1 *Review*

2 Spleen, as an optimal site for islet transplantation and
3 a source of mesenchymal stem cells

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

5   1   Department of Regenerative Medicine and Transplantation, Faculty of Medicine, Fukuoka University
6   2   Center for Regenerative Medicine, Fukuoka University Hospital
7   3   Research institute for Regenerative Medicine, Fukuoka University;
8   7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan.
9   naoakisakata@fukuoka-u.ac.jp, gyoshimatsu@fukuoka-u.ac.jp, skodama@fukuoka-u.ac.jp
10 *   Correspondence: skodama@fukuoka-u.ac.jp; Tel.: +81-92-801-1011 (ext. 3631); Fax: +81-92-801-1019

12 **Abstract:** In this review, we show the unique potential of spleen as an optimal site for islet
13 transplantation and a source of mesenchymal stem cells. Islet transplantation is a cellular
14 replacement therapy to treat severe diabetes mellitus, but its clinical outcome is unsatisfactory at
15 present. One factor in clinical success of this therapy is selection of the most appropriate
16 transplantation site. The spleen has been studied for a long time as a candidate site for islet
17 transplantation. Its advantages include physiological insulin drainage and regulation of immunity.
18 Recently it has also been shown that the spleen contributes to the regeneration of transplanted
19 islets. The efficacy of transplantation is not as high as that obtained with intraportal
20 transplantation, which is the current representative method of clinical islet transplantation. Safer
21 and more effective methods of islet transplantation need to be established before the spleen can be
22 effectively used in the clinic. Spleen also has an interesting aspect as a mesenchymal stem cell
23 reservoir. The splenic mesenchymal stem cells contribute to tissue repair in damaged tissue, and
24 thus, the infusion can be a promising therapy for autoimmune diseases, including type 1 diabetes
25 mellitus and Sjogren's syndrome.

26 **Keywords:** keyword 1; Spleen 2; Islet Transplantation 3; Transplant Site 4; Immunity 5; Tolerance 6;
27 Regeneration 7; Diabetes Mellitus 8; Mesenchymal Stem Cell 9; Sjogren's Syndrome 10; HOX11

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29 1. Introduction

30   Spleen is an abdominal organ which is located in the left upper quadrant. It is a peripheral
31 lymphoid organ which plays an important role in immnune system including maturation of B cells,
32 T cells and plasma cells, and production of immunoglobulin M (IgM) [1]. Furthermore, spleen acts as
33 a hematopoietic organ at prenatal period and in the case of massive hemorrhage and bone marrow
34 deficiency. Removal of old red blood cells and reserve of blood are also important functions of the
35 spleen. On the other hands, spleen has been considered as an "unnecessary organ" for a long time
36 because these functions of the spleen can be substituted by other organs and loss of spleen does not
37 lead to patient's death directly. Thus, splenectomy has been widely performed in the case of splenic
38 injury and pancreatic malignant tumor. Recently overwhelming postsplenectomy infection (OPSI) is
39 focused on as a severe complication with high mortality of splenectomy. Loss of spleen causes
40 immunological hypofunction, which lead to exacerbation of the bacteria infection by *Streptococcus*
41 *pneumoniae*, *Haemophilus influenzae*, or *Neisseria meningitides* [2]. The OPSI remembers us the
42 importance of spleen.
43   The importance of spleen is not limited within immunological aspect. In this review, we show
44 the unique potential of spleen as an optimal site for islet transplantation and a source of
45 mesenchymal stem cells.

2. 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 [3-5].

Although islet transplantation was first established in the clinic in the 1970s [6], 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 [7]. 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" [8]. 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 woman with type 1 diabetes mellitus, who received transplantation of approximately 800,000 islet equivalents, achieved normoglycemia for 22 days without insulin treatment [9].

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) [10]. 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 [11, 12]. 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 [13]. 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 [14, 15]. 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 [16]. 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 [17].

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 [18]. 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 ($\geq$ 0.3 ng/mL) were also significantly higher in the period 2007 – 2010 versus 1999 – 2002 (90 % vs. 60 % at three years after transplantation) [19]. Moreover, it was observed that approximately 80 % of recipients who had received $\geq$600,000 total islet equivalents achieved insulin independence, compared to 55 % who had received <600,000 islet equivalents [20]. Islet transplantation is therefore now considered a practical option for treating

98 severe diabetes mellitus in order to improve endocrinal function and to prevent hypoglycemic attack,
99 but the current clinical outcome is still not satisfactory. The key points in obtaining a positive
100 outcome are the acquisition of large numbers of islets from the donor pancreas, prevention of graft
101 loss in the early stage of transplantation and maintaining engraftment for long period. Another key
102 factor influencing engraftment is the transplant site, and the outcome of clinical islet transplantation
103 could be further improved by utilizing a more optimal transplant site.

