Islets 5:2, 79–86; March/April 2013; © 2013 Landes Bioscience

Murine animal models for preclinical islet transplantation

No model fits all (research purposes)

Elisa Cantarelli,[1,*] Antonio Citro,[1,2] Simona Marzorati,[1] Raffaella Melzi,[1] Marina Scavini[1] and Lorenzo Piemonti[1]

[1]San Raffaele Diabetes Research Institute (OSR-DRI); San Raffaele Scientific Institute; Milan, Italy; [2]Department of Surgery; University of Pavia; Pavia, Italy

Keywords: islet transplantation, preclinical model, type 1 diabetes, hyperglycemia, equivalent islet, intra-hepatic islet infusion

Abbreviations: T1D, type 1 diabetes; IBMIR, immediate blood-mediated inflammatory reaction; STZ, streptozotocin; ROS, reactive oxygen species; MLD-STZ, multiple low doses of streptozotocin; NOD, non-obese diabetic; RIP, rat insulin promoter; GP, glycoprotein; NP, nucleoprotein; LCMV, Lymphocytic ChorioMeningitis Virus; HA, hemoagglutinin; IEQ, Islet Equivalent; minor H Ag, minor histocompatibility antigens; minor HY Ag, male-specific Y chromosome

Advances in islet transplantation research have led to remarkable improvements in the outcome in humans with type 1 diabetes. However, pitfalls, mainly linked both to early liver-specific inflammatory events and to pre-existing and transplant-induced auto- and allo-specific adaptive immune responses, still remain. In this scenario, research into pancreatic islet transplantation, essential to investigate new strategies to overcome open issues, needs very well-designed preclinical studies to obtain consistent and reliable results and select only promising strategies that may be translated into the clinical practice. This review discusses the main shortcomings of the mouse models currently used in islet transplantation research, outlining the main factors and variables to take into account for the design of new preclinical studies. Since several parameters concerning both the graft (i.e., islets) and the recipient (i.e., diabetic mice) may influence transplant outcome, we recommend considering several critical points in designing future bench-to-bedside islet transplantation research.

Introduction

Type 1 diabetes (T1D) is an autoimmune disease mediated by a combination of genetic and environmental triggers resulting in the lymphocytic infiltration of pancreatic islets with selective and progressive loss of the insulin-producing β cells. Even if over the years the autoimmune events have been well elucidated—at least in the mouse model—the etiology and pathogenesis of T1D involve poorly understood genetic risk variants and still (almost) unidentified environmental factors.[1]

Since the '90s, pancreatic islet transplantation, by replacing β cell function, is the only cure for patients with T1D. Islet transplantation is a relatively easy surgical procedure, consisting in the infusion of islets into the liver after percutaneous transhepatic catheterization of the portal vein. The primary outcomes of the clinical trials include rate and severity of hypoglycaemic events, improvement of HbA1c levels and stabilization or regression of secondary chronic complications of diabetes. Advances in islet transplantation research have led to remarkable improvement in the outcome of islet transplantation as documented by the International Islet Transplant Registry (http://www.citregistry.org): considering the 2007–2010 period, the reported insulin independence rates were 66% at 1 y, 55% at 2 y and 44% at 3 y.[2]

As reported by many studies an estimated 60–80% of the transplanted islet mass is lost within hours to days after intrahepatic islet infusion, mainly because of an immediate blood-mediated inflammatory reaction (IBMIR), thrombosis and hepatic tissue ischemia with release of liver enzymes.[3,4] Evidence of the inflammatory and innate immune reactions influencing islet engraftment and survival mainly comes from quantitative positron emission tomography imaging of labeled islets in humans[3,4] and non-invasive magnetic resonance imaging studies in mouse models.[5] Moreover, both pre-existing and transplant-induced auto- and allo-specific adaptive immune responses play a major role in islet loss.[6]

Recognizing these problems has increased the efforts in the search for (1) anti-inflammatory strategies to control and/or modulate the early innate immune response and favor islet survival and engraftment, (2) immunosuppressive or tolerogenic therapies to prevent and/or inhibit auto- and allo-specific adaptive immune responses, (3) alternative sites for islet implantation to avoid liver-specific hurdles and (4) alternative sources of insulin-producing cells to overcome both cadaveric donor shortage and variability of islet isolation. Many mouse models of diabetes are available to investigators, including the spontaneous (i.e., both immune- and not immune-mediated), chemically-induced (i.e., not immune-mediated) and transgenic or knockout derived (i.e., both immune- and not immune-mediated) model.

