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REVIEW

Intraportal islet transplantation: the impact of the liver microenvironment

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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.

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Key words
gut–liver axis, instant blood-mediated inflammatory reaction, ischemia/reperfusion, islet transplantation, liver microenvironment

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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.

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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, liver dendritic cell, hepatocyte, and gut–liver axis, together with islet transplantation. Crossed references were also selected. Only studies written in English and published in peer-reviewed journals were considered. Studies were included according to their ability to provide insight into the link between islet transplantation and the selected areas of interest, and were grouped by chapters in the text below.

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, XIa-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], C5a 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

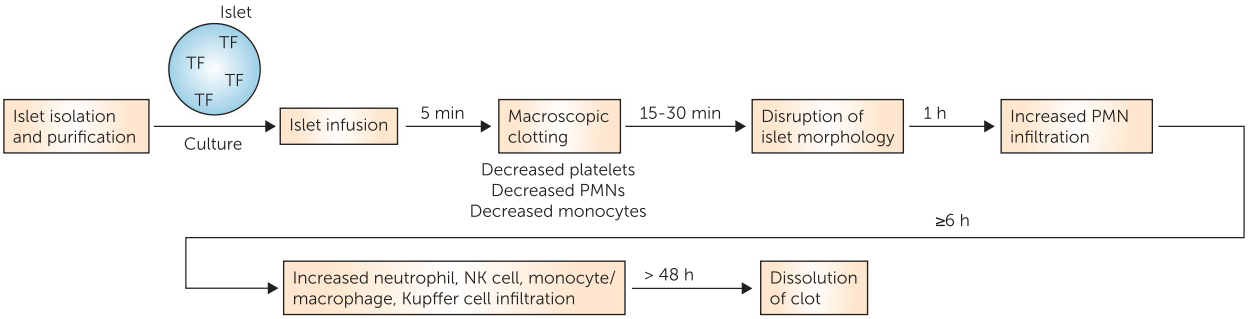

Figure 1 Instant blood-mediated inflammatory reaction (IBMIR) time line as summarized from references [8–12]. TF, tissue factor; PMN, polymorphonuclear leukocytes; MPO, myeloperoxidase; NK, natural killer; min, minutes.

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].

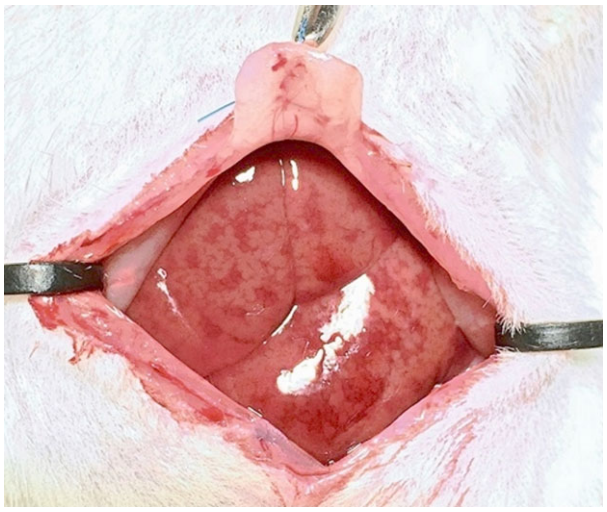

Figure 2 Patches of liver ischemia after intraportal islet transplantation in *Rattus norvegicus*.

