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Review

Spleen, as an optimal site for islet transplantation

Naoaki Sakata [1], Gumpei Yoshimatsu [2] and Shohta Kodama [1, 2, 3*]

1 Department of Regenerative Medicine and Transplantation, Faculty of Medicine, Fukuoka University
2 Center for Regenerative Medicine, Fukuoka University Hospital
3 Research institute for Regenerative Medicine, Fukuoka University;
7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan.
naoakisakata@fukuoka-u.ac.jp, gyoshimatsu@fukuoka-u.ac.jp, skodama@fukuoka-u.ac.jp
* Correspondence: skodama@fukuoka-u.ac.jp; Tel.: +81-92-801-1011 (ext. 3631); Fax: +81-92-801-1019

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Abstract: Islet transplantation is a cellular replacement therapy to treat severe diabetes mellitus, but its clinical outcome is unsatisfactory at present. One factor in clinical success of this therapy is selection of the most appropriate transplantation site. In this review, we review evidence showing the advantages of the spleen as a transplantation site for islets. The spleen has been studied for a long time as a candidate site for islet transplantation. Its advantages include physiological insulin drainage and regulation of immunity. Recently it has also been shown that the spleen contributes to the regeneration of transplanted islets and that splenic stem cells have the potential to differentiate into islet cells. The spleen also has some disadvantages associated with the transplantation procedure itself (bleeding, thrombosis and splenic infarction). The efficacy of transplantation is not as high as that obtained with intraportal transplantation, which is the current representative method of clinical islet transplantation. Safer and more effective methods of islet transplantation need to be established before the spleen can be effectively used in the clinic to support the engraftment of multiple transplanted islets.

Keywords: keyword 1; Spleen 2; Islet Transplantation 3; Transplant Site 4; Immunity 5; Tolerance 6; Regeneration 7; Diabetes Mellitus 8; Liver 9; Intrasplenic 10; Stem Cell

1. Introduction: Islet Transplantation and the Hurdles

Islet transplantation is a cellular replacement therapy to treat severe diabetes mellitus in patients who are unable to control their blood glucose even with intensive insulin treatment. Islet transplantation enables patients to receive an appropriate supply of insulin in response to changes in blood glucose levels. Islet transplantation also can prevent severe hypoglycemia and life-threatening complications including cardiomyopathy, nephropathy, retinopathy and neuropathy [1-3].

Although islet transplantation was first established in the clinic in the 1970s [4], the early therapeutic outcome was inadequate, and islet transplantation is still regarded as an "experimental therapy". At the end of the 1990s, fewer than 50% of patients achieved insulin independence at two months after islet transplantation and less than 10 % after one year [5]. However, a turning point in islet transplantation was the development of an automated method for islet isolation in the mid-1980s. This method involves the progressive chemical and mechanical digestion of the pancreas in a warm collagenase solution using a digestion chamber known as a "Ricordi chamber" [6]. Purification of islets from the digested pancreatic tissue is performed by density – gradient separation using a blood cell processor IBM 2991 device (sold as COBE 2991®, Terumo BCT, Inc., Lakewood, CO, USA). This advance in digestion and islet purification has enabled the harvesting of large numbers of islets with high purity, and was important to achieving the first clinical success in islet transplantation in 1989 at Washington University in St Louis. This was a thirty six year old

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woman with type 1 diabetes mellitus, who received transplantation of approximately 800,000 islet equivalents, achieved normoglycemia for 22 days without insulin treatment [7].

The other turning point was the development of an effective immunosuppressive regimen for islet transplantation. In 1990, the Pittsburg group achieved success in prolonging insulin independence for over 3 months in a clinical allogeneic islet transplantation study using tacrolimus (FK506) [8]. Tacrolimus is an inhibitor of calcineurin, which is required for T-cell receptor induction of interleukin-2 (IL-2) and for T cell proliferation. Tacrolimus has a superior safety profile compared to cyclosporine, an earlier calcineurin inhibitor [9, 10]. At the end of 1990s, the Edmonton group developed an islet transplantation protocol using the steroid-free immunosuppressive agents sirolimus, daclizumab and tacrolimus. In a study involving seven patients with severe type 1 diabetes, all were able to function without insulin treatment and no episodes of hypoglycemic coma were reported [11]. Sirolimus (rapamycin) inhibits the activation of T and B cells by suppressing the multifunctional serine-threonine kinase mTOR (mammalian target of rapamycin), which is required for efficient production of IL-2 [12, 13]. Daclizumab is a monoclonal antibody directed against CD25, a component of IL-2 receptor, and thereby blocks the formation of the high-affinity IL-2 receptor. Daclizumab can prevent acute rejection by inhibiting the expansion of cytotoxic T cells [14]. The recommended protocol employed today uses antithymocyte globulin (ATG) plus the recombinant soluble tumor necrosis factor receptor protein etanercept as induction immunosuppressant agents, followed by tacrolimus or cyclosporine along with mycophenolate mofetil (an inhibitor of purine biosynthesis) for immunosuppression maintenance. The Minnesota group tested this protocol on six recipients and four of them became insulin-independent for a mean of 3 years [15].

The outcome for clinical islet transplantation has dramatically improved over the past 50 years due to technological improvements. A report in 2005 by the Edmonton group analyzing the long-term outcomes of their 65 patients showed that approximately 80 percent of them achieved successful islet engraftment at five years after transplantation (i.e. detection of serum C-peptide and reactivity to glucose stimulation), but only 10 % of the patients remained free from insulin treatment [16]. A recent report from the Collaborative Islet Transplant Registry (CITR: a registry of clinical islet transplant cases performed in USA, Europe or Australia) indicated that the rates of insulin independence at three years after transplantation have been improving (44 % in 2007 – 2010 era vs. 27 % in 1999 – 2002 era). The positive fasting C-peptide levels (≥ 0.3 ng/mL) were also significantly higher in the period 2007 – 2010 versus 1999 – 2002 (90 % vs. 60 % at three years after transplantation) [17]. Moreover, it was observed that approximately 80 % of recipients who had received ≥600,000 total islet equivalents achieved insulin independence, compared to 55 % who had received <600,000 islet equivalents [18]. Islet transplantation is therefore now considered a practical option for treating severe diabetes mellitus in order to improve endocrinal function and to prevent hypoglycemic attack, but the current clinical outcome is still not satisfactory. The key points in obtaining a positive outcome are the acquisition of large numbers of islets from the donor pancreas, prevention of graft loss in the early stage of transplantation and maintaining engraftment for long period. Another key factor influencing engraftment is the transplant site, and the outcome of clinical islet transplantation could be further improved by utilizing a more optimal transplant site.

2. Candidate Transplantation Sites for Islets

What would be an optimal site for islet transplantation? We would define it by the following three criteria: 1) sites with an abundant, oxygen- and nutrient-rich blood flow, 2) sites that are privileged immunologically to minimize transplant graft loss, and 3) sites where transplantation can be performed with minimum invasiveness. To date, many organs have been assessed including the liver [19-21], renal subcapsular space [19, 20], omental pouch [22, 23], mesentery [24], gastrointestinal tract [25], skeletal muscle [26], subcutaneous tissue [26], eye [27], brain [28], testis [29, 30], bone marrow [31], thymus [32], and spleen [33]. However, it has been difficult to find a site that meets all three criteria (Table 1).

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Table 1. Candidate Islet Transplantation Sites other than Spleen.

Transplant sites	Merits	Demerits
Liver	✓ Representative site for clinical transplantation ✓ Relatively easy to access ✓ Physiological insulin secretion	✓ IBMIR ✓ Innate immunity ✓ Portal thrombosis and hypertension
Kidney	✓ The highest transplant efficacy in rodent models	✓ Difficulty in transplantation due to tight capsule in large animals ✓ Systemic insulin release
Omental pouch	✓ Potential to accommodate large numbers of islets ✓ Rich vascularity ✓ Physiological insulin secretion	✓ No reports ✓ No clinical trials ✓ Possibility of risk associated with surgery including adhesion and ileus
Mesentery	✓ Rich vascularity ✓ Physiological insulin drainage	✓ Impossibility of graft removal without sacrificing intestinal tract
Gastrointestinal tract	✓ Rich vascularity ✓ Physiological insulin secretion ✓ Possibility of endoscopic approach	✓ Impossibility of graft removal without sacrificing intestinal tract
Muscle and subcutaneous tissue	✓ Easiest access with minimum invasion	✓ Poorest in transplant efficacy ✓ Systemic insulin release
Immune privilege site (brain, testis, eye, thymus)	✓ Prevention, reduction or suppression of immunity	✓ Difficulty of clinical setting

The liver has been used as a site for clinical islet transplantation for a long time. It is the largest organ that can accommodate large numbers of islets following a simple transplant procedure (percutaneous infusion into intrahepatic portal vein using ultrasonography under local anesthesia) [34]. On the other hand, the liver also has some problems as a transplant site. Many islets are destroyed in the early stages of transplantation due, in part, to hypoxia caused by ischemia. The isolated islets are in an avascular state throughout the process of preparation [35] and suffer from hypoxia in the hypo-oxygenized portal venous blood (the mean PO_2 of approximately 5 mmHg [36]) until revascularization occurs. Moreover, the islets themselves can be a cause of liver ischemia by embolizing the peripheral portal vein [37, 38]. Another issue is inflammation and immunity. The transplanted islets are frequently the subject of an innate immune response and are attacked by Kupffer cells, tissue macrophages in the liver [39, 40] as well as by natural killer cells [41], and this

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may in turn induce an adaptive immune response. Furthermore, infusion of islets into the blood stream can trigger the instant blood-mediated inflammatory reaction (IBMIR), which can damage intraportal transplanted islets [42]. The IBMIR is triggered by the exposure of islet surface molecules during the process of islet isolation and purification [43, 44]. One such surface molecule is tissue factor (coagulation factor III), which causes the rapid binding of platelets, leading to coagulation and activation of complement systems. Most of the islets are destroyed by this reaction within 1 hour after transplantation [43]. Some immunosuppressants can be more toxic to islets in the liver, as their concentration is higher in the portal vein than in peripheral vessels [45]. Other complications of intraportal islet transplantation include portal hypertension and portal vein thrombosis. Portal hypertension can be a risk factor for post-transplant bleeding, portal vein thrombosis and sepsis [46, 47]. Portal vein thrombosis is a critical complication in islet transplantation and can cause esophageal varices, splenomegaly, mesenteric ischemia, sepsis and death [48].