104 **3. Candidate Transplantation Sites for Islets**

105 What would be an optimal site for islet transplantation? We would define it by the following
106 three criteria: 1) sites with an abundant, oxygen- and nutrient-rich blood flow, 2) sites that are
107 privileged immunologically to minimize transplant graft loss, and 3) sites where transplantation can
108 be performed with minimum invasiveness. To date, many organs have been assessed including the
109 liver [21-23], renal subcapsular space [21, 22], omental pouch [24, 25], mesentery [26],
110 gastrointestinal tract [27], skeletal muscle [28], subcutaneous tissue [28], eye [29], brain [30], testis
111 [31, 32], bone marrow [33], thymus [34], and spleen [35]. However, it has been difficult to find a site
112 that meets all three criteria (Table 1).

113   **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

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115   The liver has been used as a site for clinical islet transplantation for a long time. It is the largest
116   organ that can accommodate large numbers of islets following a simple transplant procedure
117   (percutaneous infusion into intrahepatic portal vein using ultrasonography under local anesthesia)
118   [36]. On the other hand, the liver also has some problems as a transplant site. Many islets are
119   destroyed in the early stages of transplantation due, in part, to hypoxia caused by ischemia. The
120   isolated islets are in an avascular state throughout the process of preparation [37] and suffer from
121   hypoxia in the hypo-oxygenized portal venous blood (the mean PO_2 of approximately 5 mmHg [38])
122   until revascularization occurs. Moreover, the islets themselves can be a cause of liver ischemia by
123   embolizing the peripheral portal vein [39, 40]. Another issue is inflammation and immunity. The
124   transplanted islets are frequently the subject of an innate immune response and are attacked by
125   Kupffer cells, tissue macrophages in the liver [41, 42] as well as by natural killer cells [43], and this

126 may in turn induce an adaptive immune response. Furthermore, infusion of islets into the blood
127 stream can trigger the instant blood-mediated inflammatory reaction (IBMIR), which can damage
128 intraportal transplanted islets [44]. The IBMIR is triggered by the exposure of islet surface molecules
129 during the process of islet isolation and purification [45, 46]. One such surface molecule is tissue
130 factor (coagulation factor III), which causes the rapid binding of platelets, leading to coagulation and
131 activation of complement systems. Most of the islets are destroyed by this reaction within 1 hour
132 after transplantation [45]. Some immunosuppressants can be more toxic to islets in the liver, as their
133 concentration is higher in the portal vein than in peripheral vessels [47]. Other complications of
134 intraportal islet transplantation include portal hypertension and portal vein thrombosis. Portal
135 hypertension can be a risk factor for post-transplant bleeding, portal vein thrombosis and sepsis [48,
136 49]. Portal vein thrombosis is a critical complication in islet transplantation and can cause
137 esophageal varices, splenomegaly, mesenteric ischemia, sepsis and death [50].