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],

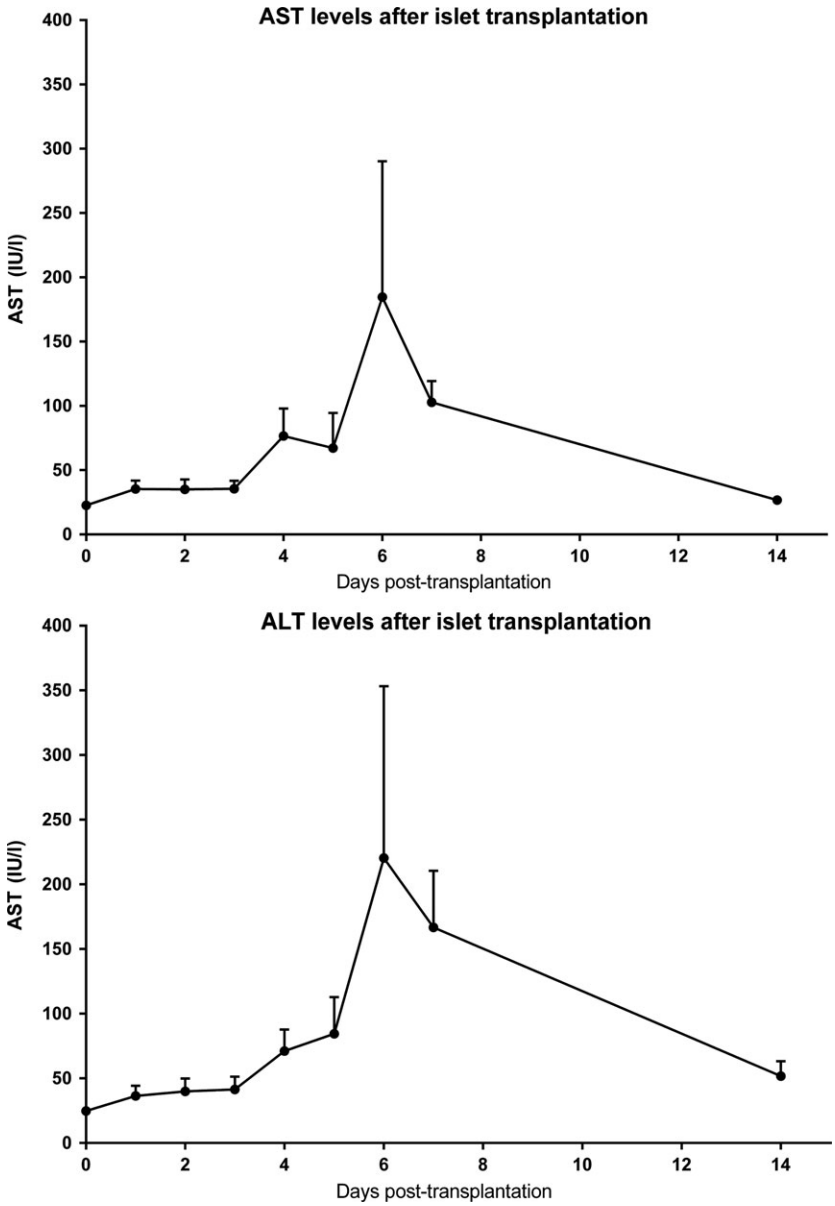

Figure 3 Human aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels after first intraportal islet transplantation, Geneva results. We can observe a peak in AST and ALT levels between days 5–7 after islet transplantation.

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 intraportally transplanted islets has only rarely been studied thus far. The available studies have all been conducted on animal models.

Kupffer cells

Kupffer cells phagocyte harmful components passing through the liver. In the setting of intraportal islet transplantation, Kupffer cells are activated through the complement pathway, due to the hepatic ischemia/reperfusion lesions surrounding transplanted islets [47];

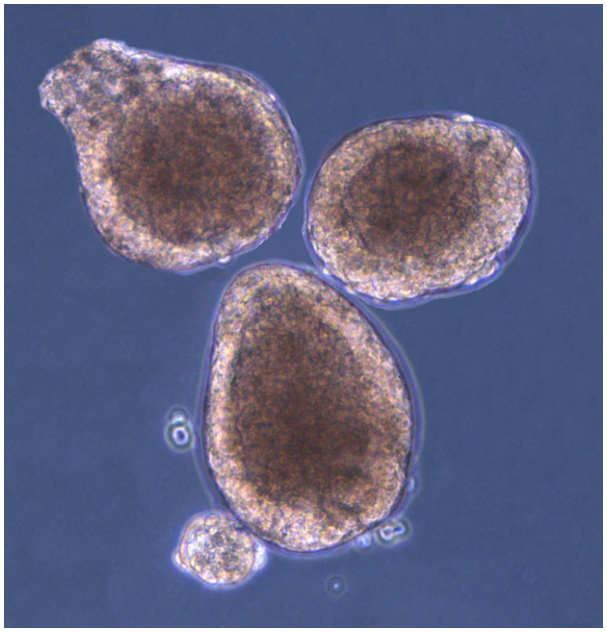

Figure 4 *Rattus norvegicus* islets at day 5 of culture. The cultured islets have a dark, hypoxic, core.