In this review, we will focus on the main methodological aspects of islet transplantation research in mouse models of

*Correspondence to: Elisa Cantarelli; Email: cantarelli.elisa@hsr.it
Submitted: 01/18/13; Revised: 04/11/13; Accepted: 04/13/13
http://dx.doi.org/10.4161/isl.24698

Table 1. Practical guidelines for preclinical islet transplantation studies

KEY POINTS			LITERATURE SEARCH ON PUBMED (from June 1, 2008 to June 1, 2012)
GRAFT	Islet Equivalent normalization	yes	23%
		no	77%
RECIPIENT	Diabetes mouse models	chemically-induced	88%
		spontaneous	9%
		others/no info	3%
	Recipients pre-transplant glycemia	> 200 mg/dl	2%
		> 250 mg/dl	6%
		> 300 mg/dl	23%
		> 350 mg/dl	20%
		> 400 mg/dl	8%
		> 450 mg/dl	4%
		> 500 mg/dl	2%
		no info	35%
	Site for islet infusion	Liver	23%
		Kidney capsule	70%
		Other alternative sites	7%

Critical points that we firmly recommend to consider are spotlighted in red. A literature search yielded 153 studies in which islet transplantation was performed in T1D mouse models published between June 1, 2008, and May 31, 2012.

diabetes. It is crucial to appreciate whether and how transplantation outcomes are affected by the graft (i.e., islets) or the recipient (i.e., diabetic mice) selected for a given experiment. Translational research, including islet transplantation, requires controlled preclinical studies of good quality to generate reliable results before starting clinical trials in humans.

Diabetes Mouse Models

A critical component of islet transplantation research are the many mouse models of diabetes, including chemically-induced, spontaneous and transgenic or knockout.

Chemically-induced diabetes mouse models. For decades alloxan or streptozotocin (STZ) have been used to induce diabetes in transplant recipient animals.[7] A literature search covering the last four years (June 2008–May 2012) documents that 88% of the mouse studies in islet transplantation have used chemically-induced diabetes recipients (**Table 1**).

Alloxan and STZ are glucose analogs that are internalized into the β cells via GLUT2 and induce necrosis and insulin deficiency.[7,8] Their cytotoxicity is achieved through different intracellular pathways: induction of reactive oxygen species (ROS) for alloxan and DNA alkylation for STZ.[8] Different doses and administration routes are used according to the rodent strains and gender. A mouse strain-dependent hierarchical response to STZ exists: DBA/2 > C57BL/6 > MRL/MP > 129/SvEv > BALB/c. Moreover, in all these mouse strains, males have a more robust STZ-induced hyperglycemia than females, possibly because estradiol protects β cells from oxidative stress-induced apoptosis.[9,10]

Methods to induce STZ-diabetes can be categorized as (1) single high dose of STZ or (2) multiple low doses of STZ (MLD-STZ) over a period of several consecutive days. A single high dose of STZ is used to induce severe diabetes. In our experience, nearly 100% of C57BL/6 mice develop severe hyperglycemia (i.e., non-fasting glycemia > 450 mg/dl) at 1–3 d after STZ injection. In this model the chemical direct toxicity of STZ is responsible for β cell death. MLD-STZ is used to induce mild diabetes. In our experience, 50% of C57BL/6 mice develop mild hyperglycemia (i.e., non-fasting glycemia > 300 mg/dl) at 9–10 d after the first STZ injection. In this model β cell apoptosis (occurring 4–5 d after the first injection) and immune-mediated damage (leucocytes infiltration of islets at 10–12 d after the first STZ injection) are both responsible for β cell death.[10,11] Supporting the role of the immune system in the development of diabetes after MLD-STZ, Ihm et al. have described that macrophages presents β cell auto-antigen to T-helper cells leading to the development of β cell-specific cytotoxic cells.[11,12] Moreover, splenocytes transfer from MLD-STZ mice to recipient mice pre-treated with a single sub-diabetogenic dose of STZ resulted in increased lymphocytes rolling and endothelial adhesion toward islets, demonstrating that splenic lymphocytes contain immunologically activated islet-specific T cells.[13-15] Strain variability in STZ-induced hyperglycemia was attributed to differences in the immune response: C57BL/6 and BALB/c mice are Th1- and Th2-prone, respectively.[9] Islets of MLD-STZ C57BL/6 mouse produce IFN-γ and TNF-α (i.e., Th1-type cytokine) and have reduced IL-4 and IL-10 levels (i.e., Th2-type cytokines), while opposite findings were reported for the BALB/c mouse. Apparently, reduction and upregulation of Th2-type cytokines are more strongly associated,

with susceptibility and resistance to MLD-STZ induced diabetes than upregulation of Th1-type cytokine levels.[16]

An often underestimated critical point in chemically-induced diabetes mouse models is that both alloxan and STZ produce toxic side effects on multiple organs including liver, kidney, bone marrow and spleen. For example, the immunosuppressive effects of STZ-induced diabetes may result in absolute lymphopenia and a relative increase of T regulatory cells, introducing confounding variables in the model.[10,17] Toxic side effects sometimes complicate the interpretations of results and should be taken in consideration when designing the study and analyzing the results.