138   A common islet transplantation site in experimental studies, especially rodent, is the kidney (i.e.
139 renal subcapsular space). There are some reports of islet transplantation into the kidney that have
140 led to the restoration of normoglycemia. These studies have used relatively small numbers of islets,
141 as it is difficult to transplant large amounts of islets into the human renal subcapsular space because
142 it is rather inelastic and tight [51]. This may be why clinical progress in renal subcapsular islet
143 transplantation has lagged [52]. Muscle and subcutaneous tissues have also been examined as
144 candidate transplantation sites, as the transplantation procedure and biopsies can be performed
145 easily with minimal invasion and few complications. These sites suffer from hypovascularity and
146 hypoxia, and transplantation efficacy could be improved if these obstacles were overcome,
147 especially in subcutaneous tissue [28]. Another problem with these sites is systemic insulin release.
148 In general, secreted insulin from the pancreas flows into the liver via the portal vein, and therefore
149 smaller amounts of insulin are needed to control blood glucose. This is referred to as physiological
150 insulin secretion, as opposed to systemic insulin release. When islets are transplanted into
151 intramuscular and subcutaneous sites, resulting in systemic insulin release, a much larger amount of
152 insulin needs to be produced, as the insulin does not enter the portal system directly. The large
153 amount of insulin required to be produced by the transplanted islets in order to control blood
154 glucose is similar that required by insulin injection therapy. Another favorable islet transplantation
155 site is the omental pouch. It has advantages in that the insulin drainage is via the portal vein, thus
156 closer to physiological, and this site is highly vascularized [53]. There has been much progress in
157 intra-omental pouch islet transplantation in rodent [25], dog [54] and nonhuman primate models
158 [55]. In particular, because the omental is highly vascularized, this site has been proposed as an
159 alternative site for encapsulated islet transplantation [56-58], but to date no clinical trials have been
160 performed. The mesentery is also considered a candidate islet transplant site due to its rich
161 vascularization and ability to accommodate a large number of islets, however one disadvantage is
162 that if there is any trouble with the graft it would be difficult to remove it without damage to the
163 intestinal tract [59]. The submucosal space of the gastrointestinal tract is another candidate site that
164 has a rich vascular supply providing oxygen and nutrients and connects to the same portal system as
165 the liver, spleen and pancreas [53]. Hara and colleagues have studied transplantation into this
166 location by endoscopy in a pig model [27, 60], but there has been limited demonstration of this
167 concept in large animal models. The brain, testis, the anterior to chamber of the eye, and the thymus
168 are the organs where the immunological response is suppressed and are thus considered "immune
169 privileged" sites. The "immune privilege" of these sites was once assumed to be due to lack of
170 cellular infiltration and lymphatic drainage [61], but more recently it has been shown that this is
171 provided by a complex of immune responses [62]. For example, the brain, testis and retina-blood
172 barrier are maintained in an immunosuppressed condition due to a cellular physical shield [62-64].
173 In some cases, regulatory T cells (Tregs) also contribute to immune privilege. Larocque and
174 colleagues showed that the immune response in the brain could be normally activated when
175 CD4+CD25+ Tregs were depleted [65]. Hedger further revealed that rodent testes contain significant
176 numbers of immunoregulatory cells, including Tregs [66]. And recently, Farooq showed that Tregs
177 contribute to immune tolerance in the rodent anterior chamber when challenged by myelin antigen

[67]. 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 [29, 30, 32, 34], little has been done in a human clinical setting. In particular, the brain or eye is problematic site for transplantation, as it would be difficult to remove a graft without damage in case of graft failure.