A common islet transplantation site in experimental studies, especially rodent, is the kidney (i.e. renal subcapsular space). There are some reports of islet transplantation into the kidney that have led to the restoration of normoglycemia. These studies have used relatively small numbers of islets, as it is difficult to transplant large amounts of islets into the human renal subcapsular space because it is rather inelastic and tight [49]. This may be why clinical progress in renal subcapsular islet transplantation has lagged [50]. Muscle and subcutaneous tissues have also been examined as candidate transplantation sites, as the transplantation procedure and biopsies can be performed easily with minimal invasion and few complications. These sites suffer from hypovascularity and hypoxia, and transplantation efficacy could be improved if these obstacles were overcome, especially in subcutaneous tissue [26]. Another problem with these sites is systemic insulin release. In general, secreted insulin from the pancreas flows into the liver via the portal vein, and therefore smaller amounts of insulin are needed to control blood glucose. This is referred to as physiological insulin secretion, as opposed to systemic insulin release. When islets are transplanted into intramuscular and subcutaneous sites, resulting in systemic insulin release, a much larger amount of insulin needs to be produced, as the insulin does not enter the portal system directly. The large amount of insulin required to be produced by the transplanted islets in order to control blood glucose is similar that required by insulin injection therapy. Another favorable islet transplantation site is the omental pouch. It has advantages in that the insulin drainage is via the portal vein, thus closer to physiological, and this site is highly vascularized [51]. There has been much progress in intra-omental pouch islet transplantation in rodent [23], dog [52] and nonhuman primate models [53]. In particular, because the omental is highly vascularized, this site has been proposed as an alternative site for encapsulated islet transplantation [54-56], but to date no clinical trials have been performed. The mesentery is also considered a candidate islet transplant site due to its rich vascularization and ability to accommodate a large number of islets, however one disadvantage is that if there is any trouble with the graft it would be difficult to remove it without damage to the intestinal tract [57]. The submucosal space of the gastrointestinal tract is another candidate site that has a rich vascular supply providing oxygen and nutrients and connects to the same portal system as the liver, spleen and pancreas [51]. Hara and colleagues have studied transplantation into this location by endoscopy in a pig model [25, 58], but there has been limited demonstration of this concept in large animal models. The brain, testis, the anterior to chamber of the eye, and the thymus are the organs where the immunological response is suppressed and are thus considered “immune privileged” sites. The “immune privilege” of these sites was once assumed to be due to lack of cellular infiltration and lymphatic drainage [59], but more recently it has been shown that this is provided by a complex of immune responses [60]. For example, the brain, testis and retina-blood barrier are maintained in an immunosuppressed condition due to a cellular physical shield [60-62]. In some cases, regulatory T cells (Tregs) also contribute to immune privilege. Larocque and colleagues showed that the immune response in the brain could be normally activated when CD4+CD25+ Tregs were depleted [63]. Hedger further revealed that rodent testes contain significant numbers of immunoregulatory cells, including Tregs [64]. And recently, Farooq showed that Tregs contribute to immune tolerance in the rodent anterior chamber when challenged by myelin antigen

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[65]. Many experimental trials have investigated allo- and xenogeneic islet transplantation into immune privileged sites in using non-human animals. While such studies in animals have demonstrated the effectiveness of transplantation into immune privileged sites [27, 28, 30, 32], little has been done in a human clinical setting. In particular, the brain or eye are problematic sites for transplantation, as it would be difficult to remove a graft without damage in case of graft failure.

3. Characteristics of the Spleen as an Islet Transplant Site

Among the candidate islet transplant sites, the spleen may come closest to being an ideal site. The spleen is a highly vascularized organ which receives blood from the splenic artery and drains into the portal venous system. Vascularization is the most important factor determining the success of transplantation, and the spleen provides a rich oxygen and nutrition supply. Another advantage is that islets transplanted into the spleen can achieve physiological levels of insulin secretion, as insulin produced by pancreatic β cells flows into the portal – splenic vein (portal venous circulation) [66]. In contrast, insulin provided by a subcutaneous pump or by injection is delivered directly into systemic circulation. Recent advances in these insulin injection systems enable them to achieve close to physiological insulin release profiles (i.e. in the portal system), but there is still a limitation in day-to-day changes in insulin sensitivity [67]. As the spleen connects to the portal venous system, as does the liver and pancreas, insulin released from transplanted islets flows into the splenic vein.

The spleen is the site responsible for immune tolerance, and tends to be somewhat immunosuppressed, although this suppression is weaker than that found in immune privileged sites such as the testis or thymus. Previous studies have revealed that the spleen is involved in the suppression of T cell proliferation and antibody production following the induction of immune tolerance [68, 69]. Other studies have shown that splenic dendritic cells are a good source of suppressor cytokines, including transforming growth factor-β (TGFβ). The splenic T cell population was shown to include suppressor T cells [70], a cell type rebranded today as Tregs [71]. Tregs in the spleen prevent antigen presentation by dendritic cells to effector T cells, and suppress proliferation of effector T cells via production of suppressor cytokines including TGFβ, interleukin (IL) 10 and IL-35 [72]. Horton and colleagues performed intrasplenic allo-transplantation of islets into lymphoid-irradiated dogs that had received donor bone marrow transplantation before transplantation. In this study, the authors observed that islet graft function was maintained after total pancreatectomy without the use of immunosuppressants [73]. Moreover, splenocytes themselves may help regulate autoimmunity. In a previous study, we found that we could rescue non-obese (NOD) mice from a severe diabetic condition by injection of live donor splenocytes with complete Freund's adjuvant (CFA) to eliminate autoimmunity. In contrast, NOD mice that received irradiated splenocytes all became diabetic. Attack against lymphoid cells was minimal when live splenocytes were injected into CFA-infused mice [74, 75]. Thus it is not too surprising that the spleen can also protect transplanted islets from innate inflammatory responses, which are a major factor contributing to islet graft failure, as are acquired immune responses. Previously, we reported that several kinds of inflammatory cytokines, including monocyte chemotactic protein-1 (MCP-1), granulocyte-colony stimulating factor (G-CSF), and high-mobility group box 1 (HMGB1), were increased in the plasma after intraportal islet transplantation [76-78]. We also confirmed that these cytokines were significantly lower in intrasplenic transplantation in comparison with intraportal transplantation [79].

Interestingly, the spleen has been shown to be a reservoir of islet stem cells in diabetic mice. We confirmed that CD45- (nonlymphoid) splenocytes could develop into stem cells and further differentiate into islet progenitor cells, thus contributing to islet regeneration [74]. Moreover, we found in a subsequent study that adult mice spleens contained putative mesenchymal stem cells expressing Hox11 (known as Tlx1, a marker of splenic stem cell [80]) but not Pdx1, an early pancreatic regeneration marker, and that were CD45- in origin [81]. Lee and colleagues have provided additional evidence showing that removal of the spleen in children with severe thalassemias leads to the eventual development of insulin-dependent diabetes [82]. Thus, the spleen may facilitate the proliferation of intrasplenic transplanted islets. In 1989, Wohlrab and colleagues

