Intraportal islet transplantation: the impact of the liver microenvironment

Vaihere Delaune¹,², Thierry Berney²,³, Stéphanie Lacotte¹ & Christian Toso¹,²

SUMMARY

The portal vein remains the preferred site for pancreatic islet transplantation due to its easy access and low morbidity. However, despite great progress in isolation and transplantation protocols over the past few years, it is still associated with the early loss of some 50–70% of transplanted islets. The complex liver microenvironment itself presumably plays an important role in this loss. The present review focuses on the specifics of the liver microenvironment, notably the localized hepatic ischemia/reperfusion injury following transplantation, the low oxygenation of the portal vein, the instant blood-mediated inflammatory reaction, the endogenous liver immune system, and the gut–liver axis, and how they can each have an impact on the transplanted islets. It identifies the potential, or already applied, clinical interventions for improving intraportal islet survival, and pinpoints those promising areas still lacking preclinical research. Future interventions on clinical intraportal islet transplantation need to take into account the global context of the liver microenvironment, with multi-point interventions being most likely to improve early islet survival and engraftment.

Introduction

The liver is currently the preferred site for clinical islet transplantation. It can be accessed by a minimally invasive procedure, and presents a low morbidity profile with rates of bleeding and thrombosis <10%. However, more than one donor is needed in order to reach insulin independence in clinical islet transplantation. This observation is linked to the subacute/chronic multifactorial impact of alloimmunity, recurrent autoimmunity, and drug toxicity [1]. In addition, early events also have a significant impact, as suggested by rodent and human positron-emission tomography studies with a potential loss of up to 50–70% of islets immediately after transplantation [2,3]. While early events are likely also related to mechanical injuries, the liver microenvironment is believed to play an important role, and other transplantation sites are currently explored [4], including the immune-privileged eye, the striated muscle, the omentum [5], and the bone marrow [6]. They each have their own drawbacks, notably the risk of vision impairment, a decreased efficiency of immunosuppression in the bone marrow [7], a lower oxygen and nutrient supply, a location distant from the physiologic release of insulin, and the need for invasive surgery.
While investigators are still exploring alternative transplantation sites, the pitfalls associated with the intraportal location must be solved. The present review explores selected key events affecting islets in the liver microenvironment, including the instant blood-mediated inflammatory reaction, islet and localized liver ischemia/reperfusion injury, islet hypoxia, the activation of endogenous liver immune cells, and the impact of the gut–liver axis. It identifies clinical interventions that could decrease the risk of early islet losses after intraportal transplantation, and discusses areas where preclinical studies are still needed.

**Methods**

The current article is based on a narrative (nonsystematic) review. Although a systematic review might have been a preferred option, the multitude of topics addressed in this review made us undertake a narrative approach. The literature search was performed in Medline, using the following keywords: instant blood-mediated inflammatory reaction (IBMIR), ischemia–reperfusion, hypoxia, Kupffer cell, liver sinusoidal endothelial cell (LSEC), stellate cell, liver lymphocyte, reperfusion, hypoxia, Kupffer cell, liver sinusoidal endothelial cell (LSEC), stellate cell, liver lymphocyte, liver dendritic cell, hepatocyte, and gut–liver axis. It identifies clinical interventions that could decrease the risk of early islet losses after intraportal transplantation, and discusses areas where preclinical studies are still needed.

**The instant blood-mediated inflammatory reaction (IBMIR)**

Instant blood-mediated inflammatory reaction is the most studied consequence of the intraportal route on the transplanted islets. It is a complex nonspecific response of the innate immune system that takes place early after transplantation, with the constitution of thrombi and a dense lymphocyte and macrophage infiltrate [8].

Instant blood-mediated inflammatory reaction is initiated by a strong activation of the coagulation cascade, which peaks 6–12 h after clinical and large animal transplantation experiments [9,10]. Both coagulation pathways are recruited; the intrinsic pathway is triggered by the negatively charged islet surface [11], and the extrinsic pathway is triggered by the expression of tissue factor (TF) by the cultured islets [12]. It is associated with increased levels of pro-coagulating factors XIIa-antithrombin, Xla-antithrombin and the thrombin–antithrombin III complex, and the generation of D-dimer [9,12]. Macroscopic clots develop as early as 5 min after infusion, with a high consumption of platelets, neutrophils, and monocytes. A time line, based on in vitro and in vivo large animal data, can be established for the constitution of this thrombo-inflammatory infiltrate (Fig. 1).

Instant blood-mediated inflammatory reaction also includes the activation of the complement [13]. C1q, C4, C3, nad C9 can be found in and on the islets, together with IgG and IgM deposition [14,15]. This results in the formation of the inflammation-promoting anaphylatoxins C3a and C5a [16].