4. Characteristics of the Spleen as an Islet Transplant Site

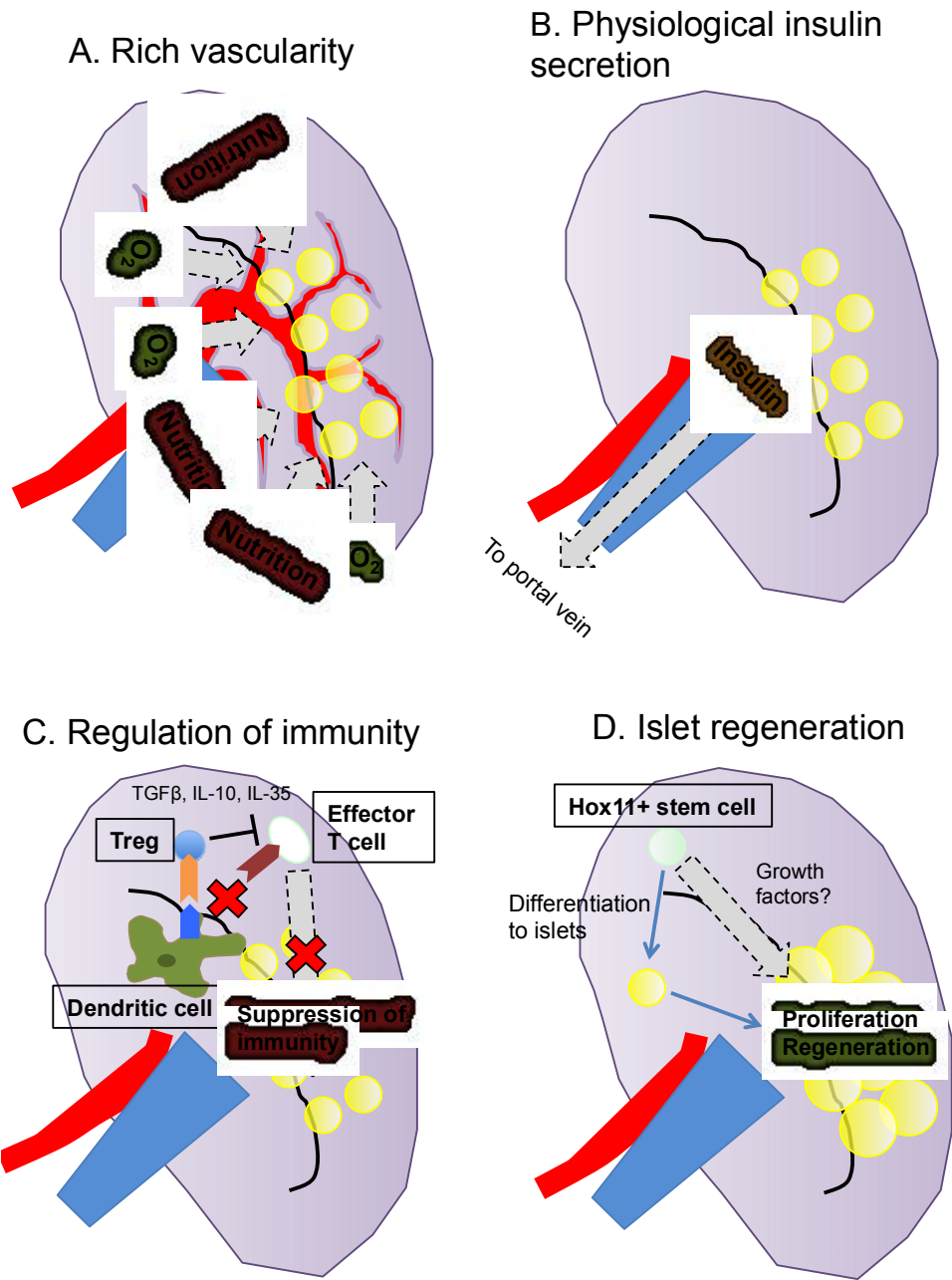
Among the candidate islet transplant sites, the spleen may come closest to being an ideal site. The spleen is a highly vascularized organ which receives blood from the splenic artery and drains into the portal venous system. Vascularization is the most important factor determining the success of transplantation, 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) [68]. 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 [69]. 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 [70, 71]. 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 [72], a cell type rebranded today as Tregs [73]. 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 [74]. 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 [75]. Moreover, splenocytes themselves may help regulate autoimmunity. In a previous study, we found that we could rescue non-obese (NOD) mice (representative type 1 DM animal model) 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 [76, 77]. 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 [78-80]. We also confirmed that these cytokines were significantly lower in intrasplenic transplantation in comparison with intraportal transplantation [81].

Interestingly, the spleen has been shown to be a reservoir of islet stem cells in diabetic mice (review in detail later). We confirmed that CD45- (nonlymphoid) splenocytes could develop into stem cells and further differentiate into islet progenitor cells, thus contributing to islet regeneration [76]. 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 [82]) but not Pdx1, an early pancreatic regeneration marker, and that were CD45- in origin [83]. 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 [84]. Thus, the spleen may facilitate the proliferation of intrasplenic transplanted islets. In 1989, Wohlrab and

colleagues 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 [85]. 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) [81]. 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 [86].

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.

5. Outcomes of Intrasplenic Islet Transplantation

246   The major studies on intrasplenic islet transplantation are summarized in Table 2. Historically,
247 intrasplenic islet transplantation has been performed since the late 1970s, when a number of trials
248 looking at intrasplenic islet autotransplantation into pancreatomized dogs demonstrated that this
249 method could result in the recovery of endocrine function [87-90]. This model has been used not
250 only for the assessment of transplantation efficacy [87, 88, 90-100], but also for the assessment of the
251 transplantation of cold or cryopreserved islets [101-104] and the toxicity of immunosuppressants [93,
252 105-108]. Other animals such as pig [109] and monkey [110-112] have also been used for islet
253 autotransplantation and have shown acceptable outcomes.
254   In the 1980s, some groups worked with allo- [113] and xenogeneic [114] islet transplant models.
255 Du Toit and colleagues performed intrasplenic allogeneic islet transplantation in pancreatomized
256 dogs treated with cyclosporin and showed that survival was extended in comparison with
257 non-immunosuppressed dogs [113]. Moreover, this allogeneic transplant dog model helped
258 demonstrate the usefulness of rapamycin in transplantation [115]. Andersson reported survival of
259 allogeneic grafts from cultured islets for several weeks without the use of any immunosuppressants
260 [116]. In a xenograph model, the Washington group (Paul Lacy) succeeded in prolonging graft
261 survival for more than 100 days using cultured islets in a rat to mouse transplant model where the
262 recipients were treated with anti-rat and/or anti-mouse lymphocyte sera [114]. These findings
263 demonstrated the possibility of using the spleen for transplantation of allo- and xenogeneic islets.
264

265   **Table 2.** Outcomes of Intrasplenic Islet Transplantation.