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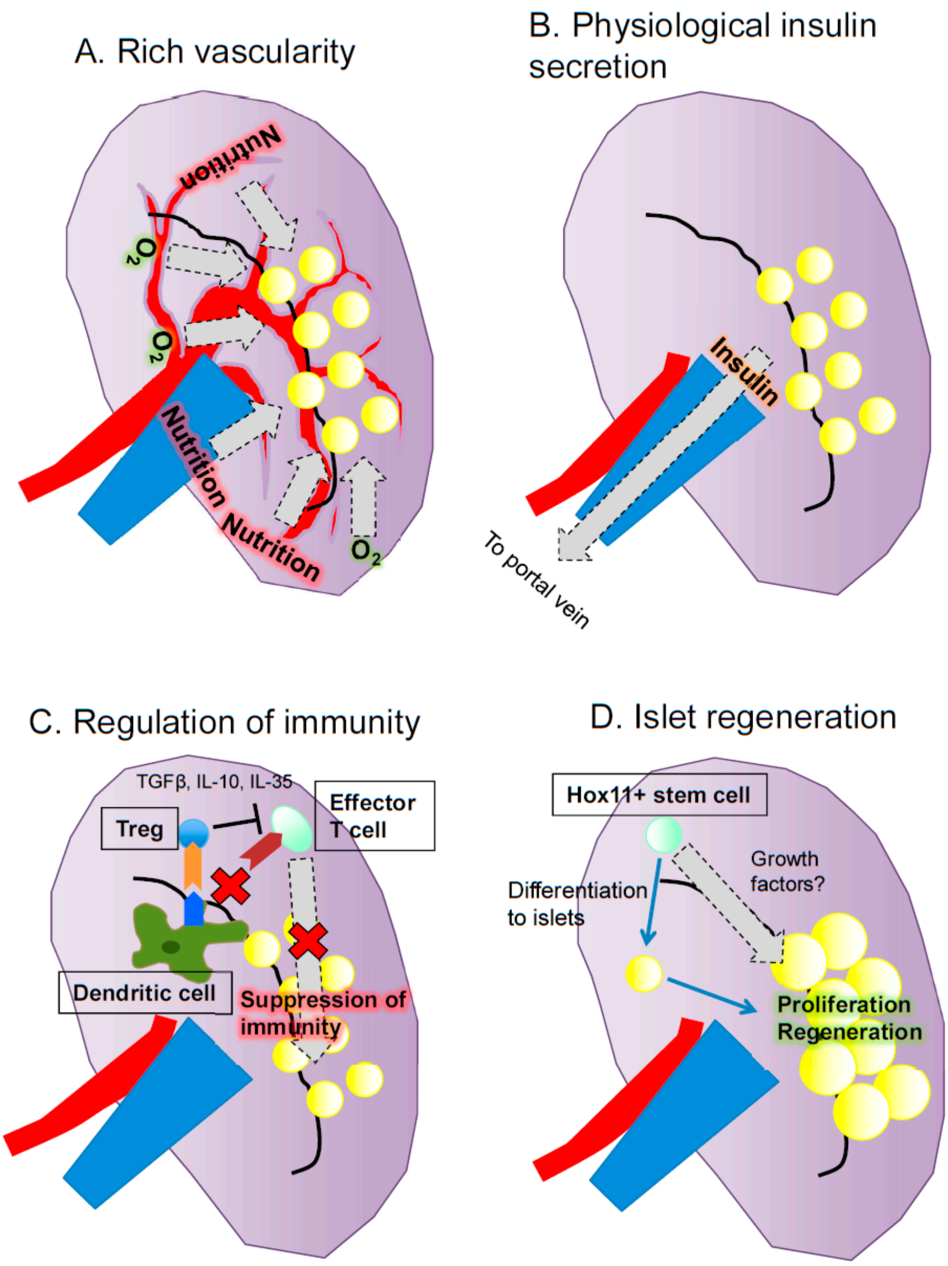
first observed proliferation of β cells in intrasplenic transplanted islets at 200 days post-transplantation. They speculated that the proliferative response was the result of a long-term stimulation by slightly enhanced plasma glucose levels at the transplantation site [83]. We also observed proliferation of intrasplenic islets transplanted into the renal subcapsule, and these transplanted islets expressed both insulin and ribonucleoside-diphosphate reductase subunit M2 b (Rrm2b) [79]. The Rrm2b gene encodes the small subunit of a p53-inducible ribonucleotide reductase. Expression of Rrm2b may therefore contribute to proliferation of the transplanted islets, as this gene has a role in DNA synthesis [84].

In summary, the spleen may be close to an optimal site for islet transplantation due to its rich vascularity, physiological insulin secretion, regulation of immunity including autoimmunity, and potential for islet regeneration (Figure 1).

Figure 1. Summary of the Characteristics of the Spleen as a Transplantation Site for Islets. The spleen has four advantages as a site of islet transplantation: (A) rich vascularity, (B) physiological insulin secretion, (C) regulation of immunity, and (D) potential for islet regeneration.



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4. Outcomes of Intrasplenic Islet Transplantation

The major studies on intrasplenic islet transplantation are summarized in Table 2. Historically, intrasplenic islet transplantation has been performed since the late 1970s, when a number of trials looking at intrasplenic islet autotransplantation into pancreatomized dogs demonstrated that this method could result in the recovery of endocrinal function [85-88]. This model has been used not only for the assessment of transplantation efficacy [85, 86, 88-98], but also for the assessment of the transplantation of cold or cryopreserved islets [99-102] and the toxicity of immunosuppressants [91, 103-106]. Other animals such as pig [107] and monkey [108-110] have also been used for islet autotransplantation and have shown acceptable outcomes.

In the 1980s, some groups worked with allo- [111] and xenogeneic [112] islet transplant models. Du Toit and colleagues performed intrasplenic allogeneic islet transplantation in pancreatomized dogs treated with cyclosporin and showed that survival was extended in comparison with non-immunosuppressed dogs [111]. Moreover, this allogeneic transplant dog model helped demonstrate the usefulness of rapamycin in transplantation [113]. Andersson reported survival of allogeneic grafts from cultured islets for several weeks without the use of any immunosuppressants [114]. In a xenograph model, the Washington group (Paul Lacy) succeeded in prolonging graft survival for more than 100 days using cultured islets in a rat to mouse transplant model where the recipients were treated with anti-rat and/or anti-mouse lymphocyte sera [112]. These findings demonstrated the possibility of using the spleen for transplantation of allo- and xenogeneic islets.

Table 2. Outcomes of Intrasplenic Islet Transplantation.

Authors and References	Published Year	Transplant model	Comments
Kolb E, et al. [85]	1977	Auto (dog)	✓ Achieved normoglycemia, but glucose tolerance was impaired
Feldman SD, et al. [86]	1977	Auto (dog)	✓ Achieved normoglycemia, but glucose tolerance was impaired. ✓ Implantation into splenic pulp.
Gray BN, et al. [88]	1979	Auto (dog)	✓ Response of insulin and glucagon to arginine stimulation. ✓ Implantation into splenic pulp.
Mehigan DG, et al. [115]	1981	Auto (dog)	✓ Assessment of quality of collagenase.
Andersson A, et al. [116]	1981	Iso (mouse)	✓ Achieved normoglycemia after transplantation of 500 islets
Steffes MW, et al. [117]	1981	Iso, Allo (mouse)	✓ A minimum of 13 weeks of nearly normal glucose levels after receiving skin grafts and spleen cells.
Du Toit DF, et al. [111]	1982	Allo (dog)	✓ Extended survival, but normoglycemia not achieved.
Janney CG, et al. [112]	1982	Xeno (rat to mouse)	✓ Prolongation of more than up to 100 days graft survival using cultured islets and

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			administration of anti-mouse and/or anti-rat lymphocyte sera.
Andersson A. [114]	1982	Allo (mouse)	✓ Graft survival of several weeks with cultured islets but without immunosuppressants.
Toledo-Pereyra LH, et al. [99]	1983	Allo (dog)	✓ Graft using cryopreserved islets was not rejected for more than 60 days.
Warnock GL, et al. [118]	1983	Iso (dog)	✓ Five month graft survival. ✓ Implantation via splenic vein.
Andersson A [119]	1983	Iso (mouse)	✓ Implantation of 500 islets was sufficient to achieve normoglycemia, while implantation of 150 islets was not.
Merrell RC, et al. [89, 90]	1985	Auto (dog)	✓ Achieved normoglycemia. ✓ Implantation via splenic vein.
Kneteman NM, et al. [120]	1985	Allo (dog)	✓ Prolongation of graft survival (approximately 20 days) using cyclosporine.
Gray DW, et al. [109]	1986	Auto (monkey)	✓ Achieved normoglycemia for 6 months. ✓ The first report of a monkey model.
Gores PF, et al. [91]	1987	Auto (dog)	✓ Achieved normoglycemia for more than 30 days.
Kneteman NM, et al [105]	1987	Allo (dog)	✓ Achieved normoglycemia for more than 100 days using cyclosporine.
Hayek A, et al. [121]	1988	Iso (rat)	✓ Partially achieved normoglycemia by transplantation of 1,000 neonatal islets.
Sutton R, et al. [110]	1989	Auto (monkey)	✓ Achieved normoglycemia with reduced insulin response.
Evans MG, et al. [92]	1989	Auto (dog)	✓ The normoglycemic rate was 90 % at one month after transplantation.
van der Vliet JA, et al. [93, 94]	1989	Auto (dog)	✓ The normoglycemic rate was 63 %.
Warnock GL, et al. [95]	1990	Auto (dog)	✓ The normoglycemic rate was 63 %. ✓ Comparison between splenic

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				vein and pulp as the route of transplantation. Intravenous route was superior (The normoglycemia rate was 86 %, vs. 33 %).
Ziegler B, et al. [122]	1990	Iso (rat)	✓	Achieved normoglycemia by transplantation of 1,200 islets
Korsgren O, et al. [123]	1990	Iso (mouse)	✓	Achieved normoglycemia by transplantation of 500 islets
Scharp DW, et al. [96]	1992	Auto (dog)	✓	The normoglycemic rate was 86 % at 1 year after transplantation.
Motojima K, et al. [97]	1992	Auto (dog)	✓	Normoglycemia was not achieved.
Marchetti P, et al. [98]	1993	Auto (dog)	✓	The normoglycemic rate was 90 %, and decreased to 71 % at 1 year after transplantation.
Ao Z, et al. [52]	1993	Auto (dog)	✓	The normoglycemic rate was 67 %.
Yakimets WJ, et al. [113]	1993	Allo (dog)	✓	Approximate 20 days graft survival using cyclosporine and rapamycin.
Hesse UJ, et al. [107]	1994	Auto (pig)	✓	The normoglycemic rate was 50 %.
Eizirik DL, et al. [124]	1997	Xeno, Allo (human and mouse to nude mouse)	✓	Normoglycemia was achieved by transplantation of 300 human islets into renal subcapsular space or 200 mouse islets into pulp of the spleen.
Horton PJ, et al. [73]	2000	Allo (dog)	✓	Normoglycemia achieved by pre-transplant irradiation of total lymphocytes and donor-specific bone marrow transplantation.