A panel of cytokines leads to the recruitment and activation of inflammatory cells. The activated thrombin promotes the secretion of adhesion factors such as P-selectin by endothelial cells, thus activating platelet aggregation. The endothelial cells also secrete the pro-inflammatory interleukin (IL)-6 and IL-8, which help recruit neutrophilic granulocytes and macrophages on site. The monocytes/macrophages secrete interferon gamma (IFNγ), IL-1β, IL-6, and IL-8, thus upholding the inflammatory response [13]. Due to the hypoxia and stress induced by isolation, the islets themselves promote this inflammation by not only secreting TF, but also expressing other pro-inflammatory and danger signals, such as the high-mobility group box 1 (HMGB1), IFNγ, IL-6, IL-8, IL-1β, IFNγ-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1 [17], tumor necrosis factor (TNF)-α, nuclear factor-kappa B (NF-κB), macrophage migration inhibitory factor, and nitric oxide (NO), among others [13,18].

Much effort has been made to prevent IBMIR in preclinical models. Heparin and low-molecular-weight dextran sulfate (LMW-DS) have been shown to decrease IBMIR in various in vitro and in vivo animal models [19–21]. Alternative molecules have been tested, such as nicotinamide [22], thrombin inhibitors [23], complement inhibitor sCR1 [11], C5α inhibitors [24,25], with varying levels of success. Alternative ways of protecting the islets against IBMIR are also under study, such as PEGylation [26] or endothelial cell [27] coating of the islets. In humans, only heparin is routinely used [28].

**Ischemia/reperfusion injury**

During organ recovery, preservation, and implantation, grafts undergo a transient deprivation of their oxygen supply, with subsequent restoration. This process leads
to ischemia/reperfusion injury and altered early graft function.

Ischemia/reperfusion injury is difficult to characterize after islet transplantation because of the lack of easy access to biopsy [29]. Such lesions are expected both in the islets themselves and in the surrounding liver tissue, due to the microembolization of islets in the presinusoidal veins.

Rodent (especially mouse) models of intraportal islet transplantation do not perfectly reflect the human situation because of the higher islet-to-portal vein diameter ratio, with a more proximal embolization of rodent islets. However, they allowed the identification of liver ischemia (Fig. 2) and necrosis as contributors of early islet failure [30].

In humans, these events can be detected by the, transient, increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) seen in half of recipients, and peaking one week after transplantation (Fig. 3). Although the systemic impact of ischemia/reperfusion is relatively minor in humans, it is likely more significant locally at the islet level, contributing to the early loss of islets. As a further note, the increase in AST and ALT is lower when patients have been previously transplanted, an observation which remains poorly understood, but may be related to a better stability of immunosuppression serum levels [31,32].

The use of alternating cycles of liver flow interruption and restoration, known as ischemia preconditioning, protects both the liver and transplanted islets from ischemic lesions [30]. This proof of principle opens the way for other clinical interventions known to prevent ischemia/reperfusion injury.

### Intraportal islet hypoxia

Native islets are extremely well oxygenated, using 5–15% of the blood flow destined to the whole pancreas, with an oxygen tension of about 40 mmHg [33]. However, in culture conditions, large isolated islets suffer from hypoxia with central necrosis and apoptosis [34] (Fig. 4). During the first few days after intraportal transplantation, the islets are only oxygenated via diffusion, in the low oxygen tension portal vein system, which is further impaired by the activation of the coagulation cascade in the portal system during the “instant blood-mediated inflammatory reaction.” It takes 7–14 days for the islets to develop a functional circulatory system [35–39]. Even after 3 months, the islets chronically keep a low endogenous oxygen tension of 5 mmHg [40]. These observations do not appear to be solely due to the intraportal location, as islet grafts transplanted in better oxygenated sites face similar issues [33].

A number of therapeutic options have been tested in animal models either to directly improve islet oxygenation by hyperoxic housing of animals [41],
hyperbaric oxygen therapy of transplanted rodents [42], and intraperitoneal oxygenation [43], or indirectly by increasing VEGF expression [44]. Interestingly, some authors have demonstrated that pancreatic islets develop a better resistance to hypoxia after in vitro ischemic preconditioning [45,46], once more highlighting the potential benefit of in vivo ischemic preconditioning.

These observations reflect the importance of having an adequate oxygen supply early after islet transplantation. In the clinical setting, this could perhaps be achieved by avoiding low hemoglobin levels and ensuring enough systemic oxygen delivery. The clinical value of the above-mentioned interventions still needs to be defined.