Authors and References	Published Year	Transplant model	Comments
Kolb E, et al. [87]	1977	Auto (dog)	✓ Achieved normoglycemia, but glucose tolerance was impaired
Feldman SD, et al. [88]	1977	Auto (dog)	✓ Achieved normoglycemia, but glucose tolerance was impaired. ✓ Implantation into splenic pulp.
Gray BN, et al. [90]	1979	Auto (dog)	✓ Response of insulin and glucagon to arginine stimulation. ✓ Implantation into splenic pulp.
Mehigan DG, et al. [117]	1981	Auto (dog)	✓ Assessment of quality of collagenase.
Andersson A, et al. [118]	1981	Iso (mouse)	✓ Achieved normoglycemia after transplantation of 500 islets
Steffes MW, et al. [119]	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. [113]	1982	Allo (dog)	✓ Extended survival, but normoglycemia not achieved.
Janney CG, et al. [114]	1982	Xeno (rat to mouse)	✓ Prolongation of more than up to 100 days graft survival using cultured islets and administration of anti-mouse and/or anti-rat lymphocyte sera.

Andersson A. [116]	1982	Allo (mouse)	✓	Graft survival of several weeks with cultured islets but without immunosuppressants.
Toledo-Pereyra LH, et al. [101]	1983	Allo (dog)	✓	Graft using cryopreserved islets was not rejected for more than 60 days.
Warnock GL, et al. [120]	1983	Iso (dog)	✓ ✓	Five month graft survival. Implantation via splenic vein.
Andersson A [121]	1983	Iso (mouse)	✓	Implantation of 500 islets was sufficient to achieve normoglycemia, while implantation of 150 islets was not.
Merrell RC, et al. [91, 92]	1985	Auto (dog)	✓ ✓	Achieved normoglycemia. Implantation via splenic vein.
Kneteman NM, et al. [122]	1985	Allo (dog)	✓	Prolongation of graft survival (approximately 20 days) using cyclosporine.
Gray DW, et al. [111]	1986	Auto (monkey)	✓ ✓	Achieved normoglycemia for 6 months. The first report of a monkey model.
Gores PF, et al. [93]	1987	Auto (dog)	✓	Achieved normoglycemia for more than 30 days.
Kneteman NM, et al [107]	1987	Allo (dog)	✓	Achieved normoglycemia for more than 100 days using cyclosporine.
Hayek A, et al. [123]	1988	Iso (rat)	✓	Partially achieved normoglycemia by transplantation of 1,000 neonatal islets.
Sutton R, et al. [112]	1989	Auto (monkey)	✓	Achieved normoglycemia with reduced insulin response.
Evans MG, et al. [94]	1989	Auto (dog)	✓	The normoglycemic rate was 90 % at one month after transplantation.
van der Vliet JA, et al. [95, 96]	1989	Auto (dog)	✓	The normoglycemic rate was 63 %.
Warnock GL, et al. [97]	1990	Auto (dog)	✓ ✓	The normoglycemic rate was 63 %. Comparison between splenic vein and pulp as the route of transplantation. Intravenous

				route was superior (The normoglycemia rate was 86 %, vs. 33 %).
Ziegler B, et al. [124]	1990	Iso (rat)	✓	Achieved normoglycemia by transplantation of 1,200 islets
Korsgren O, et al. [125]	1990	Iso (mouse)	✓	Achieved normoglycemia by transplantation of 500 islets
Scharp DW, et al. [98]	1992	Auto (dog)	✓	The normoglycemic rate was 86 % at 1 year after transplantation.
Motojima K, et al. [99]	1992	Auto (dog)	✓	Normoglycemia was not achieved.
Marchetti P, et al. [100]	1993	Auto (dog)	✓	The normoglycemic rate was 90 %, and decreased to 71 % at 1 year after transplantation.
Ao Z, et al. [54]	1993	Auto (dog)	✓	The normoglycemic rate was 67 %.
Yakimets WJ, et al. [115]	1993	Allo (dog)	✓	Approximate 20 days graft survival using cyclosporine and rapamycin.
Hesse UJ, et al. [109]	1994	Auto (pig)	✓	The normoglycemic rate was 50 %.
Eizirik DL, et al. [126]	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. [75]	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 [94] (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 [127, 128]. Many other studies have reported the superiority of spleen compared to liver [98, 109] or omental pouch [54, 129], although some groups have reported the opposite [95-97] (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 [88, 90]. After various trials, the Warnock 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 [120] (Table 2). Intravenous transplantation is

281 generally regarded as preferable to intrasplenic transplantation, in part because intrasplenic
282 transplantation carries the risk of IBMIR that can damage the transplanted islets, similar to
283 intraportal transplantation [53].
284
285 **Table 3.** Transplant Efficacy of Intrasplenic Islet Transplantation.