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-α, IL-1β, 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 $Gr\text{-}1^+CD11b^+$ neutrophils, resulting in the production of IFNγ and IL-1β [62,63]. Some investigators have shown improved intraportal islet survival after adenosine administration, thus inactivating the NKT cell-mediated IFNγ 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

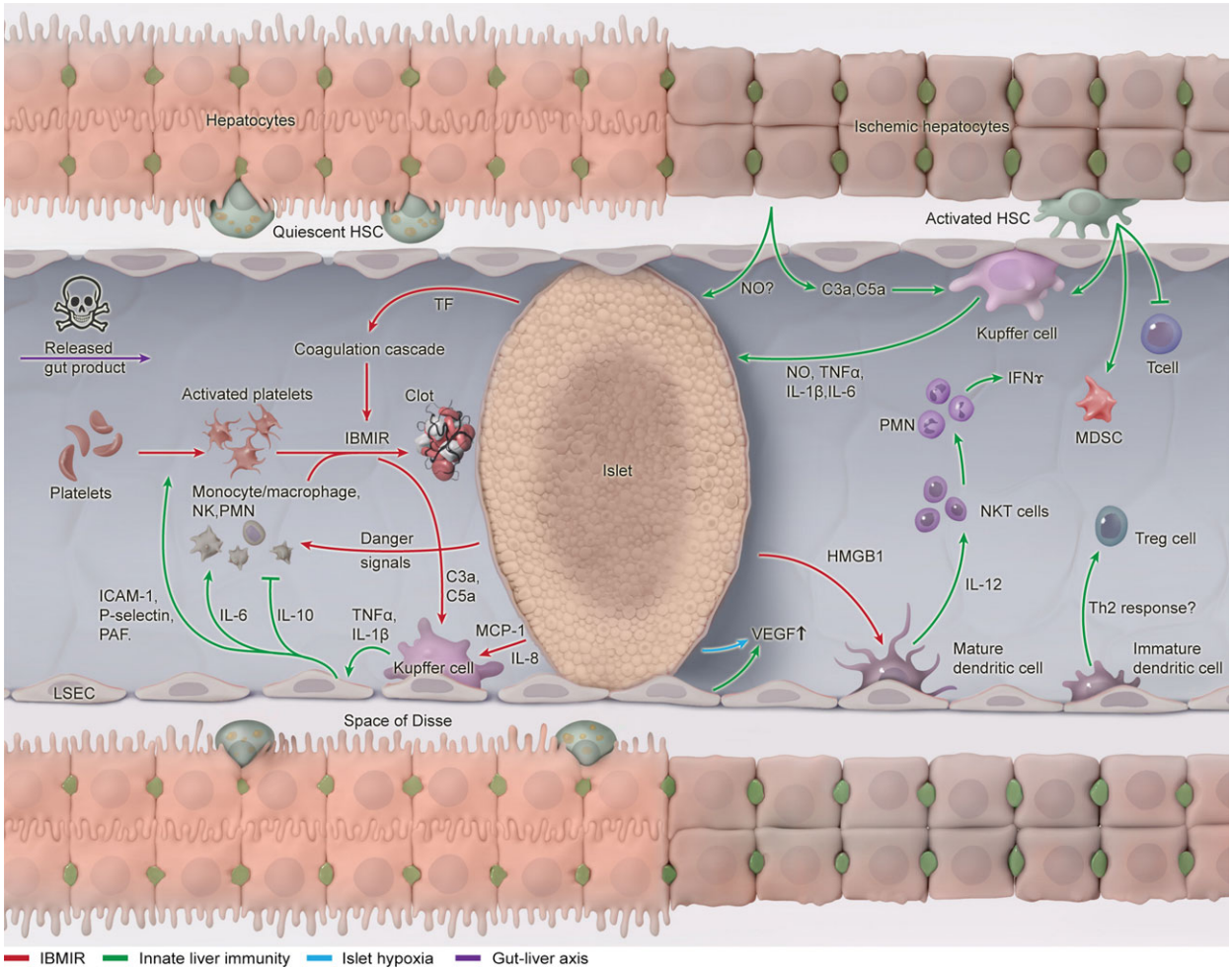

Figure 5 A simplified representation of the main events occurring after intraportal islet transplantation. HSC, hepatic stellate cell; LSEC, liver sinusoidal endothelial cell; MDSC, myeloid-derived suppressor cell; PMN, polymorphonuclear cell; NK, natural killer; NKT, natural killer T cell; TF, tissue factor; NO, nitric oxide; VEGF, vascular endothelial growth factor; IBMIR, instant blood-mediated inflammatory response; ICAM-1, intercellular adhesion molecule 1; PAF, platelet-activating factor; HMGB1, high-mobility group box 1; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; C3a, C5a, complement-induced anaphylatoxins; IFNγ, interferon gamma; *Danger signals*: MIF (migration inhibitory factor), TNFα (tumor necrosis factor α), IP10 (IFNγ-inducing protein 10), NFκB (nuclear factor-kappa B), *HMGB1*, *MCP-1*, *IL-1*β, *IL-6*, *IL-8*, *IFN*γ, *NO*.

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.

Table 1. Current clinical practice influencing the liver microenvironment.

Current clinical practice
• Heparin supplementation of culture medium [19]
• Nicotinamide supplementation of culture medium [22]
• Aggressive treatment by intravenous heparin [28]
• Twenty-four-hour culture of islets before transplantation, decreases tissue factor expression [92]
• Systematic antibiotic prophylaxis following islet transplantation; this could have an impact on circulating pathogen-associated molecular patterns
• Most current immunosuppressive molecules are based on controlling lymphocyte-mediated immunity, this also acts on resident liver lymphocytes
• The use of drugs such as anakinra and etanercept to directly target pro-inflammatory cytokines
• The use of pentoxifylline, which has antioxidant, anti-inflammatory, and antithrombotic properties

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 *Bacteroides* spp., and a decrease in *Bifidobacterium* spp. and butyrate-producing bacteria [73,74]. The *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 *Bacteroidetes* associated with a decrease in *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