The endogenous liver immune system

The impact of the endogenous liver immune system on intraportal islet transplantation, Kupffer cells are activated through the complement pathway, due to the hepatic ischemia/reperfusion lesions surrounding transplanted islets [47];
and more specifically by the anaphylatoxins C3a and C5a, expressed during IBMIR [16,48]. Once activated, Kupffer cells can have two modes of action against islets, phagocytosis and the secretion of inflammatory mediators and free radicals [49].

Until now, most investigators have looked at macrophages, and rarely specifically at Kupffer cells. Macrophages are cytotoxic to pancreatic islets in culture [50], notably through their secretion of NO, TNF-\(\alpha\), IL-1\(\beta\), IL-6, and prostaglandins [51]. Similarly, Kupffer cells are activated by cultured islets, and even more so by unpurified islets, with the secretion of eicosanoids (thromboxane and prostaglandins) [52]. In addition to activating macrophages/Kupffer cells, isolated islets secrete MCP-1 and IL-8, further attracting more macrophages [17]; MCP-1 may be linked to a lower rate of posttransplant insulin independence [17]. In vivo, macrophage depletion improves graft survival after allogeneic islet transplantation in Lewis rats [51]. Although Kupffer cells are difficult to specifically target, they appear to be key players, and further animal investigations should be performed in the islet transplantation setting.

Liver sinusoidal endothelial cells

Activated Kupffer cells interact with LSEC [51]. LSEC have a pro-inflammatory action, with the expression of intercellular adhesion molecule-1 (ICAM-1) and platelet-activating factor, allowing for the adherence of leukocytes, the recruitment of lymphocytes, and the activation of platelets [53], and with the secretion of IL-6 after contact with platelets [54]. Therefore, they probably play an important role in maintaining and exacerbating IBMIR.

Conversely, in an inflammatory environment, LSEC also have an anti-inflammatory effect by secreting IL-10, and a pro-angiogenic role by the secretion of vascular endothelial growth factor (VEGF) [53]. However, the specific role of LSEC on intraportally transplanted islets remains to be determined, and the current lack of appropriate blocking agents limits the potential for a clinical intervention.

Hepatic stellate cells

Hepatic stellate cells, present in the space of Disse, are mostly quiescent, but can contribute to the immune balance. After injury, they activate Kupffer cells [55], but they also have a strong immunosuppressive activity via the induction of myeloid-derived suppressor cells (MDSC) [56] and the inhibition of T cells [57,58]. When co-transplanted with islets, they promote immune tolerance [56,59]. However, these studies were conducted with islets transplanted under the kidney capsule, and not in the stellate cell natural hepatic environment. Further studies are needed to better understand their protective role on intraportally transplanted islets.

Resident liver lymphocytes

The liver is a lymphoid organ with a very high number of natural killer (NK), NKT, and CD8\(^+\) T cells and, at a lesser rate, CD4\(^+\) T cells. In this context, studies have shown that NK cells are vastly involved in early intraportal islet graft loss, even in a syngeneic setting [60]. NKT cells, via dendritic cell activation [61], are also highly involved in early graft rejection with the downstream activation of Gr-1\(^+\)CD11b\(^+\) neutrophils, resulting in the production of IFN\(\gamma\) and IL-1\(\beta\) [62,63]. Some investigators have shown improved intraportal islet survival after adenosine administration, thus inactivating the NKT cell-mediated IFN\(\gamma\) production by neutrophils [64]. No human data are available thus far on adenosine administration and islet transplantation.

Liver dendritic cells

Mature dendritic cells play an important role in allogeneic liver graft rejection, notably via their activation...
of T cells and secretion of IL-12. The “immature” liver dendritic cells, however, are known to only weakly activate T cells, and mostly induce a Th2 response [65].

Their impact on islet transplantation has been explored by a limited number of studies. Mature dendritic cells appear detrimental [66], whereas immature dendritic cells could have a tolerogenic effect on islet graft as suggested by in vitro studies [67,68]. In animal models, interventions to inhibit dendritic cell maturation, or to make them more tolerogenic, increase islet allograft acceptance [69–71]. However, these studies were all performed by transplanting islets under the kidney capsule, which does not fully reflect the immune response found in clinical islet transplantation. Further data are required on the effect of dendritic cells on intraportally transplanted islets in animal models.

**Hepatocytes**

The hepatocytes themselves could, through ischemia/reperfusion lesions, also contribute to maintaining an inflammatory environment by their known production of NO when injured [49], but no data are currently available on this topic.