Authors and References	Published Year	Transplant model		Comments
Sutton R, et al. [112]	1989	vs. Liver (Auto, monkey)	✓	Intrasplenic transplantation showed no superiority over intraportal transplantation
Evans MG, et al. [94]	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. [95, 96]	1989	vs. Liver (Auto, dog)	✓	The normoglycemic rate was 63 % for intrasplenic vs. 75 % for intraportal.
Warnock GL, et al. [97]	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. [98]	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. [99]	1992	vs. Liver (Auto, dog)	✓	Normoglycemia was not achieved with either intrasplenic or intraportal transplantation.
Ao Z, et al. [54]	1993	vs. Omental pouch (Auto, dog)	✓	The normoglycemic rate was 67 % for intrasplenic vs. 50 % for intraomental transplantation.
Hesse UJ, et al. [109]	1994	vs. Liver (Auto, pig)	✓	The normoglycemic rate was 50 % for intrasplenic vs. 25 % for intraportal transplantation.
Gustavson SM, et al. [129]	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. [127]	2017	vs. Liver, Kidney (Allo, pig)	✓	Allo-transplant model using fetal porcine islets. Transplantation efficacy was kidney > spleen > liver.
Stokes RA, et al. [128]	2017	vs. Liver, Kidney (Iso, 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).
		vs. Liver, Kidney, Portal vein, Muscle (Xeno, human to SCID mouse)	✓	Xeno: transplantation of human 2,000 islets. The normoglycemia rate was 100 % for kidney, 70 % for muscle, and 60 % for portal vein.

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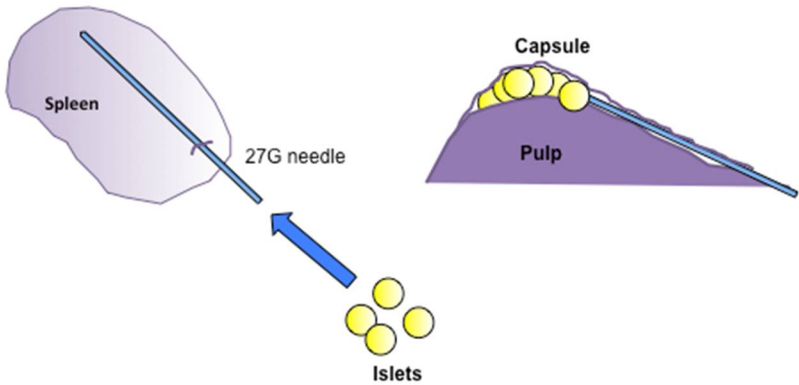
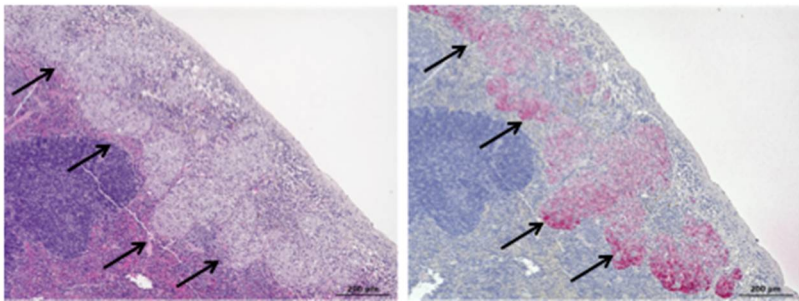
288   To examine the potential usefulness of the spleen as an islet transplantation site and to try to
289 develop a better procedure for intrasplenic transplantation, we explored the "splenic subcapsular
290 implantation technique" using a rodent syngeneic transplant model and analyzed the transplant
291 efficacy of this method compared to intrahepatic and renal subcapsular transplantation [81]. This
292 procedure involved direct puncture from the surface with a 27-gauge needle and implantation of
293 islets under the splenic surface without venous or pulp injury (Figure 2).

294
295 **Figure 2.** A. Procedure of splenic subcapsular implantation technique. B. Engrafted islets (indicated by arrows)
296 under the capsule of spleen at 28 days after transplantation. Left: hematoxylin and eosin staining, Right:
297 immunostaining for insulin.