While the spleen has many advantages over other transplant sites, the efficacy of transplantation has been somewhat unclear. For example, Evans and colleagues showed that transplantation efficacy into spleen was better than that of the liver or kidney in an islet autotransplantation dog model: 90% of animals achieved normoglycemia at one month for spleen compared to 33% for liver and 0% for kidney [92] (Table 3). Using fetal porcine allotransplantation and murine transplantation models, Stokes et al. showed higher transplantation efficacy for spleen compared to liver, although kidney was better [125, 126]. Many other studies have reported the superiority of spleen compared to liver [96, 107] or omental pouch [52, 127], although some groups have reported the opposite [93-95] (Table 3).

Next, the route of transplantation into the spleen needs to be considered. In the earliest studies, the pulp was used as the transplant site in the spleen [86, 88]. After various trials, the Warnock

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group tested intrasplenic islet transplantation via the splenic vein using islet autotransplanted pancreatomized dog model, and observed greater effectiveness versus transplantation into pulp, achieving normoglycemia in 86 %, vs. 33 % of animals [118] (Table 2). Intravenous transplantation is generally regarded as preferable to intrasplenic transplantation, in part because intrasplenic transplantation carries the risk of IBMIR that can damage the transplanted islets, similar to intraportal transplantation [51].

Table 3. Transplant Efficacy of Intrasplenic Islet Transplantation.

Authors and References	Published Year	Transplant model	Comments
Sutton R, et al. [110]	1989	vs. Liver (Auto, monkey)	✓ Intrasplenic transplantation showed no superiority over intraportal transplantation
Evans MG, et al. [92]	1989	vs. Liver, Kidney (Auto, dog)	✓ The transplantation efficacy was best in the intrasplenic transplanted dog model: 90% achieved normoglycemia at one month, compared to 33% for intraportal and 0% for renalsubcapsular.
van der Vliet JA, et al. [93, 94]	1989	vs. Liver (Auto, dog)	✓ The normoglycemic rate was 63 % for intrasplenic vs. 75 % for intraportal.
Warnock GL, et al. [95]	1990	vs. Liver (Auto, dog)	✓ The normoglycemic rate was 63 % for intraspenic vs. 80 % for intraportal. ✓ Hyperglycemia after transplantation was less severe and onset was delayed.
Scharp DW, et al. [96]	1992	vs. Liver (Auto, dog)	✓ The normoglycemic rate was 86 % for intrasplenic vs. 50% for intraportal at 1 year after transplantation.
Motojima K, et al. [97]	1992	vs. Liver (Auto, dog)	✓ Normoglycemia was not achieved with either intrasplenic or intraportal transplantation.
Ao Z, et al. [52]	1993	vs. Omental pouch (Auto, dog)	✓ The normoglycemic rate was 67 % for intrasplenic vs. 50 % for intraomental transplantation.
Hesse UJ, et al. [107]	1994	vs. Liver (Auto, pig)	✓ The normoglycemic rate was 50 % for intrasplenic vs. 25 % for intraportal transplantation.

Gustavson SM, et al. [127]	2005	vs. Omental pouch (Auto, dog)	✓ Transplantation efficacy was better for intrasplenic versus intraomental pouch transplantation as assessed by glucose tolerance test.
Stokes RA, et al. [125]	2017	vs. Liver, Kidney (Allo, pig)	✓ Allo-transplant model using fetal porcine islets. Transplantation efficacy was kidney > spleen > liver.
Stokes RA, et al. [126]	2017	vs. Liver, Kidney (Iso, mouse) vs. Liver, Kidney, Portal vein, Muscle (Xeno, human to SCID mouse)	✓ Iso: transplantation of 220-250 islets. The normoglycemia rate was 100 % in kidney, 29 % in spleen, 0 % in liver (subcapsular space was used in the spleen and liver transplant models). ✓ Xeno: transplantation of human 2,000 islets. The normoglycemia rate was 100 % for kidney, 70 % for muscle, and 60 % for portal vein.

To examine the potential usefulness of the spleen as an islet transplantation site and to try to develop a better procedure for intrasplenic transplantation, we explored the “splenic subcapsular implantation technique” using a rodent syngeneic transplant model and analyzed the transplant efficacy of this method compared to intrahepatic and renal subcapsular transplantation [79]. This procedure involved direct puncture from the surface with a 27-gauge needle and implantation of islets under the splenic surface without venous or pulp injury (Figure 2).

Figure 2. A. Procedure of splenic subcapsular implantation technique. B. Engrafted islets (indicated by arrows) under the capsule of spleen at 28 days after transplantation. Left: hematoxylin and eosin staining, Right: immunostaining for insulin.

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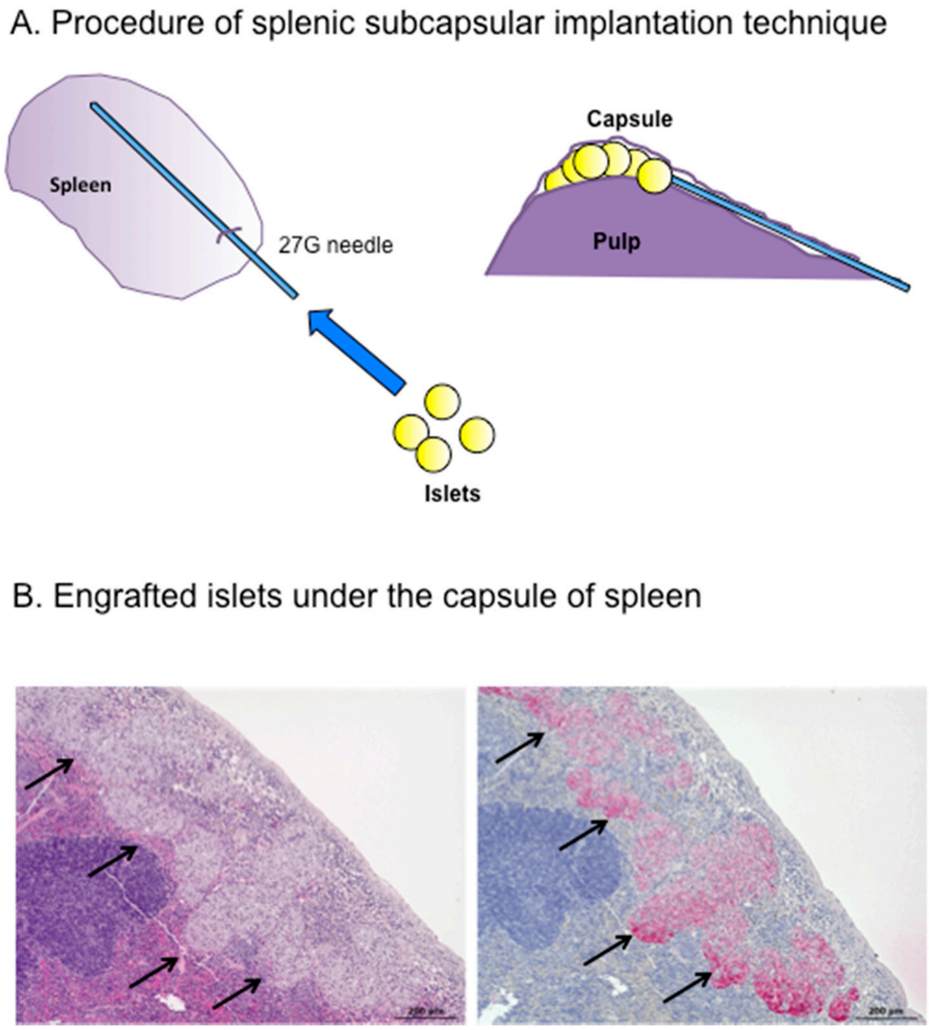

Amazingly, all of the mice (n = 10) achieved normoglycemia for two months despite having received only 50 islets by intrasplenic transplantation. In contrast, none of the mice achieved normoglycemia when islets were transplanted into the liver or kidney. Thus, not only was transplantation efficacy superior to transplantation into other sites, but three to four diabetic mice could be treated by a single donor mouse using this method (i.e. 150 – 200 islets can be harvested from one donor mouse). Normoglycemia could also be achieved using as few as 25 islets transplanted into the spleen when glucose levels were also rigorously managed. By histological assessment, we observed that intrasplenic transplanted islets were enlarged in size. The transplantation efficacy of this model clearly exceeded those previously reported for intrapulp and intravenous transplantation models [95, 116]. We speculate that this is because intrapulp and intravenous transplantation involves greater tissue damage and consequent exposure of islets to blood, thus inducing IBMIR, compared to intraspenic transplantation. In addition to preventing graft loss, intraspenic transplantation allows the engrafted islets to access a rich oxygen and nutrition supply due to an abundant blood flow. These factors, plus the privileged immune status of this site, may be responsible for a greater success of engraftment and regeneration.

5. Conclusion: for the Future Clinical Intrasplenic Islet Transplantation

The first intrasplenic islet transplantation was performed in a clinical setting at the University of Leicester 20 years ago. Five chronic pancreatitis patients underwent spleen-preserving total pancreatectomy and intrasplenic islet autotransplantation, and of these, two acquired insulin independence for over a year. However, the patients who underwent this procedure suffered from high morbidity, including splenic infarction and portal thrombosis [33]. Du Toit reported that

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intrasplenic islet transplantation was accompanied by some life-threatening complications including subcapsular hematoma, intrasplenic necrosis and cavitation, capsular perforation, and arteriolar thrombosis [111]. However, we believe these complications could be overcome with advances in surgical procedures. In our opinion, implantation into the splenic subcapsular region may minimize the risk of necrosis, thrombosis and hemorrhage by preventing venous and pulp injury. Laparoscopic surgery could also minimize the surgical stress of the transplantation, as can intraportal transplantation. We would suggest that with the combination of techniques described here, intraspenic transplantation may offer the most optimal approach to islet transplantation among the approaches currently available.