Spontaneous autoimmune mouse model. In islet transplantation research the non-obese diabetic (NOD) mouse model is used less frequently than the chemically-induced diabetes mouse model. This model mimics many features of human T1D, involving both genetic (i.e., 50–100 genes in total) and environmental factors that ultimately lead to an histological pictures of islet similar to that described in human T1D at onset. NOD mice have relatively mild defects of immune tolerance (when compared with FoxP3 or AIRE mutant mice), suggesting both the polygenic nature of T1D and the small influence of more than one single locus. T1D develops because of an immune response involving β cell-specific T cells that leads to autoimmunity. Islets mononuclear cell infiltration (insulitis), involving $CD4^+$ and $CD8^+$ T cells, macrophages, NK cells and B cells, is detected at 4–6 weeks of age in both male and female mice. β cell killing in the islets is asynchronous, with different islets destroyed over time until enough destruction has occurred for hyperglycemia to develop.[11] Islet insulitis leads to spontaneous T1D onset at 12–16 weeks of age, more frequently among females (70–90%) than males (10–40%). However, the finding that the lower incidence of spontaneous T1D in males occurs despite of similar levels of early insulitis than in females suggests that the intra-pancreas autoimmune process includes (1) an insulitis that is completely penetrant, well-tolerated for a long period of time and does not completely destroy target tissue and (2) an overt T1D that is not completely penetrant.[12] Furthermore, T1D incidence is highly variable among different NOD colonies, mainly because of microbial environment. Thus, NOD colonies must be maintained in pathogen-free conditions to obtain a high and consistent incidence of T1D.[18] Actually NOD mouse recipients are used to fully mimic late-stage T1D patients undergoing islet transplantation, with a memory β cell-specific immune response re-activated after graft infusion. However, both the high variability in T1D incidence and the different timing of T1D onset among NOD colonies has limited the number of islet transplantation studies that have used NOD mice as transplant recipients. A literature search covering the last four years (June 2008–May 2012) documents that only 9% of studies in islet transplantation have used the spontaneous T1D mouse model as recipient (**Table 1**).

Transgenic or knockout diabetes mouse models. Transgenic and gene targeting technologies have produced many animal models of diabetes with reduced genetic and pathogenic complexity compared with the chemically-induced and spontaneous diabetes models.

In Ins2 Akita mouse,[19] pancreatic islets fail to normally develop because of a miss-sense mutation in the coding sequence of the insulin molecule. The aminoacid substitution leads to a defective folding of the pro-insulin chain that triggers a massive compensatory "quality control" mechanism chaperonine-mediated in the endoplasmic reticulum. The disruption of the normal processing of the regulated secretory pathway results in lower levels of mature insulin (either Ins2 or Ins1) and, hence, early development of hyperglycemia at 4 weeks of age. Histological analysis of islets shows the presence of atrophic β cells largely devoid of secretory granules, similarly to the chemically-induced MLD-STZ diabetes mouse model, although without intra-islet insulitis or inflammatory infiltrate. After the onset of diabetes, the heterozygous mutant mice may survive relatively long periods without insulin treatment thanks to the secretion of subnormal levels of fully processed Ins1 that escapes intracellular degradation. Because of the stable hyperglycemia this model may be useful for β cell replacement studies.

Islet transplantation studies have taken advantage of the introduction in β cells of neo antigens by transgenesis under the control of rat insulin promoter (RIP) to establish neo (self) antigen expression that will be the target of immunity. These transgenic mouse models combined with T-cell receptor transgenic provides an elegant system to evaluate and measure (self) antigen-specific T-cell response. Many viral antigens have been inserted under RIP control: glycoprotein (GP) or nucleoprotein (NP) of Lymphocytic ChorioMeningitis Virus (LCMV)[20,21] and hemoagglutinin (HA) of Influenza Virus.[22] These transgenic mouse models are called pathogen-induced models and diabetes development is driven by single (self) antigen-specific T cells.[23] Expression of the neo (self) antigen per se does not lead to diabetes or islet dysfunction, while virus infection, by eliciting a neo (self) antigen-specific immune response, induces antigen-specific cross-response against β cells.[23-25] Disease develops after (auto)-reactive T cells are activated systemically and reach the pancreatic islets, where they have to be further driven by local antigen-presenting cells that uptake β cell antigens and provide costimulation causing β cell destruction and hyperglycemia. The introduction of these transgenic mouse models in islet transplantation research is quite recent. This model fully mimics patients with late-stage T1D undergoing islet transplantation, with no residual β cells mass (i.e., with hyperglycemia) in the presence of memory β cell-specific immune response. Moreover, the possibility to track an antigen-specific T-cell response provides the opportunity to identify factors involved in the immune attack and assess the efficacy of different immune suppression protocols.

Pancreatic Islet Transplantation: Critical Parameters

Well-designed preclinical studies are pivotal to obtain high quality data and to safely translate islet transplantation research into clinical care. Various transplant settings are available to investigators: syngeneic (i.e., islet cell transplantation from a genetically MHC-identical donor), allogeneic (i.e., islet cell transplantation from a genetically MHC-non identical donor of the same species) and xenogeneic (i.e., islet cell transplantation from one species to

another). In all these settings it is crucial to set the best conditions considering the specific aims of the experiment.