**The gut–liver axis**

Due to its specific anatomic location, the liver is directly exposed to antigens and toxins released from the bowel. In islet transplantation, the gut–liver cross talk can be altered through (i) changes in the microbiota profile, (ii) alterations of the gut barrier, and (iii) an increased release and action of toxic gut products.
Changes in the microbiota profile

The intestinal microbiota are made of a fragile equilibrium of some $10^{14}$ microorganisms that can change with age, close human relationships, cultural environment, geographic location, and location in the digestive tract. It is altered in patients with type 1 diabetes, and appears involved in autoimmunity [72]. Finnish clinical studies found that children and adolescents at risk of T1DM tend to have a less diverse microbiota, with an increase in \textit{Bacteroides} spp., and a decrease in \textit{Bifidobacterium} spp. and butyrate-producing bacteria [73,74].

\textit{Bacteroides} spp. is a large family of Gram-negative bacteria, known to express lipopolysaccharide (LPS) [75].

The impact of gut microbiota has been studied after various organ transplantations in animal models. In the case of liver transplantation, an increase in \textit{Bacteroidetes} associated with a decrease in \textit{Firmicutes} predicted acute graft rejection in a rat model [76].

The impact of pre-existing diabetes-linked microbiota alterations on the success or failure of islet transplantation and the possible modifications of the microbiome after islet transplantation have not been explored thus far. Nevertheless, one can speculate that at least some of the observed diabetes-linked microbiota features contribute to islet injury. Total intestinal decontamination partially reduces the severity of the Kupffer cell-mediated ischemia/reperfusion injury after mouse liver transplantation [77] and, in humans, efficiently prevents acute graft versus host disease after clinical hematopoietic stem cell transplantation [78]. The impact of gut decontamination and/or fecal transplantation could also be explored in islet transplantation.

Alterations of the gut barrier

An increased permeability of the gut barrier increases bacterial translocation and LPS release. In clinical islet transplantation, patients with T1DM are exposed to this risk due to (i) the impact of diabetes itself, (ii) a possible transient and moderate increase in portal pressure, and (iii) immunosuppressant side effects.

Type I diabetes mellitus

The intestinal barrier exhibits increased permeability in patients with clinical T1DM [79]. Similarly, gut permeability also increases in diabetic rats; this can be reversed by inhibiting zonulin, an intestinal tight junction modulator [80]. A similar strategy could be tested in preclinical models of intraportal islet transplantation.

Portal congestion

Intraportal islet transplantation leads to a transient, moderate increase in portal vein pressure, which leads to small bowel congestion, and increases the risk of LPS release.

Immunosuppression

Rapamycin and tacrolimus, the most commonly used drugs after islet transplantation, increase intestinal permeability [81], with higher levels of systemic LPS [82]. This leads to a persistent engagement of the LPS/Toll-like receptor (TLR) 4 pathway and a chronic inflammatory state.

Increased release and action of toxic gut products

Lipopolysaccharide release

Circulating pathogen-associated molecular patterns, such as LPS, stimulate the professional antigen-presenting cells, and are part of the danger model, subsequently promoting allogeneic rejection [83]. Animal studies have found that the inhibition of the LPS/TLR4 pathway, through a deficiency in MyD88, protects germ-free nonobese diabetic (NOD) mice from the onset of autoimmune diabetes after fecal transplantation of commensal microbiota [84]. This protection is further conveyed to wild-type NOD mice having received...
microbiota from pathogen-free MyD88-deficient NOD mice [85]. Islets express TLR4, and the LPS/TLR4 pathway has been shown to have a negative effect on the survival of transplanted islets [86,87]. Reciprocally, TLR4 blockade increases mouse islet allograft survival [86,88]. While waiting for clinical-grade blocking antibodies, TLR4 inhibition appears as a promising therapeutic option in the clinical setting.

The immunosuppressive storm

Orally taken drugs have a first hepatic passage, with the highest drug levels found in this organ [89]. This can contribute to islet injury as currently used immunosuppressive drugs are islet-toxic [90]. In this regard, perhaps immunosuppressive drugs should be given intravenously during the first few days after islet transplantation (thus preventing the high hepatic levels).

Conclusion

A major drawback in identifying a potential clinical translation from ongoing animal studies is the fact that the majority of studies performed on rodents include subcapsular kidney islet transplantation. Due to the widely different microenvironments, some promising
interventions may not have as high an impact on intraportally transplanted islets, and data should be analyzed with caution, while waiting for confirmatory experiments.

Although alternative sites and techniques, such as subcutaneous polymeric scaffolding [91], should continue to be explored, the liver remains the location most used for clinical islet transplantation. Early after transplantation, the islets initiate a complex cascade of interconnected harmful events, which are schematized in Fig. 5. Many of them are specific to the liver site, because of the islet–blood contact, the presence of specific liver immune cells, and the anatomic location of the liver downstream of the gut.