In conclusion, the spleen has historically been an important site for islet transplantation, but its utility could be greatly improved by the application of some recent novel findings and techniques. We would advocate the development of clinical methods to optimize the safe and effective transplantation of islets into the spleen.

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Abbreviations

ATG	antithymocyte globulin
CFA	complete Freund's adjuvant
CITR	Collaborative Islet Transplant Registry
G-CSF	granulocyte-colony stimulating factor
HMGB1	high-mobility group box 1
IBMIR	instant blood-mediated inflammatory reaction
IL	Interleukin
MCP-1	monocyte chemotactic protein-1
mTOR	mammalian target of rapamycin
NOD	non-obese
Rrm2b	ribonucleoside-diphosphate reductase subunit M2 b
TGFβ	transforming growth factor-β
Tregs	regulatory T cells

References

1. Fiorina, P.; Shapiro, A. M.; Ricordi, C.; Secchi, A., The clinical impact of islet transplantation. *Am J Transplant* **2008,** 8, (10), 1990-7.
2. Fiorina, P.; Folli, F.; Bertuzzi, F.; Maffi, P.; Finzi, G.; Venturini, M.; Socci, C.; Davalli, A.; Orsenigo, E.; Monti, L.; Falqui, L.; Uccella, S.; La Rosa, S.; Usellini, L.; Properzi, G.; Di Carlo, V.; Del Maschio, A.; Capella, C.; Secchi, A., Long-term beneficial effect of islet transplantation on diabetic macro-/microangiopathy in type 1 diabetic kidney-transplanted patients. *Diabetes Care* **2003,** 26, (4), 1129-36.
3. Fiorina, P.; Folli, F.; Zerbini, G.; Maffi, P.; Gremizzi, C.; Di Carlo, V.; Socci, C.; Bertuzzi, F.; Kashgarian, M.; Secchi, A., Islet transplantation is associated with improvement of renal function among uremic patients with type I diabetes mellitus and kidney transplants. *J Am Soc Nephrol* **2003,** 14, (8), 2150-8.

4. Sutherland, D. E.; Radosevich, D. M.; Bellin, M. D.; Hering, B. J.; Beilman, G. J.; Dunn, T. B.; Chinnakotla, S.; Vickers, S. M.; Bland, B.; Balamurugan, A. N.; Freeman, M. L.; Pruett, T. L., Total pancreatectomy and islet autotransplantation for chronic pancreatitis. *J Am Coll Surg* **2012,** 214, (4), 409-24; discussion 424-6.
5. Ricordi, C.; Strom, T. B., Clinical islet transplantation: advances and immunological challenges. *Nat Rev Immunol* **2004,** 4, (4), 259-68.
6. Ricordi, C.; Lacy, P. E.; Finke, E. H.; Olack, B. J.; Scharp, D. W., Automated method for isolation of human pancreatic islets. *Diabetes* **1988,** 37, (4), 413-20.
7. Scharp, D. W.; Lacy, P. E.; Santiago, J. V.; McCullough, C. S.; Weide, L. G.; Falqui, L.; Marchetti, P.; Gingerich, R. L.; Jaffe, A. S.; Cryer, P. E.; et al., Insulin independence after islet transplantation into type I diabetic patient. *Diabetes* **1990,** 39, (4), 515-8.
8. Tzakis, A. G.; Ricordi, C.; Alejandro, R.; Zeng, Y.; Fung, J. J.; Todo, S.; Demetris, A. J.; Mintz, D. H.; Starzl, T. E., Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. *Lancet* **1990,** 336, (8712), 402-5.
9. Ekmekcioglu, O.; Turkan, S.; Yildiz, S.; Gunes, Z. E., Comparison of tacrolimus with a cyclosporine microemulsion for immunosuppressive therapy in kidney transplantation. *Turk J Urol* **2013,** 39, (1), 16-21.
10. Bergmann, T. K.; Isbel, N. M.; Barraclough, K. A.; Campbell, S. B.; McWhinney, B. C.; Staatz, C. E., Comparison of the influence of cyclosporine and tacrolimus on the pharmacokinetics of prednisolone in adult male kidney transplant recipients. *Clin Drug Investig* **2014,** 34, (3), 183-8.
11. Shapiro, A. M.; Lakey, J. R.; Ryan, E. A.; Korbutt, G. S.; Toth, E.; Warnock, G. L.; Kneteman, N. M.; Rajotte, R. V., Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* **2000,** 343, (4), 230-8.
12. Kahan, B. D., Sirolimus: a comprehensive review. *Expert Opin Pharmacother* **2001,** 2, (11), 1903-17.
13. Mukherjee, S.; Mukherjee, U., A comprehensive review of immunosuppression used for liver transplantation. *J Transplant* **2009,** 2009, 701464.
14. Milo, R.; Osherov, M., Daclizumab and its use in multiple sclerosis treatment. *Drugs Today (Barc)* **2017,** 53, (1), 7-18.
15. Bellin, M. D.; Kandaswamy, R.; Parkey, J.; Zhang, H. J.; Liu, B.; Ihm, S. H.; Ansite, J. D.; Witson, J.; Bansal-Pakala, P.; Balamurugan, A. N.; Papas, K. K.; Sutherland, D. E.; Moran, A.; Hering, B. J., Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant* **2008,** 8, (11), 2463-70.
16. Ryan, E. A.; Paty, B. W.; Senior, P. A.; Bigam, D.; Alfadhli, E.; Kneteman, N. M.; Lakey, J. R.; Shapiro, A. M., Five-year follow-up after clinical islet transplantation. *Diabetes* **2005,** 54, (7), 2060-9.
17. Barton, F. B.; Rickels, M. R.; Alejandro, R.; Hering, B. J.; Wease, S.; Naziruddin, B.; Oberholzer, J.; Odorico, J. S.; Garfinkel, M. R.; Levy, M.; Pattou, F.; Berney, T.; Secchi, A.; Messinger, S.; Senior, P. A.; Maffi, P.; Posselt, A.; Stock, P. G.; Kaufman, D. B.; Luo, X.; Kandeel, F.; Cagliero, E.; Turgeon, N. A.; Witkowski, P.; Naji, A.; O'Connell, P. J.; Greenbaum, C.; Kudva, Y. C.; Brayman, K. L.; Aull, M. J.; Larsen, C.; Kay, T. W.; Fernandez, L. A.; Vantyghem, M. C.; Bellin, M.; Shapiro, A. M., Improvement in outcomes of clinical

islet transplantation: 1999-2010. *Diabetes Care* **2012,** 35, (7), 1436-45.

18. Balamurugan, A. N.; Naziruddin, B.; Lockridge, A.; Tiwari, M.; Loganathan, G.; Takita, M.; Matsumoto, S.; Papas, K.; Trieger, M.; Rainis, H.; Kin, T.; Kay, T. W.; Wease, S.; Messinger, S.; Ricordi, C.; Alejandro, R.; Markmann, J.; Kerr-Conti, J.; Rickels, M. R.; Liu, C.; Zhang, X.; Witkowski, P.; Posselt, A.; Maffi, P.; Secchi, A.; Berney, T.; O'Connell, P. J.; Hering, B. J.; Barton, F. B., Islet product characteristics and factors related to successful human islet transplantation from the Collaborative Islet Transplant Registry (CITR) 1999-2010. *Am J Transplant* **2014,** 14, (11), 2595-606.
19. Mellgren, A.; Schnell Landstrom, A. H.; Petersson, B.; Andersson, A., The renal subcapsular site offers better growth conditions for transplanted mouse pancreatic islet cells than the liver or spleen. *Diabetologia* **1986,** 29, (9), 670-2.
20. Hiller, W. F.; Klempnauer, J.; Luck, R.; Steiniger, B., Progressive deterioration of endocrine function after intraportal but not kidney subcapsular rat islet transplantation. *Diabetes* **1991,** 40, (1), 134-40.
21. Kemp, C. B.; Knight, M. J.; Scharp, D. W.; Ballinger, W. F.; Lacy, P. E., Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. *Diabetologia* **1973,** 9, (6), 486-91.
22. Yasunami, Y.; Lacy, P. E.; Finke, E. H., A new site for islet transplantation--a peritoneal-omental pouch. *Transplantation* **1983,** 36, (2), 181-2.
23. Kin, T.; Korbutt, G. S.; Rajotte, R. V., Survival and metabolic function of syngeneic rat islet grafts transplanted in the omental pouch. *Am J Transplant* **2003,** 3, (3), 281-5.
24. Osama Gaber, A.; Chamsuddin, A.; Fraga, D.; Fisher, J.; Lo, A., Insulin independence achieved using the transmesenteric approach to the portal vein for islet transplantation. *Transplantation* **2004,** 77, (2), 309-11.
25. Fujita, M.; McGrath, K. M.; Bottino, R.; Dons, E. M.; Long, C.; Kumar, G.; Ekser, B.; Echeverri, G. J.; Hata, J.; Haruma, K.; Cooper, D. K.; Hara, H., Technique of endoscopic biopsy of islet allografts transplanted into the gastric submucosal space in pigs. *Cell Transplant* **2013,** 22, (12), 2335-44.
26. Sakata, N.; Aoki, T.; Yoshimatsu, G.; Tsuchiya, H.; Hata, T.; Katayose, Y.; Egawa, S.; Unno, M., Strategy for clinical setting in intramuscular and subcutaneous islet transplantation. *Diabetes Metab Res Rev* **2014,** 30, (1), 1-10.
27. Mojibian, M.; Harder, B.; Hurlburt, A.; Bruin, J. E.; Asadi, A.; Kieffer, T. J., Implanted islets in the anterior chamber of the eye are prone to autoimmune attack in a mouse model of diabetes. *Diabetologia* **2013,** 56, (10), 2213-21.
28. Xin, Z. L.; Ge, S. L.; Wu, X. K.; Jia, Y. J.; Hu, H. T., Intracerebral xenotransplantation of semipermeable membrane- encapsuled pancreatic islets. *World J Gastroenterol* **2005,** 11, (36), 5714-7.
29. Bobzien, B.; Yasunami, Y.; Majercik, M.; Lacy, P. E.; Davie, J. M., Intratesticular transplants of islet xenografts (rat to mouse). *Diabetes* **1983,** 32, (3), 213-6.
30. Ferguson, J.; Scothorne, R. J., Extended survival of pancreatic islet allografts in the testis of guinea-pigs. *J Anat* **1977,** 124, (Pt 1), 1-8.
31. Cantarelli, E.; Citro, A.; Pellegrini, S.; Mercalli, A.; Melzi, R.; Dugnani, E.; Jofra, T.; Fousteri, G.; Mondino, A.; Piemonti, L., Transplant Site Influences the Immune Response After Islet