The graft: normalization of islet preparations. As already stated for human islet isolation in 1989 during the workshop on the 2nd Congress on International Pancreas and Islet Transplantation Association, an accurate and consistent method to quantify the amount of islets infused is of crucial relevance for transplantation research. Murine islets size is quite heterogeneous, with their diameter ranging from 50 to 350 μm.[24] In order to quantify transplanted islet mass, both number and size need to be taken into consideration.[25] Preclinical islet transplantation research may borrow from islet transplantation in humans the well-accepted "islet equivalent" concept. Islet equivalent (IEQ) is the standard estimate of isolated islet volume: an IEQ is defined as the mass of an islet with a diameter of 150 μm, assuming that islets are spherical. Although recently reconsidered taking into account the criticism that islets are not perfectly rounded,[26] IEQ remains the most used method to normalize islet preparations. A literature search covering the past four years (June 2008–May 2012) documents that only 23% of the studies in murine islet transplantation have used the "islet equivalent" normalization (**Table 1**), with over 75% of the studies non reporting any normalization of the transplanted islet mass.

From a practical point of view the number and size distribution of the isolated islets in a preparation are estimated in a small aliquot. The absolute islet number should be counted by optical microscope and both maximum and minimum islet diameters should be measured using an image software. The isolation index is calculated by dividing the absolute islet number in the preparation by the volume of an IEQ with a diameter of 150 μm. The use of the "islet equivalent" concept in preclinical experiments makes islet mass homogeneous between different experiment, and this is crucial for comparing experiments. Moreover, the use of the equivalent islet normalization defines two different models of islet transplantation: (1) the marginal islet mass model and (2) the full islet mass model.

The marginal mass model is used to evaluate islet survival and/or engraftment in syngeneic transplantations. In this model implantation of a marginal mass of islets achieves of normoglycemia in less than 100% of recipients and/or reversal of hyperglycemia is delayed. For the intrahepatic site, in our experience, the marginal mass of islets ranges from 100 to 250 IEQ. Manipulations resulting in a higher percentage of successful grafts and/or shortening of the time lag to achieve normoglycemia are interpreted as favoring islet survival and/or engraftment. This is the best model in preclinical islet research to discriminate and select promising strategies to improve islet engraftment (i.e., anti-inflammatory drugs, alternative site for islet infusion).

The full mass model is used to evaluate islet survival in allogeneic transplantations. In this model islet infusion (in our experience for intrahepatic site more than 300 IEQ) achieves normoglycemia in a high percentage of recipient mice (nearly 100%). This model allows to clearly define the time to rejection, as loss of graft function (i.e., non-fasting glycemia > 300 mg/dl). This is the best model in preclinical islet research to discriminate and select promising strategies to modulate auto- or allo-immune responses (i.e., immunosuppressive drugs, tolerance induction strategies). The full mass model can also be used in syngeneic setting for histological, morphological or imaging studies.

The recipient: only severely diabetic mice. Hyperglycemia is the results of β cell destruction because of chemical, immune or biological mechanism according to the mouse model used. Severity of STZ-induced diabetes is critical for islet transplantation studies, especially those aiming to reproduce human transplantation conditions. Late-stage patients with T1D undergoing islet transplantation have no measurable C-peptide (i.e., an estimate of endogenous insulin secretion) and ideally also islet transplantation recipient mice should have no residual endogenous insulin secretion. Furthermore, in preclinical mouse models diabetes must be severe enough to confidently detect changes in islet graft function over time due to different experimental conditions and settings.

In islet transplantation preclinical research, there is no commonly accepted threshold to define diabetes in recipient mice. Very heterogeneous thresholds are used by different investigators and the majority of studies use recipient mice with only moderate diabetes, and, therefore, with a sizeable residual β cell mass. A literature search covering the past four years (June 2008–May 2012) yielded 153 studies in which islet transplantation was performed in the diabetes mouse models. The definition of diabetes was not reported in 35% of the articles, was reported as glucose levels > 200 mg/dl in 2%, > 250 mg/dl in 6%, > 300 mg/dl in 23%, > 350 mg/dl in 20%, > 400 mg/dl in 8%, > 450 mg/dl in 4% and > 500 mg/dl in 2% (**Table 1**).

Transplant recipient mice receive islet grafts few days after diabetes onset. To eliminate the confounding of residual endogenous insulin secretion and/or β cell recovery due to an incomplete β cell destruction, we suggest to define diabetes in an islet transplant recipient mouse as blood glucose levels > 450 mg/dl.[27] Recently, it has been shown that the degree of hyperglycemia of the recipient influences the engraftment of allogeneic islets (isolated from C57BL/6) transplanted under the kidney capsule of non-autoimmune (BALB/c) and autoimmune (NOD) diabetic mice. An increased incidence of primary non-function was observed when a marginal number of islets were transplanted into severely (i.e., glycemia between 351 and 550 mg/dl) in comparison with moderately (i.e., glycemia between 240 and 350 mg/dl) diabetic mice.[28] Furthermore, we have previously demonstrated that pre-transplant blood glucose levels inversely correlate with the time to loss of β cell function in allogeneic islets transplanted under the kidney capsule of STZ-induced diabetes mice.[27] The negative influence of hyperglycemia on islet engraftment and allograft function may simply be explained by the lower residual β cell mass in mice with higher blood glucose levels. Conversely, moderately diabetic mice require low number of islets in order to maintain normoglycemia and have an increased probability of achieving normoglycemia with their residual endogenous β cell mass. A second hypothesis may be the deleterious effects of high glucose levels on transplanted islets, which has been well-documented in many studies. Transplanted islets, because of the limited oxygen supply, experience both β cell mass loss[29] and impaired insulin secretion.[30,31] Hyperglycemia may worsen this