A number of clinical interventions have been established or explored with a desire to prevent these events. Current clinical practice and research agenda concerning the liver microenvironment are summarized in Tables 1 and 2. The most relevant one is the use of aggressive intravenous anticoagulation early after transplantation [28], in order to lessen IBMIR. Anticoagulation should be combined with a strict blood glucose control via intensive intravenous insulin administration during at least 5 days after transplantation. Other interventions include the use of antioxidants, such as pentoxifylline, and anti-inflammatory drugs, such as anakinra (anti-IL1R) and etanercept (anti-TNF).

Alternative areas of action should be explored. Islets are expected to undergo more ischemia/reperfusion lesions than whole organs, because not only do they undergo the oxygen deprivation/restoration during recovery and transplantation, but they also undergo an added warm hypoxic phase during isolation and culture. As such, the prevention of ischemia/reperfusion lesions through drugs (i.e. sevoflurane), ischemia preconditioning, or by using mechanical pancreas perfusion prior to isolation, deserves (pre)clinical assessment. Also, one should maintain an appropriate oxygen supply to the islets early after transplantation, at least by avoiding low hemoglobin levels. Finally, interventions on the gut–liver axis also appear to be of interest. They could include the use of a clinical-grade anti-TLR4 antibody, or interventions on the microbiota, including the possible administration of topical antibiotics, such as rifaximin, prior to transplantation in order to decrease the release of LPS.

Overall, these established or explored interventions appear pivotal, not only in preventing the early islet losses, but also in decreasing the danger signals released during IBMIR and via the gut–liver axis.

The liver site should still be favored, but it should be re-explored taking into account the global intraportal islet microenvironment. Multi-point interventions are likely to further improve early islet engraftment and their long-term survival.