A. Procedure of splenic subcapsular implantation technique

B. Engrafted islets under the capsule of spleen

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 [97, 118]. 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.

6. 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 [35]. Du Toit reported that intrasplenic islet transplantation was accompanied by some life-threatening complications including subcapsular hematoma, intrasplenic necrosis and cavitation, capsular perforation, and arteriolar thrombosis [113]. 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.

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.

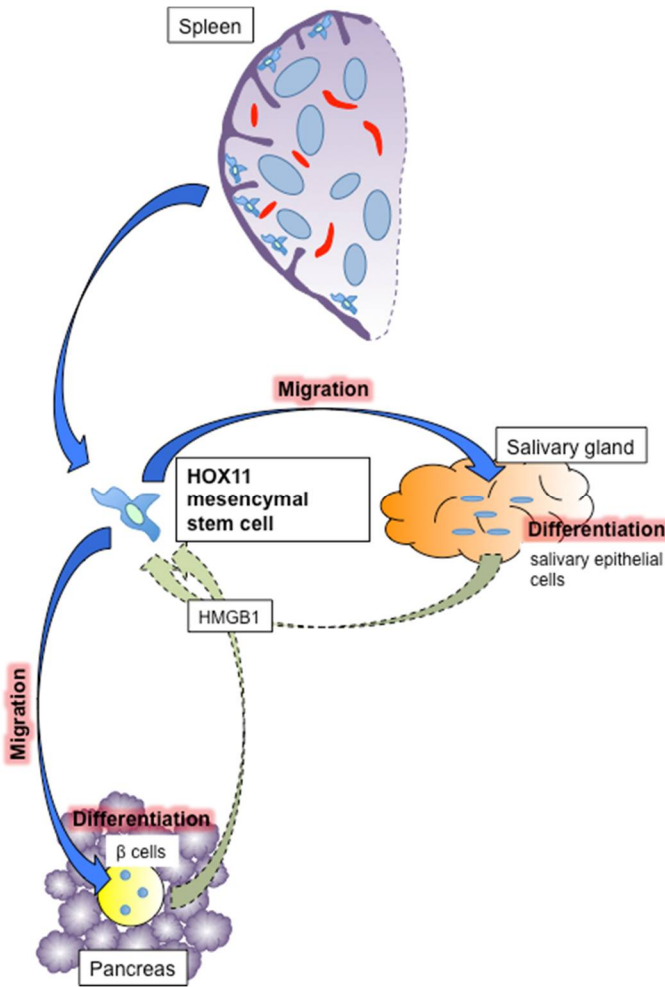
7. Spleen, as a Source of Mesenchymal Stem Cells

Finally, we close this review by introducing about splenic mesenchymal stem cell. As mentioned in the Chapter 4, spleen has an interesting aspect as a stem cell reservoir, as same as islet transplant site. In spleen, both hematopoietic and mesenchymal stem cells, which are labeled or not in CD45 respectively, are harbored. CD45- splenic mesenchymal stem cells have an important role in tissue repairing in damaged tissue, like the cells in other organs including bone marrow and adipose tissue. The first step of the tissue repairing is migration to damaged tissue. The mesenchymal stem cells migrate into the damaged tissue specifically when the damage occurred. It is considered that mesenchymal stem cells catch some kinds of signals, released from the damaged tissue, and migrate and accumulate in the tissue selectively. The representative signal is HMGB1. HMGB1 is a protein which interacts with nucleosome, transcription factors and histones. It influences chromatin structure and remodeling by binding to the internucleosomal linker regions in chromatin, and facilitating nucleosome sliding [130]. It has been clarified that HMGB1 released from damaged and apoptotic tissues activates nuclear factor-kappa B (NF-κB) by binding to the toll-like receptor 4 (TLR4) and receptor for advanced glycation endproducts (RAGE) expressed on the surface of immune and inflammatory cells, causes immune response and inflammatory reaction, and removes the damaged and apoptotic tissues [131]. This sequential reaction needs binding between HMGB1 and damaged tissue derived DNA and histone protein [132]. On the other hand, free HMGB1 released from damaged tissue contributes to the migration of mesenchymal stem cells to damaged tissue and to the tissue repairing [133] (Figure 3). It is considered that migration of splenic stem cells are controlled by HMGB1, especially in the case of inadequately functioning bone marrow due to disease [134].