loss by increasing β cell function and, consequently, their exhaustion. In addition, chronic hyperglycemia has deleterious effects on insulin secretion, which may also contribute to poor graft performance. Finally, in this scenario we cannot exclude that pre-transplant blood glucose levels may influence the magnitude of the anti-graft immune reaction by inducing graft overfunction, which indeed enhances the immunogenicity of the transplanted islets.

The recipient: the liver as first choice for islet transplantation. Although many alternative sites have been used in mouse models, the liver is the gold standard in preclinical studies designed to collect preliminary data for islet transplantation in humans. It is worthwhile mentioning that intrahepatic islet infusion in the mouse model has some biological pitfalls also described in the human counterpart: thrombosis,[32,33] liver ischemia with elevated blood liver enzymes,[34] IBMIR[35] and innate immune cells recruitment.[36] However, a literature search covering the past four years (June 2008–May 2012) documents that only 23% of the published studies have used the liver as a site for islet transplant, whereas 70% have used the kidney capsule and 7% other alternative sites (i.e., bone marrow, pancreas, omentum, testis, subcutaneous, muscle, submandibular gland and epididimal fat pad) (**Table 1**).

The kidney capsule has been a preferred site for experimental islet transplants in mouse models because (1) the surgical procedure is relatively easy, minimally-invasive with low mortality rates, (2) hyperglycemia is reverted in few days by transplanting a small number of islet[37] and (3) allows graft retrieval by nephrectomy for both histological studies and proof of function of the islet graft. These technical advantages have led to the use of the kidney capsule also as a site for co-cellular transplant studies including islet co-transplanted with endothelial cells,[38] mesenchymal[39,40] and bone marrow stem cells,[41] neuronal stem cells,[42] and Sertoli cells.[43] However, the kidney capsule does not represent a suitable site for islet infusion in preclinical studies for several reasons. First, the kidney capsule is an extravascular site and the liver an intravascular site for islet transplantation. The differences both in surgical procedures and microenvironment truly have a significant impact on the early immune and non-immune events affecting graft survival, engraftment and function. Numerous evidences exist that islets in the renal subcapsular space are not exposed to the non-specific immune response mediated predominantly by innate and inflammatory events that mostly influence intra-liver islet engraftment. These complex biological phenomena triggered by the contact between islets and blood are liver-specific (i.e., specific of an intravascular site). Second, the site of islet infusion may influence the magnitude of the allo-specific cellular immune response. Differences between the two sites both in immunosuppressive drug levels and kinetics of allo-antigen specific adaptive immunity have been reported in the literature. Moreover, site-dependent cell activation of the innate immune system, triggered by tissue injury during islet isolation and ischemia reperfusion, may initiate and amplify the allo-specific adaptive immune response.[44] For these reasons, preclinical islet transplantation under the kidney capsule (i.e., extravascular site) does not represent a good model for proof-of-principle or translational studies, and, therefore, new immunosuppressive and/or anti-inflammatory strategies need to be tested in the intra-hepatic mouse model of islet transplantation before translation into the clinic. Peri-transplant treatments targeting the innate inflammatory immune events need to be tested in the intra-liver islet infusion mouse model because site-specific differences clearly exist in islet engraftment. On this basis the routine peri-transplant prescription of treatments to reduce the inflammatory response often lacks appropriate and robust supportive preclinical data. For instance the addition of drugs targeting TNF-α and IL-1β to the standard immunosuppressive regimen of T1D recipients receiving islet transplantation was based on experiments in a mouse model of islet transplantation performed under the kidney capsule.[45-47] On the contrary, a phase 2 pilot study to test safety and efficacy of the peri-transplant administration of CXCR1/2 inhibitor and a planned multicenter clinical trial on a large number of patients[36] are based on consistent and reliable preclinical results obtained in the intrahepatic mouse model. Moreover, alternative sites for islet infusion should be carefully compared with the intrahepatic site which is the standard site used in clinical practice. Appropriate preclinical studies are essential to prove safety and efficacy of islet transplant in the alternative site compared with the liver. In this context, after promising preclinical data, a pilot clinical study is ongoing at the San Raffaele Scientific Institute (Milan) to test safety and feasibility of the bone marrow as an alternative site for islet auto- and allo-transplantation.[48]

The recipient: choosing a suitable control group. As in human islet transplant, also in the mouse model the performances of transplanted islets are affected by the isolation process. It has been shown that mouse islet preparations may differ in pro-inflammatory molecule release, viability, insulin secretion and purity. Although a correlation between quality of the islet preparation and islet function is well known, islet transplantation in the mouse model is usually performed the day after islet isolation, without further information on islet quality. Several methods are currently used to correlate in vitro data with in vivo function, but results are usually not available at the time of transplant because these tests are time-consuming. Because of the variability of islet quality in different preparations, when planning an experiment in a mouse model we should include an internal control group that will be crucial when analyzing and interpreting results, both for intra-experiment normalization and mice stratification. For this reason we strongly suggest to always pair treated and control recipients to insure that islets from the same isolation are injected in an equal number of mice in each study arm.

