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## Islet Transplantation in Bone Marrow

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#### Abstract

*Islet transplantation can be an important therapeutic option for adults with unstable type 1 diabetes-individuals who, despite their best efforts, have wide, unpredictable fluctuations in blood sugar levels or who are no longer able to sense that their blood sugar is dangerously low. For these people, transplantation may be a viable solution. Since the first report of successful pancreatic islet transplantation to reverse hyperglycaemia in diabetic rodents, there has been great interest in determining the optimal site for implantation. Although the portal vein remains the most frequently used site clinically, it is not ideal. About half of the islets introduced into the liver die during or shortly after transplantation. Complications associated with intraportal islet injection and the progressive functional decline of intrahepatic islets encourage the exploration of alternative sites. In tests in animals, scientists from the Diabetes Research Institute in Milan showed that bone marrow is a promising alternative site for islet transplantation. This review considers bone marrow as site of islet transplantation and metabolic, immunological and technical aspects are discussed.*

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#### Introduction

Despite the substantial improvements in insulin therapy thanks to new commercial drugs and the adoption of intensive treatment regimens able to improve glycemic control, exogenous insulin administration cannot avoid the long-term complications of diabetes and the life expectancy of diabetic patients is still shorter compared to that of the general population<sup>1,2</sup> In principle, the treatment for type-1 diabetes and many cases of type-2 diabetes lies in the possibility of finding a beta cell mass substitute capable of performing two essential functions: assessing blood sugar levels and secreting appropriate levels of insulin in the vascular bed. Currently, the only available clinical therapy capable of restoring beta cell mass in diabetic patients is the allogenic/autologous transplantation of beta cells (somatic cell therapy with pancreas, Langerhans islets or beta cell transplantation).

Replacement of the whole gland re-establishes long-term normoglycemia, with a success rate of 80%<sup>3</sup>, and is especially successful for patients who undergo simultaneous pancreas and kidney transplantation. However, because of the risk of surgical complications, this procedure will never be a viable option for most type 1 diabetic patients. Those offered this treatment are patients who have already developed many of the secondary complications, including end-stage renal failure, and still have a quality of life that is adequate for undergoing such a difficult treatment. Since the breakthrough made by Shapiro *et al.*<sup>4</sup> islet transplantation has emerged as an attractive alternative to whole pancreas transplantation. Despite advances in recent years<sup>5</sup> allogenic somatic therapy is still problematic.

A non-specific immune response mediated predominantly by innate inflammatory processes related to mechanics and site, and pre-existing and transplant-induced auto- and allo-specific cellular immune responses (possibly promoted by the initial inflammation) play a major role in the loss of islets and islet function from the liver. Although significantly improved by the implementation of the Edmonton protocol, our capacity of achieving long-lasting insulin independence in patients with T1D undergoing portal vein islet transplantation remains limited.<sup>6,7</sup> This indicates that the detrimental impact of innate and adaptive immune responses is not fully contained by the Edmonton protocol-associated regimen of generalized immunosuppression (i.e. induction with daclizumab [anti-IL-2R $\alpha$  mAb] and maintenance with rapamycin [mTOR activation blocker] plus tacrolimus [calcineurin inhibitor] in a steroid-free treatment).

Prolong intrahepatic islet survival by increasing the potency of such regimen is not practicable, due to the likelihood of enhancing susceptibility to cancer and infections, and the toxicity that some of these drugs may have towards kidney functions and transplanted islets. Rather, it is intuitive that alternative strategies aimed at selectively inhibiting undesired islet-specific or non specific immune responses represent an ideal step towards a better management (i.e. weaning/withdrawal of generalized immune suppression) and outcome (i.e. long-lasting insulin independence) of islet transplanted T1D patients.

The liver was suggested as an optimal site for islet transplantation by Lacy *et al.*, using a rat model of diabetes.<sup>8</sup> By the 1980s, successful transplantation of islet autografts was reported in humans by using infusion of cells into the patient's liver through the portal venous circulation.<sup>9-11</sup> Subsequently, the publication of the first case of insulin independence in a diabetic patient after infusion of islets through the portal vein consecrated the liver as the site of choice for the islet transplantation in humans.<sup>12</sup> Because of this early success, the