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REFERENCES
1. Harlan DM, Kenyon NS, Korsgren O, Roep BO. Society for the I of D. Current Advances and Travails in Islet Transplantation. Diabetes 2009; 58: 2175.
2. Toso C, Zaidi H, Morel P, et al. Positron-emission tomography imaging of early events after transplantation of islets of Langerhans. Transplantation 2005; 79: 353.
3. Eich T, Eriksson O, Sundin A, et al. Positron emission tomography: a real-time tool to quantify early islet engraftment in a preclinical large animal model. Transplantation 2007; 84: 893.
4. Merani S, Toso C, Emamouille J, Shapiro AMJ. Optimal implantation site for pancreatic islet transplantation. Br J Surg 2008; 95: 1449.
5. Espes D, Lau J, Quach M, Ullsten S, Christoffersson G, Carlson P-O. Rapid restoration of vascularity and oxygenation in mouse and human islets transplanted to omentum may contribute to their superior function compared to intraportally transplanted islets. Am J Transplant 2016; 16: 3246.
6. Cantarelli E, Melzi R, Mercalli A, et al. Bone marrow as an alternative site for islet transplantation. Blood 2009; 114: 4566.
7. Cantarelli E, Citro A, Pellegrini S, et al. Transplant site influences the immune response after islet transplantation: bone marrow vs liver. Transplantation DOI: 10.1097/TP.0000000000001462 [Epub ahead of print]
8. Sever CE, Demetris AJ, Zeng Y, et al. Islet cell allotransplantation in diabetic patients. Histologic findings in four adults simultaneously receiving kidney or liver transplants. Am J Pathol 1992; 140: 1255.
9. Johansson H, Lukinius A, Moberg L, et al. Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. Diabetologia 2005; 48: 1755.
10. Lambin A, Tournoys A, Gmyr V, et al. Activation of the coagulation pathway by the graft intraportal d’îlots de Langerhans chez le porc âgé. Ann Chir 2001; 126: 743.
11. Bennet W, Groth C-G, Larson R, Nilsson B, Korsgren O. Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with Type 1 diabetes. Ups J Med Sci 2000; 105: 125.
12. Moberg L, Johansson H, Lukinius A, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. Lancet 2002; 360: 2039.
13. Härstedt M, Lindblom S, Karlsson-Parr A, Nilsson B, Korsgren O. Characterization of innate immunity in an extended whole blood model of human islet allotransplantation. Cell Transplant 2016; 25: 503.
14. Titus TT, Horton PJ, Badet L, et al. Adverse outcome of human islet-allogeneic blood interaction. Transplantation 2003; 75: 1317.
15. Tjernberg J, Ekdahl KN, Lambris JD, Korsgren O, Nilsson B. Acute antibody-mediated complement activation mediates lysis of pancreatic islet cells and may cause tissue loss in clinical islet transplantation. Transplantation 2008; 85: 1193.
16. Bennet W, Sundberg B, Groth CG, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? Diabetes 1999; 48: 1907.
17. Piemonti L, Leone BE, Nano R, et al. Human pancreatic islets produce and secrete MCP-1/CCL2: relevance in human islet transplantation. Diabetes 2002; 51: 55.
18. Johansson U, Olsson A, Gabrielson S, Nilsson B, Korsgren O. Inflammatory mediators expressed in human islets of Langerhans: implications for islet transplantation. Biochem Biophys Res Commun 2003; 308: 474.
19. Cabric S, Sanchez J, Lundgren T, et al. Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. Diabetes 2007; 56: 2008.
20. Goto M, Johansson H, Maeda A, Elgue G, Korsgren O, Nilsson B. Low molecular weight dextran sulfate prevents the instant blood-mediated inflammatory reaction induced by adult porcine islets. Transplantation 2004; 77: 741.
21. Johansson H, Goto M, Dufrane D, et al. Low molecular weight dextran sulfate: a strong candidate drug to block IBMR in clinical islet transplantation. Am J Transplant 2006; 6: 305.
22. Jung D-Y, Park JB, Joo S-Y, et al. Effect of nicotinamide on early graft failure following intraportal islet transplantation. Exp Mol Med 2009; 41: 782.
23. Ozmen L, Ekdahl KN, Elgue G, Larson R, Korsgren O, Nilsson B. Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: possible application of the thrombin inhibitor melagatran in clinical islet transplantation. Diabetes 2002; 51: 1779.
24. Tokodai K, Goto M, Inagaki A, et al. C5α-inhibitory peptide combined with gabezate mesilate prevents the instant blood-mediated inflammatory reaction in a rat model of islet transplantation. Transplant Proc 2010; 42: 2102.
25. Tokodai K, Goto M, Inagaki A, et al. C5a-blockade improves early outcomes after intraportal islet transplantation. Transplantation 2010; 90: 1358.
26. Teramura Y, Iwata H. Surface modification of islets with PEG-lipid for improvement of graft survival in intraportal transplantation. Transplantation 2009; 88: 624.
27. Johansson U, Elgue G, Nilsson B, Korsgren O. Composite islet-endothelial cell grafts: a novel approach to counteract innate immunity in islet transplantation. Am J Transplant 2005; 5: 2632.
28. Koh A, Senior P, Salam A, et al. Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success. Transplantation 2010; 89: 465.
29. Tosco C, Isse K, Demetris AI, et al. Histologic graft assessment after clinical islet transplantation. Transplantation 2009; 88: 1286.
30. Yin D, Ding JW, Shen J, Ma L, Hara M, Chong AS. Liver ischemia contributes to early islet failure following intraportal transplantation: benefits of liver ischemic-preconditioning. Am J Transplant 2006; 6: 60.
31. Barshes NR, Lee TC, Goodpastor SE, et al. Transaminitis after pancreatic islet transplantation. J Am Coll Surg 2005; 200: 333.
32. Rafał E, Ryan EA, Paty BW, et al. Changes in liver enzymes after clinical islet transplantation. Transplantation 2003; 76: 1280.
33. Karlsson PO, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. Diabetes 2001; 50: 489.
34. Giuliani M, Moritz W, Bodmer E, et al. Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. Cell Transplant 2005; 14: 67.
35. Menger MD, Jaeger S, Walter P, Feifel G, Hammersen F, Messmer K. Angiogenesis and hemodynamics of microvasculature of transplanted islets of Langerhans. Diabetes 1989; 38(Suppl 1): 199.
36. Vajkoczy P, Olofsson AM, Lehr HA, et al. Histogenesis and ultrastructure of pancreatic islet graft microvasculature. Evidence for graft revascularization by endothelial cells of host origin. Am J Pathol 1995; 146: 1397.
37. Vajkoczy P, Menger MD, Simpson E, Messmer K. Angiogenesis and vascularization of murine pancreatic islet isografts. Transplantation 1995; 60: 123.
38. Jansson L, Karlsson P-O. Graft vascular function after transplantation of pancreatic islets. Diabetologia 2002; 45: 749.
39. Jones GL, Juszczak MT, Hughes SJ, Kooner P, Powis SH, Press M. Time course and quantification of pancreatic islet revascularization following intraportal transplantation. Cell Transplant 2007; 16: 505.
40. Olsson R, Olerud J, Pettersson U, Karlsson P-O. Increased numbers of low-oxygenated pancreatic islets after intraportal islet transplantation. Diabetes 2011; 60: 2350.
41. Hughes SJ, Davies SE, Powis SH, Press M. Hyperoxia improves the survival of intraportally transplanted syngeneic pancreatic islets. Transplantation 2003; 75: 1954.
42. Sakata N, Chan NK, Ostrowski RP, et al. Hyperbaric oxygen therapy improves early posttransplant islet function. Pediatr Diabetes 2010; 11: 471.
43. Sakai T, Li S, Kuroda Y, Tanaka Y, Fujino Y, Suzuki Y. Oxygenation of the portal vein by intraperitoneal administration of oxygenated perfluorochemical improves the engraftment and transplanted function of intraportally transplanted islets. Pancreas 2011; 40: 403.
44. Langlois A, Bietiger W, Seyfritz E, et al. Improvement of rat islet viability during
transplantation: validation of pharmacological approach to induce VEGF overexpression. Cell Transplant 2011; 20: 1333.

