and 3) genetic alteration of SCs to express essential therapeutic proteins to alleviate diseases. Just over the past three decades scientist have began to realize and exploit SCs unique qualities and perform the necessary animal studies to establish a role for SCs in the clinical setting. However, the ability of SCs to protect co-transplanted tissue in large animal models (e.g. primates) needs to be carried out before clinical trials are performed. When Enrico Sertoli discovered SCs, describing them as "mother cells" in 1865 (Sertoli, 1865), he was clearly ahead of his time.

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