subsequent clinical experience of islet transplantation has been developed almost exclusively using the intra-hepatic infusion through the portal vein. In the last years, however, it has becoming increasingly recognized that the liver may not be the optimal environment as a recipient site for pancreatic islets, owing not only to immunologic<sup>13,14</sup> but also anatomic<sup>15,16</sup> and physiologic factors that likely contribute to the decline of islet mass after implantation.<sup>17-19</sup> Intrahepatic islet infusion in man is associated with an immediate blood-mediated inflammatory reaction, thrombosis and hepatic tissue ischemia with elevated blood liver enzymes.<sup>20-30</sup> Loss of as many as 50-75% of islets during engraftment in the liver has been suggested to be a prime factor necessitating the very large number of islets needed to achieve normoglycemia. Furthermore, the necessity for cannulation of the portal system to seed the islets produces an increase in portal pressure proportional to the islet mass administered by infusion<sup>31</sup> thus restricting the total mass that can be implanted. As a consequence, a highly purified suspension of islets is needed to transplant sufficient cells to achieve insulin independence. Because the purity of the suspension is inversely proportional to the islet yield per donor<sup>32</sup>, fewer islets can be isolated from the already scarce donor pool, further limiting broad clinical applicability of pancreatic islet transplantation. The recognition of these problems has renewed the interest in the search for an alternative site for implantation such as the intramuscular site and the omental pouch.<sup>33</sup>

Bone marrow (BM) may represent an ideal alternative site for pancreatic islet transplantation, thanks to its protected and extravascular (but well-vascularized) microenvironment.<sup>34-42</sup> Because of its broad distribution and easy access, BM has the potential to overcome not only the physiologic loss of islets, but also the technical limitations and complications encountered with the intraportal infusion.<sup>31</sup> To address the potential of BM as an alternative site for pancreatic islet transplanta-

tion, we recently implanted syngeneic pancreatic islet isografts (C57BL/6 islets to C57BL/6 mice) into BM of diabetic recipients and assessed short- and long-term graft survival, function and safety in comparison with the liver site.<sup>43</sup> The results show that the BM is a more suitable site than the liver for the implantation of islets. In our study, both the percentage and the timing in reversal of hyperglycemia were superior after BM infusion as compared to intrahepatic infusion using the minimal mass model. Moreover, with the exception of a small delay in gaining normal glucose tolerance after OGTT, the quality of glucose metabolism in mice that reached normoglycemia via intra-BM islet infusion was similar to that achieved by islet transplant into the liver for all the parameters evaluated (fasting and not fasting glycemia, blood insulin, HOMA-B and glucose tolerance after IVGTT). Based on our results we can conclude that the BM site for islet transplantation has a higher probability to reach euglycemia (2.5 fold increase) than the liver without compromising the quality of glucose metabolism. This is relevant because the process of intra-hepatic infusion was traditionally considered optimal due to the supposition that insulin is delivered more physiologically after intraportal transplantation.<sup>44-47</sup> However this argument has recently been challenged by experimental studies showing that intra-portal transplanted islets respond to glucose stimulation only when perfused via the hepatic artery; no response is observed after challenge via the portal vein.<sup>48</sup> There are also reports on alterations in islet function after intra-portal islet transplantation, such as a defective glucagon response to hypoglycemia<sup>19, 49, 50</sup> and defective glucose-stimulated insulin release.<sup>51</sup>

Since it was suggested that hyperinsulinemia might contribute to cancer development through the growth-promoting effect of elevated levels of insulin, it is possible that intra-BM islet transplantation could increase the risk of proliferative disease. For this reason we evaluated the impact of islets on hematopoietic

activity of BM. After islet infusion the cellularity, the histological appearance, the analysis of cell subpopulation and the progenitor cell frequency were unaffected by the presence of islets in the BM. We also took into consideration the consequences of BM islet infusion on the capacity to respond to virus-induced aplasia and the bone structure. Islets in BM of LCMV infected mice did not affect hematopoietic activity consequent to aplasia nor CTL-mediated viral clearance. These results also suggest that islets in BM are capable of sustaining those metabolic changes that are likely to occur during the rapid expansion of a very robust adaptive immune response (i.e. by day 8 post-infection secondary lymphoid organs of LCMV-infected mice are much larger in size and about 50%-70% of all CD8<sup>+</sup> T cells are LCMV-specific.<sup>52</sup>

In conclusion, we show that pancreatic islets can be engrafted into the BM, thus opening a research line with potentially significant clinical impact not only for the treatment of diabetes but for other diseases amenable to treatment with cellular transplantation. Because the BM as a site for pancreatic islet grafts can be clinically applicable and, in theory, can solve many of the problems encountered with the intrahepatic location, further research is warranted by the initial findings presented here to determine whether the results can be reproduced in large animals and eventually in humans.

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