45. Lo JF, Wang Y, Blake A, et al. Islet preconditioning via multimodal microfluidic modulation of intermittent hypoxia. Anal Chem 2012; 84: 1987.

46. Hogan AR, Doni M, Ribeiro MM, et al. Ischemic preconditioning improves islet recovery after pancreas cold preservation. Transplant Proc 2009; 41: 354.

47. Caldwell-Kenkel JC, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ. Kupffer cell activation and endothelial cell damage after storage of rat livers: effects of reperfusion. Hepatology 1991; 13: 83.

48. Tokodai K, Goto M, Inagaki A, Imura T, Nakashima W, Satomi S. Expression of receptors for anaphylatoxins C3a and C5a on rat islet preparations. Transplant Proc 2011; 43: 3179.

49. Barshes NR, Wyllie S, Goss JA. Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts. J Leukoc Biol 2005; 77: 587.

50. Appels B, Burkart V, Kantwerk-Funke G, Funda J, Kolb-Bachofen V, Kolb H. Spontaneous cytotoxicity of macrophages against pancreatic islet cells. J Immunol 1989; 142: 3803.

51. Bottino R, Fernandez LA, Ricordi C, et al. Transplantation of allogeneic islets of Langerhans in the rat liver: effects of macrophage depletion on graft survival and microenvironment activation. Diabetes 1998; 47: 316.

52. Clayton HA, Davies JE, Sutton CD, Bell PRF, Dennison AR. Coculture model of intrahepatic islet transplantation: activation of Kupffer cells by islets and acinar tissue. Cell Transplant 2001; 10: 101.

53. Poisson J, Lemoine S, Boulanger C, et al. Liver sinusoidal endothelial cells: physiology and role in liver diseases. J Hepatol 2017; 66: 212.

54. Kawasaki T, Murata S, Takahashi K, et al. Activation of human liver sinusoidal endothelial cell by human platelets induces hepatocyte proliferation. J Hepatol 2010; 53: 648.

55. Weiskirchen R, Tacke F. Cellular and molecular functions of hepatic stellate cells in inflammatory responses and liver immunology. Hepato-Biliary-Pancreatic Disease Int 2014; 3: 344.

56. Choi H-S, Hsieh C-C, Yang H-R, et al. Hepatic stellate cells regulate immune response by way of induction of myeloid suppressor cells in mice. Hepatology 2011; 53: 1007.

57. Yu M-C, Chen C-H, Liang X, et al. Inhibition of T-cell responses by hepatic stellate cells via β7-H1-mediated T-cell apoptosis in mice. Hepatology 2004; 40: 1312.

58. Maher JI. Interactions between hepatic stellate cells and the immune system. Semin Liver Dis 2001; 21: 417.

59. Zhang Z-Y, Zhou Z-Q, Song K-B, Kim S-C, Zhou G-W. Hepatic stellate cells induce immunotolerance of islet allografts. Transplant Proc 2014; 46: 1594.

60. 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: 952.

61. Matsuoka N, Itoh T, Watarai H, et al. High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice. J Clin Invest 2010; 120: 735.

62. Yasunumi Y, Kojo S, Kitamura H, et al. Vβ14 NK T cell–triggered IFN-γ production by Gr-1+CD11b+ cells mediates early graft loss of syngeneic transplanted islets. J Exp Med 2005; 202: 913.

63. Toyofuku A, Yasunumi Y, Nabeayama K, et al. Natural killer T-Cells participate in rejection of islet allografts in the liver of mice. Diabetes 2006; 55: 34.

64. Nitta T, Itoh T, Matsuoka N, et al. Prevention of early loss of transplanted islets in the liver of mice by adenosine. Transplantation 2009; 88: 49.

65. Lau AH, Thomson AW. Dendritic cells and immune regulation in the liver. Gut 2003; 52: 307.

66. Sutherland RM, Zhan Y, Carrington EM, Londrigan SL, Lew AM. Selective depletion of cross-presenting dendritic cells enhances islet allograft survival. Cell Transplant 2011; 20: 467.

67. Castellini C, Lu L, Ricordi C, Starzl TE, Rao AS, Thomson AW. Granulocyte/ macrophage colony-stimulating factor–stimulated hepatic dendritic cell progenitors prolong pancreatic islet allograft survival. Transplantation 1995; 60: 1366.