The recipient: choosing among different murine donor-recipient strain combinations for allogeneic transplant. MHC molecules are undoubtedly the most important allo-antigens triggering graft rejection. Although they are classified as full MHC-mismatched mouse model of islet transplantation, the strength/kinetics of the allo-immune response (i.e., timing of graft loss of function) varies among different murine donor-recipient strain combinations. Some strains such as C57BL/6 (i.e., $H2^b$) are strong responders able to generate vigorous and quick allo-reactive immune responses, whereas CBA and C3H

strains (i.e., $H2^k$) are mild responders able to accept grafts more readily and, finally, BALB/C and DBA strains (i.e., $H2^d$) are low or non-responders.[49-51]

As a rule of thumb in term of immune response the more stringent preclinical model is that of C57BL/6 islets transplanted in NOD mice. In fact, as in human islet transplantation, this model is characterized not only by a strong allo-immune response, but also by the re-activation of a memory auto-immune response. Therefore, this is a valuable preclinical model because it mimics both aspects of islet transplant in humans. A second very valuable model is that of BALB/c islets transplanted in C57BL/6 mice. This model is characterized by a strong allo-immune response in the absence of a memory response (unless induced by immunization strategies like pre-transplant infusion of donor splenocytes). The reverse combination of C57BL/6 islets transplanted in BALB/c mice is a less valuable model to study new immunosuppressive drug or pro-tolerogenic strategies because is characterized by a mild allo-immune response. In this line, Melzi et al. have previously documented that C57BL/6 islets transplanted in BALB/c mice show a quicker loss of function than BALB/c islets transplanted in C57BL/6 mice, independently by the site used (i.e., liver or kidney capsule).[52]

Finally the minor histocompatibility antigen mismatched model may be useful for studying specific issues. Beyond MHC molecules, differences at other loci in the presence of MHC-identity may increase susceptibility to rejection as the result of T cell recognition of other polymorphic non-MHC antigens, called minor histocompatibility antigens (minor H Ag). Minor antigens are ubiquitously expressed peptides derived from a wide variety of proteins, and presented by host- or recipient-derived MHC molecules. One set of proteins that induce minor histocompatibility responses, are encoded on the male-specific Y chromosome (minor HY Ag). In inbred mouse strains, females grafted with syngeneic male cells can respond only to product of these genes. Both MHC class I- and class II-restricted epitopes for minor HY Ags exist and cooperation between $CD4^+$ and $CD8^+$ T cell subsets are required to generate effective minor HY Ag-specific responses.[53] In this peculiar transplant setting, tetramers-peptides complexes and the development of both in vitro and ex vivo protocols to detect an antigen-specific T-cell population have allowed the dissection of minor HY Ag-specific immune responses.[54]

Data analysis: setting glycemia thresholds for reverting hyperglycemia. In islet transplantation preclinical research blood glucose levels are used to define the success of islet engraftment, survival, rejection, tolerance according to the transplant model. Islet engraftment is usually defined as the achievement of normoglycemia, and graft rejection the subsequent development of hyperglycemia. There are no standardized and widely accepted thresholds for defining either normo- or hyperglycemia murine models, and these need to be defined to correctly evaluate different biological phenomena. In the syngeneic mouse model of islet transplantation we propose to define normoglycemia after islet transplantation as two consecutive measurements of non-fasting blood glucose levels < 200 mg/dl.[52] If more stringent definition is desirable, transplant function may be normalized for the pre-transplant glycemia and the outcome classified as (1) full function (i.e., non-fasting blood glucose concentration < 50% of the pre-transplant concentration), (2) partial function (i.e., non-fasting blood glucose concentration between 50% and 80% of the pre-transplant concentration) and (3) no function (i.e., non-fasting blood glucose concentration > 80% of the pre-transplant concentration). In the allogeneic mouse model of islet transplantation, we propose to define normoglycemia as the ability to reach non-fasting blood glucose levels under 250 mg/dl within 5 d after islet infusion and graft rejection as two consecutive measurements > 300 mg/dl in mice after normoglycemia was achieved. Both in the syngeneic and allogeneic model, islet transplantation failure should be defined either as the inability to reach non-fasting blood glucose levels < 250 mg/dl or death within the first 7 d after islet transplantation (i.e., surgical death).