Transplantation: Bone Marrow Versus Liver. *Transplantation* **2017,** 101, (5), 1046-1055.
32. Levy, M. M.; Ketchum, R. J.; Tomaszewski, J. E.; Naji, A.; Barker, C. F.; Brayman, K. L., Intrathymic islet transplantation in the canine: I. Histological and functional evidence of autologous intrathymic islet engraftment and survival in pancreatectomized recipients. *Transplantation* **2002,** 73, (6), 842-52.
33. White, S. A.; London, N. J.; Johnson, P. R.; Davies, J. E.; Pollard, C.; Contractor, H. H.; Hughes, D. P.; Robertson, G. S.; Musto, P. P.; Dennison, A. R., The risks of total pancreatectomy and splenic islet autotransplantation. *Cell Transplant* **2000,** 9, (1), 19-24.
34. Owen, R. J.; Ryan, E. A.; O'Kelly, K.; Lakey, J. R.; McCarthy, M. C.; Paty, B. W.; Bigam, D. L.; Kneteman, N. M.; Korbutt, G. S.; Rajotte, R. V.; Shapiro, A. M., Percutaneous transhepatic pancreatic islet cell transplantation in type 1 diabetes mellitus: radiologic aspects. *Radiology* **2003,** 229, (1), 165-70.
35. Schramm, R.; Scheuer, C.; Yamauchi, J.; Vollmar, B.; Menger, M. D., Heat shock preconditioning impairs revascularization of freely transplanted pancreatic islets. *Transplantation* **2002,** 74, (11), 1544-50.
36. Carlsson, P. O.; Palm, F.; Andersson, A.; Liss, P., Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* **2001,** 50, (3), 489-95.
37. Sakata, N.; Hayes, P.; Tan, A.; Chan, N. K.; Mace, J.; Peverini, R.; Sowers, L.; Pearce, W. J.; Chinnock, R.; Obenaus, A.; Hathout, E., MRI assessment of ischemic liver after intraportal islet transplantation. *Transplantation* **2009,** 87, (6), 825-30.
38. Sakata, N.; Obenaus, A.; Chan, N.; Mace, J.; Chinnock, R.; Hathout, E., Factors affecting islet graft embolization in the liver of diabetic mice. *Islets* **2009,** 1, (1), 26-33.
39. Bottino, R.; Fernandez, L. A.; Ricordi, C.; Lehmann, R.; Tsan, M. F.; Oliver, R.; Inverardi, L., Transplantation of allogeneic islets of Langerhans in the rat liver: effects of macrophage depletion on graft survival and microenvironment activation. *Diabetes* **1998,** 47, (3), 316-23.
40. Fu, Y.; Lu, X.; Yi, S.; Wu, J.; O'Hara, J. M.; Hawthorne, W. J.; Hucker, K.; O'Connell, P. J., Selective rejection of porcine islet xenografts by macrophages. *Xenotransplantation* **2008,** 15, (5), 307-12.
41. Ishiyama, K.; Rawson, J.; Omori, K.; Mullen, Y., Liver natural killer cells play a role in the destruction of islets after intraportal transplantation. *Transplantation* **2011,** 91, (9), 952-60.
42. Bennet, W.; Sundberg, B.; Groth, C. G.; Brendel, M. D.; Brandhorst, D.; Brandhorst, H.; Bretzel, R. G.; Elgue, G.; Larsson, R.; Nilsson, B.; Korsgren, O., Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes* **1999,** 48, (10), 1907-14.
43. Johansson, H.; Lukinius, A.; Moberg, L.; Lundgren, T.; Berne, C.; Foss, A.; Felldin, M.; Kallen, R.; Salmela, K.; Tibell, A.; Tufveson, G.; Ekdahl, K. N.; Elgue, G.; Korsgren, O.; Nilsson, B., Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. *Diabetes* **2005,** 54, (6), 1755-62.
44. van der Windt, D. J.; Bottino, R.; Casu, A.; Campanile, N.; Cooper, D. K., Rapid loss of intraportally transplanted islets: an overview of pathophysiology and preventive strategies. *Xenotransplantation* **2007,** 14, (4), 288-97.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 February 2018 doi:10.20944/preprints201802.0101.v1

45. Desai, N. M.; Goss, J. A.; Deng, S.; Wolf, B. A.; Markmann, E.; Palanjian, M.; Shock, A. P.; Feliciano, S.; Brunicardi, F. C.; Barker, C. F.; Naji, A.; Markmann, J. F., Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity? *Transplantation* **2003,** 76, (11), 1623-5.
46. Ryan, E. A.; Lakey, J. R.; Paty, B. W.; Imes, S.; Korbutt, G. S.; Kneteman, N. M.; Bigam, D.; Rajotte, R. V.; Shapiro, A. M., Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* **2002,** 51, (7), 2148-57.
47. Kawahara, T.; Kin, T.; Shapiro, A. M., A comparison of islet autotransplantation with allotransplantation and factors elevating acute portal pressure in clinical islet transplantation. *J Hepatobiliary Pancreat Sci* **2012,** 19, (3), 281-8.
48. Thomas, R. M.; Ahmad, S. A., Management of acute post-operative portal venous thrombosis. *J Gastrointest Surg* **2010,** 14, (3), 570-7.
49. Gray, D. W.; Sutton, R.; McShane, P.; Peters, M.; Morris, P. J., Exocrine contamination impairs implantation of pancreatic islets transplanted beneath the kidney capsule. *J Surg Res* **1988,** 45, (5), 432-42.
50. Jindal, R. M.; Sidner, R. A.; McDaniel, H. B.; Johnson, M. S.; Fineberg, S. E., Intraportal vs kidney subcapsular site for human pancreatic islet transplantation. *Transplant Proc* **1998,** 30, (2), 398-9.
51. van der Windt, D. J.; Echeverri, G. J.; Ijzermans, J. N.; Cooper, D. K., The choice of anatomical site for islet transplantation. *Cell Transplant* **2008,** 17, (9), 1005-14.
52. Ao, Z.; Matayoshi, K.; Lakey, J. R.; Rajotte, R. V.; Warnock, G. L., Survival and function of purified islets in the omental pouch site of outbred dogs. *Transplantation* **1993,** 56, (3), 524-9.
53. Berman, D. M.; O'Neil, J. J.; Coffey, L. C.; Chaffanjon, P. C.; Kenyon, N. M.; Ruiz, P., Jr.; Pileggi, A.; Ricordi, C.; Kenyon, N. S., Long-term survival of nonhuman primate islets implanted in an omental pouch on a biodegradable scaffold. *Am J Transplant* **2009,** 9, (1), 91-104.
54. McQuilling, J. P.; Sittadjody, S.; Pareta, R.; Pendergraft, S.; Clark, C. J.; Farney, A. C.; Opara, E. C., Retrieval of Microencapsulated Islet Grafts for Post-transplant Evaluation. *Methods Mol Biol* **2017,** 1479, 157-171.
55. Pareta, R.; McQuilling, J. P.; Sittadjody, S.; Jenkins, R.; Bowden, S.; Orlando, G.; Farney, A. C.; Brey, E. M.; Opara, E. C., Long-term function of islets encapsulated in a redesigned alginate microcapsule construct in omentum pouches of immune-competent diabetic rats. *Pancreas* **2014,** 43, (4), 605-13.
56. Kobayashi, T.; Aomatsu, Y.; Iwata, H.; Kin, T.; Kanehiro, H.; Hisanga, M.; Ko, S.; Nagao, M.; Harb, G.; Nakajima, Y., Survival of microencapsulated islets at 400 days posttransplantation in the omental pouch of NOD mice. *Cell Transplant* **2006,** 15, (4), 359-65.
57. Weaver, J. D.; Headen, D. M.; Aquart, J.; Johnson, C. T.; Shea, L. D.; Shirwan, H.; Garcia, A. J., Vasculogenic hydrogel enhances islet survival, engraftment, and function in leading extrahepatic sites. *Sci Adv* **2017,** 3, (6), e1700184.
58. Tanaka, T.; Fujita, M.; Bottino, R.; Piganelli, J. D.; McGrath, K.; Li, J.; Lee, W.; Iwase, H.; Wijkstrom, M.; Bertera, S.; Long, C.; Landsittel, D.; Haruma, K.; Cooper, D. K.; Hara, H., Endoscopic biopsy of islet transplants in the gastric submucosal space provides evidence of islet graft rejection in diabetic pigs. *Islets* **2016,** 8, (1), 1-12.