Table 2. A summary of selected preclinical knowledge and further steps that can be taken to study the impact of the liver microenvironment in islet transplantation.

The liver micro-environment	Current pre-clinical knowledge	Further steps
Instant blood-mediated inflammatory reaction (IBMIR)	Low-molecular weight dextran sulfate [19,20]	Clinical trial completed, results pending*
	C5a inhibitors [24,25]	Clinical trial under way with eculizumab*
	PEGylation coating of islets [26]	Clinical trials under way, not in the intraportal setting*
	Endothelial cell coating of islets [27]	Needs intraportal validation in immune competent animals
Ischemia/reperfusion injury	Ischemia preconditioning of the liver [30]	Needs further validation
Islet hypoxia	In vitro and in vivo ischemia preconditioning [45,46]	Needs to be further assessed and validated for pancreatic islet viability in vivo
	Hyperoxic housing of animals [41]	Clinical trials could be carried out with patient oxygen therapy +/– maintaining normal hemoglobin levels
	Hyperbaric oxygen therapy of animals [42]	Clinical trials could be carried out in selected patients
	Intraperitoneal oxygenation [43]	Needs validation in animals before considering clinical trials, because of the invasiveness of the method
	Increasing VEGF expression [44]	Needs an efficient method that durably increases VEGF expression
Endogenous liver immune system	Aspecific macrophage depletion improves islet graft survival [51]	Needs specific Kupffer cell study
	Hepatic stellate cell: only sub-capsular kidney transplantation data [56,58]	Needs intraportal validation in animal models
	Dendritic cells: only sub-capsular kidney transplantation data [67–70]	Needs intraportal validation in animal models
The gut–liver axis	Gut microbiota : no data in islet transplantation	Fecal transplantation could be interesting to study in animal models
	Gut barrier : no data in islet transplantation	Ischemia preconditioning could decrease gut permeability during islet transplantation; Zonulin inhibition could be tested in islet transplanted animals
	Released gut product : the TLR4 pathway is implicated in islet loss [86–88]	Needs TLR4 studies conducted in intraportal models, and TLR4 blacking molecules need to be developed far humans

*According to www.clinicaltrials.gov.

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.

Authorship

VD: wrote the paper. TB, SL, CT: critical revision and editing of the paper.

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Conflicts of interest

The authors have declared no conflicts of interest.

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