Conclusion

Translational research needs very accurate preclinical studies before starting a clinical trial in humans. Islet transplantation research, taking advantage from many diabetic mouse models, is a very dynamic field. However, some methodological considerations need to be addressed to design meticulous preclinical studies and obtain consistent and reliable results to select promising strategies that may be translated into clinical practice.

The following critical points should be taken into consideration while planning preclinical bench-to-bedside islet transplantation research: (1) in all experiments islet mass should be normalized using the equivalent islet (IEQ) number; (2) irrespective of the diabetic mouse model used, only severely diabetic mice (i.e., non-fasting glycemia > 450 mg/dl) should be considered as eligible recipients; (3) the liver should be used as the preferential site for islet infusion; (4) treated and control recipient mice should always be paired to insure that the islets from the same isolation are infused in the same number of animals in each arm of the study; (5) depending on the biological question, investigators should be knowledgeable and scrupulous when choosing the donor/recipient combination; and finally, (6) at the time of data analysis the glycemia thresholds used to define graft function should always be reported.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by the EU (HEALTH-F5-2009-241883-BetaCellTherapy). EC conducted this study as part of her PhD in Molecular Medicine, Program in Basic and Applied Immunology, San Raffaele University. AC is a PhD student with the Department of Surgery at the University of Pavia.