59. Streilein, J. W., Unraveling immune privilege. *Science* **1995,** 270, (5239), 1158-9.
60. Kaur, G.; Mital, P.; Dufour, J. M., Testisimmune privilege - Assumptions versus facts. *Anim Reprod* **2013,** 10, (1), 3-15.
61. Perez, V. L.; Saeed, A. M.; Tan, Y.; Urbieta, M.; Cruz-Guilloty, F., The eye: A window to the soul of the immune system. *J Autoimmun* **2013,** 45, 7-14.
62. Pachter, J. S.; de Vries, H. E.; Fabry, Z., The blood-brain barrier and its role in immune privilege in the central nervous system. *J Neuropathol Exp Neurol* **2003,** 62, (6), 593-604.
63. Larocque, D.; Sanderson, N. S.; Bergeron, J.; Curtin, J. F.; Girton, J.; Wibowo, M.; Bondale, N.; Kroeger, K. M.; Yang, J.; Lacayo, L. M.; Reyes, K. C.; Farrokhi, C.; Pechnick, R. N.; Castro, M. G.; Lowenstein, P. R., Exogenous fms-like tyrosine kinase 3 ligand overrides brain immune privilege and facilitates recognition of a neo-antigen without causing autoimmune neuropathology. *Proc Natl Acad Sci U S A* **2010,** 107, (32), 14443-8.
64. Hedger, M. P., Immunophysiology and pathology of inflammation in the testis and epididymis. *J Androl* **2011,** 32, (6), 625-40.
65. Farooq, S. M.; Elkhatib, W. F.; Ashour, H. M., The in vivo and in vitro induction of anterior chamber associated immune deviation to myelin antigens in C57BL/6 mice. *Brain Behav Immun* **2014,** 42, 118-22.
66. Polonsky, K. S.; Given, B. D.; Hirsch, L.; Shapiro, E. T.; Tillil, H.; Beebe, C.; Galloway, J. A.; Frank, B. H.; Karrison, T.; Van Cauter, E., Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* **1988,** 81, (2), 435-41.
67. Home, P. D., Plasma insulin profiles after subcutaneous injection: how close can we get to physiology in people with diabetes? *Diabetes Obes Metab* **2015,** 17, (11), 1011-20.
68. Thompson, H. S.; Harper, N.; Bevan, D. J.; Staines, N. A., Suppression of collagen induced arthritis by oral administration of type II collagen: changes in immune and arthritic responses mediated by active peripheral suppression. *Autoimmunity* **1993,** 16, (3), 189-99.
69. Miller, A.; al-Sabbagh, A.; Santos, L. M.; Das, M. P.; Weiner, H. L., Epitopes of myelin basic protein that trigger TGF-beta release after oral tolerization are distinct from encephalitogenic epitopes and mediate epitope-driven bystander suppression. *J Immunol* **1993,** 151, (12), 7307-15.
70. Nagatani, K.; Dohi, M.; To, Y.; Tanaka, R.; Okunishi, K.; Nakagome, K.; Sagawa, K.; Tanno, Y.; Komagata, Y.; Yamamoto, K., Splenic dendritic cells induced by oral antigen administration are important for the transfer of oral tolerance in an experimental model of asthma. *J Immunol* **2006,** 176, (3), 1481-9.
71. MacDonald, T. T., Suppressor T cells, rebranded as regulatory T cells, emerge from the wilderness bearing surface markers. *Gut* **2002,** 51, (3), 311-2.
72. Takeuchi, Y.; Nishikawa, H., Roles of regulatory T cells in cancer immunity. *Int Immunol* **2016,** 28, (8), 401-9.
73. Horton, P. J.; Hawthorne, W. J.; Walters, S. N.; Patel, A. T.; O'Connell, P. J.; Chapman, J. R.; Allen, R. D., Induction of allogeneic islet tolerance in a large-animal model. *Cell Transplant* **2000,** 9, (6), 877-87.
74. Kodama, S.; Kuhtreiber, W.; Fujimura, S.; Dale, E. A.; Faustman, D. L., Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science* **2003,** 302, (5648), 1223-7.
75. Ryu, S.; Kodama, S.; Ryu, K.; Schoenfeld, D. A.; Faustman, D. L., Reversal of established

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autoimmune diabetes by restoration of endogenous beta cell function. *J Clin Invest* **2001,** 108, (1), 63-72.
76. Itoh, T.; Iwahashi, S.; Kanak, M. A.; Shimoda, M.; Takita, M.; Chujo, D.; Tamura, Y.; Rahman, A. M.; Chung, W. Y.; Onaca, N.; Coates, P. T.; Dennison, A. R.; Naziruddin, B.; Levy, M. F.; Matsumoto, S., Elevation of high-mobility group box 1 after clinical autologous islet transplantation and its inverse correlation with outcomes. *Cell Transplant* **2014,** 23, (2), 153-65.
77. Matsuoka, N.; Itoh, T.; Watarai, H.; Sekine-Kondo, E.; Nagata, N.; Okamoto, K.; Mera, T.; Yamamoto, H.; Yamada, S.; Maruyama, I.; Taniguchi, M.; Yasunami, Y., High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice. *J Clin Invest* **2010,** 120, (3), 735-43.
78. Itoh, T.; Nitta, T.; Nishinakamura, H.; Kojima, D.; Mera, T.; Ono, J.; Kodama, S.; Yasunami, Y., HMGB1-Mediated Early Loss of Transplanted Islets Is Prevented by Anti-IL-6R Antibody in Mice. *Pancreas* **2015,** 44, (1), 166-71.
79. Itoh, T.; Nishinakamura, H.; Kumano, K.; Takahashi, H.; Kodama, S., The Spleen Is an Ideal Site for Inducing Transplanted Islet Graft Expansion in Mice. *PLoS One* **2017,** 12, (1), e0170899.
80. Faustman, D. L.; Davis, M., Stem cells in the spleen: therapeutic potential for Sjogren's syndrome, type I diabetes, and other disorders. *Int J Biochem Cell Biol* **2010,** 42, (10), 1576-9.
81. Kodama, S.; Davis, M.; Faustman, D. L., Diabetes and stem cell researchers turn to the lowly spleen. *Sci Aging Knowledge Environ* **2005,** 2005, (3), pe2.
82. Lee, B. W.; Tan, S. H.; Lee, W. K.; Yap, H. K.; Aw, S. E.; Wong, H. B., Glucose tolerance test and insulin levels in children with transfusion-dependent thalassaemia. *Ann Trop Paediatr* **1985,** 5, (4), 215-8.
83. Wohlrab, F.; Schmidt, S.; Kloting, I.; Wilke, B.; Cossel, L., Ducto-insular proliferation of beta-cells after syngeneic islet transplantation into the spleen of streptozotocin-diabetic Lewis rats. *Int J Pancreatol* **1989,** 5, (1), 77-83.
84. Tanaka, H.; Arakawa, H.; Yamaguchi, T.; Shiraishi, K.; Fukuda, S.; Matsui, K.; Takei, Y.; Nakamura, Y., A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature* **2000,** 404, (6773), 42-9.
85. Kolb, E.; Ruckert, R.; Largiader, F., Intraportal and intrasplenic autotransplantation of pancreatic islets in the dog. *Eur Surg Res* **1977,** 9, (6), 419-26.
86. Feldman, S. D.; Hirshberg, G. E.; Dodi, G.; Raizman, M. E.; Scharp, D. W.; Ballinger, W. F.; Lacy, P. E., Intrasplenic islet isografts. *Surgery* **1977,** 82, (3), 386-94.
87. Schulak, J. A.; Stuart, F. P.; Reckard, C. R., Physiologic aspects of intrasplenic autotransplantation of pancreatic fragments in the dog after 24 hours of cold storage. *J Surg Res* **1978,** 24, (3), 125-31.
88. Gray, B. N.; Caruso, G.; Alford, F.; Chisholm, D., Insulin and glucagon responses of transplanted intrasplenic pancreatic islets. *Arch Surg* **1979,** 114, (1), 96-9.
89. Merrell, R. C.; Marincola, F.; Maeda, M.; Cobb, L.; Basadonna, G., The metabolic response of intrasplenic islet autografts. *Surg Gynecol Obstet* **1985,** 160, (6), 552-6.
90. Merrell, R. C.; Maeda, M.; Basadonna, G.; Marincola, F.; Cobb, L., Suppression, stress, and accommodation of transplanted islets of Langerhans. *Diabetes* **1985,** 34, (7), 667-70.