68. Stepkowski SM, Phan T, Zhang H, et al. Immature syngeneic dendritic cells potentiate tolerance to pancreatic islet allografts depleted of donor dendritic cells in microgravity culture condition. Transplantation 2006; 82: 1756.

69. Yang D-F, Qiu W-H, Zha H-F, et al. CTLA4-Ig-modified dendritic cells inhibit lymphocyte-mediated alloimmune responses and prolong the islet graft survival in mice. Transpl Immunol 2008; 19: 197.

70. Thomas DC, Wong FS, Zaconne P, Green EA, Wallberg M. Protection of islet grafts through transforming growth factor-β-induced tolerogenic dendritic cells. Diabetes 2013; 62: 3132.

71. Yang L, Liao Y-T, Yang X-F, Reng L-W, Qi H, Li F-R. Immune protective effect of human alpha-1-antitrypsin gene during β cell transplantation in diabetic mice. Immunol Res 2015; 62: 71.

72. Knip M, Siljander H. The role of the intestinal microbiota in type I diabetes mellitus. Nat Rev Endocrinol 2016; 12: 154.

73. Davis-Richardson AG, Ardisone AN, Dias R, et al. Bacteroides dorei dominates gut microbiome prior to autoimmunity in Finnish children at high risk for type 1 diabetes. Front Microbiol 2014; 5: 678.

74. de Goffau MC, Luopajärvi K, Knip M, et al. Fecal microbiota composition differs between children with β-cell autoimmunity and those without. Diabetes 2013; 62: 1238.

75. Weder HM. Bacteroides: the good, the bad, and the Nitty-Gritty. Clin Microbiol Rev 2007; 20: 593.

76. Ren Z, Jiang J, Lu H, et al. Intestinal microbial variation may predict early acute rejection after liver transplantation in rats. Transplantation 2014; 98: 844.

77. Corbitt N, Kimura S, Ise K, et al. Gut bacteria drive kuppfer cell expansion via MAMP-mediated ICAM-1 induction on sinusoidal endothelium and influence preservation-reperfusion injury after orthotopic liver transplantation. Am J Pathol 2013; 182: 180.

78. Vossen JM, Guiot HFL, Lankester AC, et al. Complete suppression of the gut microbiome prevents acute graft-versus-host disease following allogeneic bone marrow transplantation. PLoS One 2014; 9: e105706.

79. Vaara O. Is the origin of type 1 diabetes in the gut? Immunol Cell Biol 2012; 90: 271.

80. Watts T, Berti I, Saponi A, et al. Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats. Proc Natl Acad Sci U S A 2016.

81. Liu SQ, Zhao JP, Fan XX, et al. Rapamycin, a specific inhibitor of the target of rapamycin complex I, disrupts intestinal barrier integrity in broiler chicks. J Anim Physiol Anim Nutr 2016; 100: 323.

82. Gabe SM, Bjarnason I, Tolou-Ghamari Z, et al. The effect of tacrolimus (FK506) on intestinal barrier function and cellular energy production in humans. Gastroenterology 1994; 115: 67.

83. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994; 12: 991.

84. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 2008; 455: 1109.
85. Peng J, Narasimhan S, Marchesi JR, Benson A, Wong FS, Wen L. Long term effect of gut microbiota transfer on diabetes development. J Autoimmun 2014; 53: 85.
86. Giovannoni L, Muller YD, Lacotte S, et al. Enhancement of islet engraftment and achievement of long-term islet allograft survival by Toll-like receptor 4 blockade. Transplantation 2015; 99: 29.
87. Vivot K, Langlois A, Bietiger W, et al. Pro-inflammatory and pro-oxidant status of pancreatic islet in vitro is controlled by TLR-4 and HO-1 pathways. PLoS One 2014; 9: e107656.
88. Dong H, Zhang Y, Song L, et al. Cell-permeable peptide blocks TLR4 signaling and improves islet allograft survival. Cell Transplant 2016; 25: 1319.
89. Shapiro AMJ, Gallant HL, Hao EG, et al. The portal immunosuppressive storm: relevance to islet transplantation? Ther Drug Monit 2005; 27: 35.
90. Johnson JD, Ao Z, Ao P, et al. Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. Cell Transplant 2009; 18: 833.
91. Smink AM, Hertsig DT, Schwab L, et al. A Retrievable, efficacious polymeric scaffold for subcutaneous transplantation of rat pancreatic islets. Ann Surg DOI: 10.1097/SLA.0000000000001919 [Epub ahead of print].
92. Ramnath RD, Maillard E, Jones K, et al. In vitro assessment of human islet vulnerability to instant blood-mediated inflammatory reaction (IBMIR) and its use to demonstrate a beneficial effect of tissue culture. Cell Transplant 2015; 24: 2505.