The second step is differentiation into the cellular components of the damaged tissue. Our previous studies showed that the splenic mesenchymal stem cells differentiated into islets [76] and salivary epithelial cells [135]. Regarding islets, infused syngeneic CD45- splenic stem cells into NOD mice migrated into the damaged islets due to autoimmunity and differentiated into insulin positive β cells [76] (Figure 3). Robertson and colleague also certified that dissected quail splenic tissue – they did not mention but it is considered the tissue included mesenchymal stem cells – differentiated into insulin-producing cells under co-culture with chick pancreatic epithelium [136]. Regarding salivary gland, we detected infused splenocytes migrated into damaged salivary gland of NOD mice, used as Sjogren's syndrome animal models [137], and differentiated into salivary epithelial cells [135] (Figure 3). Sjogren's syndrome is characterized as an autoimmune disease, which destructs salivary glands and lachrymal glands and finally causes dry eye and a dry mouth. These symptoms drastically impair the quality of lives (QOLs) of the patients: Dry eye can lead to vision problems including loss of light sensitivity, blurred vision and corneal damage. Dry mouth induces dental caries and oral infection. Tissue repairing in salivary gland can prevent these symptoms and improve patients' QOLs, and thus mesenchymal stem cell can be a promising therapy for Sjogren's syndrome. Recently, mesenchymal stem cell infusion therapy was applied to the Sjogren's syndrome patients as a novel clinical trial [138]. No radical treatment has been developed for Sjogren's syndrome for a long time, and thus, mesenchymal stem cell infusion therapy can be a hope for the patients.

375   The CD45- splenic stem cells express OCT3/4, SOX2, KLF4, c-MYC and NANOG proteins which
376   induces matured cells into induced pluripotent stem (iPS) cells [82], and they expressed
377   transcription factors, which are identified in embryonic stem cells, including Hox11. HOX11 (TLX1),
378   like other HOXA subgroups including HOX11L2 (TLX3), is known as an oncogene which induces
379   T-cell acute lymphoblastic leukaemia (T-ALL) by chromosomal translocation of t(10;14)(q24;q11)
380   and t(7;10)(q35;q24) [139]. According to the report of Cancer Research UK, the prognosis of T-ALL is
381   relatively good (The survival rate at 5 years after diagnosis is 70 %,
382   http://www.cancerresearchuk.org/about-cancer/acute-lymphoblastic-leukaemia-all/survival).
383   Especially, Ferrando and the colleagues revealed that the survival rate of HOX11-positive T-ALL
384   patients was significantly better than that of other T-ALL patients (88 % vs. 56 %, p = 0.019) [140].
385   That means, mutation of HOX11 is a risk factor of T-ALL but it also can be used as a marker for
386   evaluating the prognosis. HOX11 is known as an embryonic protein which contributes to embryonic
387   development including cell survival, differentiation and regeneration [141, 142]. HOX11 positive
388   mesenchymal stem cells are seen under the splenic capsule and not in the pulp of the spleen [82]
389   (Figure 3). HOX11 is expressed specifically in CD45- splenic mesenchymal stem cells, but no
390   expression in stem cells in other organs including bone marrow and liver [82, 83]. It is considered
391   that HOX11 positive cells is a major components of CD45- splenic mesenchymal stem cells which
392   contribute to tissue repairing in damaged islets and salivary glands in our models.
393

394   **Figure 3.** Mechanism of tissue repairing by splenic mesenchymal stem cells. The splenic mesenchymal stem
395   cells are seen under the splenic capsule in general condition. The splenic mesenchymal stem cells migrate into
396   damaged tissue by receiving HMGB1 when the damage occurs. They differentiate into the tissue component
397   such as β cells in pancreatic islets and salivary epithelial cells in salivary gland. It is considered that HOX11
398   positive cells are the real natures of splenic mesenchymal stem cells.

399

8. Conclusion

We showed importance of spleen, as an optimal site for islet transplantation and a source of mesenchymal stem cells in this review. Spleen has considered as an unnecessary organ for a long time, but it has many unique potentials in experimental and clinical medicines. Hope to clarify unknown potentials of the spleen in further studies.

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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
IgM	immunoglobulin M
IL	interleukin
iPS	induced pluripotent stem
MCP-1	monocyte chemotactic protein-1
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor-kappa B
NOD	non-obese
OPSI	overwhelming postsplenectomy infection
QOL	quality of life
RAGE	receptor for advanced glycation endproducts
Rrm2b	ribonucleoside-diphosphate reductase subunit M2 b
T-ALL	T-cell acute lymphoblastic leukaemia
TGFβ	transforming growth factor
TLR4	toll-like receptor 4
Tregs	regulatory T cells

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