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91. Gores, P. F.; Boudreaux, J. P.; Hesse, U. J.; Najarian, J. S.; Sutherland, D. E., Canine islet autografts with and without administration of cyclosporine. *Surgery* **1987,** 101, (5), 557-61.
92. Evans, M. G.; Warnock, G. L.; Rajotte, R. V., Comparison of sites for transplantation of canine pancreatic microfragments. *Diabetes Res* **1989,** 10, (1), 35-41.
93. van der Vliet, J. A.; Kaufman, D. B.; Meloche, R. M.; Heise, J. W.; Field, M. J.; Heil, J. E.; Najarian, J. S.; Sutherland, D. E., A simple method of canine pancreatic islet isolation and intrahepatic transplantation. *J Surg Res* **1989,** 46, (2), 129-34.
94. van der Vliet, J. A.; Kaufman, D. B.; Meloche, R. M.; van der Hem, L. G.; Field, M. J.; Sutherland, D. E., Canine pancreatic islet transplantation: a comparison of two isolation techniques. *Eur Surg Res* **1990,** 22, (1), 1-7.
95. Warnock, G. L.; Dabbs, K. D.; Evans, M. G.; Cattral, M. S.; Kneteman, N. M.; Rajotte, R. V., Critical mass of islets that function after implantation in a large mammalian. *Horm Metab Res Suppl* **1990,** 25, 156-61.
96. Scharp, D. W.; Marchetti, P.; Swanson, C.; Newton, M.; McCullough, C. S.; Olack, B., The effect of transplantation site and islet mass on long-term survival and metabolic and hormonal function of canine purified islet autografts. *Cell Transplant* **1992,** 1, (2-3), 245-54.
97. Motojima, K.; Kohara, N.; Maeda, J.; Yamaguchi, M.; Kanematsu, T., A comparison of endocrine and exocrine function after pancreatic fragment autotransplantation into splenic pulp, portal vein, and hepatic parenchyma. *Transplantation* **1992,** 53, (3), 527-32.
98. Marchetti, P.; Olack, B.; Swanson, C.; Newton, M.; Scharp, D. W., Assessment of long-term (1 year) graft survival and metabolic and hormonal changes after intrasplenic canine pancreatic microfragment transplantation. *Diabete Metab* **1993,** 19, (1), 17-24.
99. Toledo-Pereyra, L. H.; Gordon, D. A.; MacKenzie, G. H., Application of cryopreservation techniques to islet cell allotransplantation. *Cryobiology* **1983,** 20, (2), 205-10.
100. Sandler, S.; Kojima, Y.; Andersson, A., Cryopreservation of mouse pancreatic islets. Effects of fast cooling on islet B cell function and on the outcome of islet transplantation. *Transplantation* **1986,** 42, (6), 588-93.
101. Heise, J. W.; Casanova, D.; Field, M. J.; Munn, S. R.; Najarian, J. S.; Sutherland, D. E., Cold storage preservation of pancreatic tissue prior to and after islet preparation in a dog autotransplantation model. *J Surg Res* **1989,** 47, (1), 30-8.
102. Casanova, D.; Heise, J. W.; Field, M. J.; Munn, S. R.; Sutherland, D. E., Successful transplantation of canine islets of Langerhans after 24 hours cold-storage. *Diabetes Res* **1989,** 10, (1), 31-4.
103. Merrell, R. C.; Mahoney, M. E.; Basadonna, G.; Cobb, L. F.; Maeda, M., Failure of canine islet allografts and autografts with cyclosporine. *Surgery* **1985,** 98, (2), 324-9.
104. Basadonna, G.; Kakizaki, K.; Merrell, R. C., Effect of cyclosporine on established islet autografts. *J Surg Res* **1986,** 40, (5), 450-4.
105. Kneteman, N. M.; Alderson, D.; Scharp, D. W., Long-term normoglycemia in pancreatectomized dogs following pancreatic islet allotransplantation and cyclosporine immunosuppression. *Transplantation* **1987,** 44, (5), 595-9.
106. Kneteman, N. M.; Marchetti, P.; Tordjman, K.; Bier, D. M.; Santiago, J. V.; Swanson, C. J.; Olack, B. J.; Scharp, D. W., Effects of cyclosporine on insulin secretion and insulin sensitivity in dogs with intrasplenic islet autotransplants. *Surgery* **1992,** 111, (4), 430-7.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 February 2018 doi:10.20944/preprints201802.0101.v1

107. Hesse, U. J.; Meyer, G. P.; Weyer, J.; Danis, J.; Pichlmaier, H., Islet isolation and autotransplantation in pigs. *Zentralbl Chir* **1994,** 119, (9), 653-60.
108. Gray, D. W., Islet isolation and transplantation techniques in the primate. *Surg Gynecol Obstet* **1990,** 170, (3), 225-32.
109. Gray, D. W.; Warnock, G. L.; Sutton, R.; Peters, M.; McShane, P.; Morris, P. J., Successful autotransplantation of isolated islets of Langerhans in the cynomolgus monkey. *Br J Surg* **1986,** 73, (10), 850-3.
110. Sutton, R.; Gray, D. W.; Burnett, M.; McShane, P.; Turner, R. C.; Morris, P. J., Metabolic function of intraportal and intrasplenic islet autografts in cynomolgus monkeys. *Diabetes* **1989,** 38 Suppl 1, 182-4.
111. Du Toit, D. F.; Reece-Smith, H.; McShane, P.; Denton, T.; Morris, P. J., Effect of cyclosporin A on allotransplanted pancreatic fragments to the spleen of totally pancreatectomized dogs. *Transplantation* **1982,** 33, (3), 302-7.
112. Janney, C. G.; Lacy, P. E.; Finke, E. H.; Davie, J. M., Prolongation of intrasplenic islet xenograft survival. *Am J Pathol* **1982,** 107, (1), 1-5.
113. Yakimets, W. J.; Lakey, J. R.; Yatscoff, R. W.; Katyal, D.; Ao, Z.; Finegood, D. T.; Rajotte, R. V.; Kneteman, N. M., Prolongation of canine pancreatic islet allograft survival with combined rapamycin and cyclosporine therapy at low doses. Rapamycin efficacy is blood level related. *Transplantation* **1993,** 56, (6), 1293-8.
114. Andersson, A., Reversal of hyperglycemia by intrasplenic transplantation of 4-week-cultured allogeneic mouse islets. *Diabetes* **1982,** 31 Suppl 4, 55-9.
115. Mehigan, D. G.; Zuidema, G. D.; Cameron, J. L., Pancreatic islet transplantation in dogs. Critical factors in technique. *Am J Surg* **1981,** 141, (2), 208-12.
116. Andersson, A.; Eriksson, U.; Petersson, B.; Reibring, L.; Swenne, I., Failure of successful intrasplenic transplantation of islets from lean mice to cure obese-hyperglycaemic mice, despite islet growth. *Diabetologia* **1981,** 20, (3), 237-41.
117. Steffes, M. W.; Nielsen, O.; Dyrberg, T.; Baekkeskov, S.; Scott, J.; Lernmark, A., Islet transplantation in mice differing in the I and S subregions of the H-2 complex. *Transplantation* **1981,** 31, (6), 476-9.
118. Warnock, G. L.; Rajotte, R. V.; Procyshyn, A. W., Normoglycemia after reflux of islet-containing pancreatic fragments into the splenic vascular bed in dogs. *Diabetes* **1983,** 32, (5), 452-9.
119. Andersson, A., The influence of hyperglycaemia, hyperinsulinaemia and genetic background on the fate of intrasplenically implanted mouse islets. *Diabetologia* **1983,** 25, (3), 269-72.
120. Kneteman, N. M.; Rajotte, R. V.; Procyshyn, A. W., Canine pancreatic fragment allotransplantation with cyclosporine A. *J Surg Res* **1985,** 39, (4), 285-93.
121. Hayek, A.; Lopez, A. D.; Beattie, G. M., Decrease in the number of neonatal islets required for successful transplantation by strict metabolic control of diabetic rats. *Transplantation* **1988,** 45, (5), 940-2.
122. Ziegler, B.; Kohler, E.; Kloting, I.; Besch, W.; Ziegler, M., Survival of islet isografts despite cytotoxicity against pancreatic islets measured in vitro. *Exp Clin Endocrinol* **1990,** 95, (1), 31-8.

123. Korsgren, O.; Jansson, L.; Sandler, S.; Andersson, A., Hyperglycemia-induced B cell toxicity. The fate of pancreatic islets transplanted into diabetic mice is dependent on their genetic background. *J Clin Invest* **1990,** 86, (6), 2161-8.

124. Eizirik, D. L.; Jansson, L.; Flodstrom, M.; Hellerstrom, C.; Andersson, A., Mechanisms of defective glucose-induced insulin release in human pancreatic islets transplanted to diabetic nude mice. *J Clin Endocrinol Metab* **1997,** 82, (8), 2660-3.

125. Stokes, R. A.; Simond, D. M.; Burns, H.; Patel, A. T.; O'Connell, P. J.; Gunton, J. E.; Hawthorne, W. J., Transplantation sites for porcine islets. *Diabetologia* **2017,** 60, (10), 1972-1976.

126. Stokes, R. A.; Cheng, K.; Lalwani, A.; Swarbrick, M. M.; Thomas, H. E.; Loudovaris, T.; Kay, T. W.; Hawthorne, W. J.; O'Connell, P. J.; Gunton, J. E., Transplantation sites for human and murine islets. *Diabetologia* **2017,** 60, (10), 1961-1971.

127. Gustavson, S. M.; Rajotte, R. V.; Hunkeler, D.; Lakey, J. R.; Edgerton, D. S.; Neal, D. W.; Snead, W. L.; Penaloza, A. R.; Cherrington, A. D., Islet auto-transplantation into an omental or splenic site results in a normal beta cell but abnormal alpha cell response to mild non-insulin-induced hypoglycemia. *Am J Transplant* **2005,** 5, (10), 2368-77.