References

1. van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiol Rev 2011; 91:79-118; PMID:21248163; http://dx.doi.org/10.1152/physrev.00003.2010
2. Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. Diabetes Care 2012; 35:1436-45; PMID:22723582; http://dx.doi.org/10.2337/dc12-0063
3. Eriksson O, Alavi A. Imaging the islet graft by positron emission tomography. Eur J Nucl Med Mol Imaging 2012; 39:533-42; PMID:21932118; http://dx.doi.org/10.1007/s00259-011-1928-4
4. Eriksson O, Eich T, Sundin A, Tibell A, Tufveson G, Andersson H, et al. Positron emission tomography in clinical islet transplantation. Am J Transplant 2009; 9:2816-24; PMID:19845588; http://dx.doi.org/10.1111/j.1600-6143.2009.02844.x
5. Crowe LA, Ris F, Nielles-Vallespin S, Speier P, Masson S, Armanet M, et al. A novel method for quantitative monitoring of transplanted islets of langerhans by positive contrast magnetic resonance imaging. Am J Transplant 2011; 11:1158-68; PMID:21564535; http://dx.doi.org/10.1111/j.1600-6143.2011.03559.x
6. Piemonti L, Everly MJ, Maffi P, Scavini M, Poli F, Nano R, et al. Alloantibody and Autoantibody Monitoring Predicts Islet Transplantation Outcome in Human Type 1 Diabetes. Diabetes 2012; 62:1-9; PMID:23274902
7. Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, et al. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Lab Anim 2011; 45:131-40; PMID:21478271; http://dx.doi.org/10.1258/la.2010.010090
8. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008; 51:216-26; PMID:18087688; http://dx.doi.org/10.1007/s00125-007-0886-7
9. Lapierre P, Béland K, Djilali-Saiah I, Alvarez F. Type 2 autoimmune hepatitis murine model: the influence of genetic background in disease development. J Autoimmun 2006; 26:82-9; PMID:16380229; http://dx.doi.org/10.1016/j.jaut.2005.11.001
10. Muller YD, Golshayan D, Ehirchiou D, Wyss JC, Giovannoni L, Meier R, et al. Immunosuppressive effects of streptozotocin-induced diabetes result in absolute lymphopenia and a relative increase of T regulatory cells. Diabetes 2011; 60:2331-40; PMID:21752956; http://dx.doi.org/10.2337/db11-0159
11. Eisenbarth GS. Type 1 diabetes: molecular, cellular and clinical immunology. Adv Exp Med Biol 2004; 552:306-10; PMID:15622970
12. André I, Gonzalez A, Wang B, Katz J, Benoist C, Mathis D. Checkpoints in the progression of autoimmune disease: lessons from diabetes models. Proc Natl Acad Sci U S A 1996; 93:2260-3; PMID:8637860; http://dx.doi.org/10.1073/pnas.93.6.2260
13. Enghofer M, Bojunga J, Ludwig R, Oldenburg A, Bernd A, Usadel KH, et al. Lymphocyte transfer in streptozotocin-induced diabetes: adhesion of donor cells to islet endothelium. Am J Physiol 1998; 274:E928-35; PMID:9612252
14. Herold KC, Vezys V, Sun Q, Viktora D, Seung E, Reiner S, et al. Regulation of cytokine production during development of autoimmune diabetes induced with multiple low doses of streptozotocin. J Immunol 1996; 156:3521-7; PMID:8617981
15. McEvoy RC, Andersson J, Sandler S, Hellerström C. Multiple low-dose streptozotocin-induced diabetes in the mouse. Evidence for stimulation of a cytotoxic cellular immune response against an insulin-producing beta cell line. J Clin Invest 1984; 74:715-22; PMID:6088584; http://dx.doi.org/10.1172/JCI111487
16. Müller A, Schott-Ohly P, Dohle C, Gleichmann H. Differential regulation of Th1-type and Th2-type cytokine profiles in pancreatic islets of C57BL/6 and BALB/c mice by multiple low doses of streptozotocin. Immunobiology 2002; 205:35-50; PMID:11999343; http://dx.doi.org/10.1078/0171-2985-00109
17. Imaeda A, Kaneko T, Aoki T, Kondo Y, Nagase H. DNA damage and the effect of antioxidants in streptozotocin-treated mice. Food Chem Toxicol 2002; 40:979-87; PMID:12065220; http://dx.doi.org/10.1016/S0278-6915(02)00014-5
18. Leiter EH. The NOD mouse: a model for insulin-dependent diabetes mellitus. Curr Protoc Immunol 2001; Chapter 15:9; PMID:18432739
19. Mathews CE, Langley SH, Leiter EH. New mouse model to study islet transplantation in insulin-dependent diabetes mellitus. Transplantation 2002; 73:1333-6; PMID:11981430; http://dx.doi.org/10.1097/00007890-200204270-00024
20. Ohashi PS, Oehen S, Buerki K, Pircher H, Ohashi CT, Odermatt B, et al. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. Cell 1991; 65:305-17; PMID:1901764; http://dx.doi.org/10.1016/0092-8674(91)90164-T
21. Oldstone MB, Nerenberg M, Southern P, Price J, Lewicki H. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell 1991; 65:319-31; PMID:1901765; http://dx.doi.org/10.1016/0092-8674(91)90165-U
22. Lo D, Freedman J, Hesse S, Palmiter RD, Brinster RL, Sherman LA. Peripheral tolerance to an islet cell-specific hemagglutinin transgene affects both CD4+ and CD8+ T cells. Eur J Immunol 1992; 22:1013-22; PMID:1348026; http://dx.doi.org/10.1002/eji.1830220421
23. Christen U, Hintermann E, Holdener M, von Herrath MG. Viral triggers for autoimmunity: is the 'glass of molecular mimicry' half full or half empty? J Autoimmun 2010; 34:38-44; PMID:19716269; http://dx.doi.org/10.1016/j.jaut.2009.08.001
24. MacGregor RR, Williams SJ, Tong PY, Kover K, Moore WV, Stehno-Bittel L. Small rat islets are superior to large islets in in vitro function and in transplantation outcomes. Am J Physiol Endocrinol Metab 2006; 290:E771-9; PMID:16303846; http://dx.doi.org/10.1152/ajpendo.00097.2005
25. Ricordi C. Quantitative and qualitative standards for islet isolation assessment in humans and large mammals. Pancreas 1991; 6:242-4; PMID:1679542; http://dx.doi.org/10.1097/00006676-199103000-00018.
26. Huang HH, Ramachandran K, Stehno-Bittel L. A replacement for islet equivalents with improved reliability and validity. Acta Diabetol 2012; e-pub ahead of print; PMID:22302191; http://dx.doi.org/10.1007/s00592-012-0375-4
27. Melzi R, Battaglia M, Draghici E, Bonifacio E, Piemonti L. Relevance of hyperglycemia on the timing of functional loss of allogeneic islet transplants: implication for mouse model. Transplantation 2007; 83:167-73; PMID:17264813; http://dx.doi.org/10.1097/01.tp.0000250659.24286.43
28. Makhlouf L, Duvivier-Kali VF, Bonner-Weir S, Dieperink H, Weir GC, Sayegh MH. Importance of hyperglycemia on the primary function of allogeneic islet transplants. Transplantation 2003; 76:657-64; PMID:12973104; http://dx.doi.org/10.1097/01.TP.0000080881.75767.0E
29. Tanabe K, Liu Y, Hasan SD, Martinez SC, Cras-Méneur C, Welling CM, et al. Glucose and fatty acids synergize to promote B-cell apoptosis through activation of glycogen synthase kinase 3β independent of JNK activation. PLoS One 2011; 6:e18146; PMID:21541314; http://dx.doi.org/10.1371/journal.pone.0018146
30. Nichols CG, Remedi MS. The diabetic β-cell: hyperstimulated vs. hyperexcited. Diabetes Obes Metab 2012; 14(Suppl 3):129-35; PMID:22928573; http://dx.doi.org/10.1111/j.1463-1326.2012.01655.x
31. Davalli AM, Scaglia L, Zangen DH, Hollister J, Bonner-Weir S, Weir GC. Vulnerability of islets in the immediate posttransplantation period. Dynamic changes in structure and function. Diabetes 1996; 45:1161-7; PMID:8772716; http://dx.doi.org/10.2337/diabetes.45.9.1161
32. Kawahara T, Kin T, Kashkoush S, Gala-Lopez B, Bigam DL, Kneteman NM, et al. Portal vein thrombosis is a potentially preventable complication in clinical islet transplantation. Am J Transplant 2011; 11:2700-7; PMID:21883914; http://dx.doi.org/10.1111/j.1600-6143.2011.03717.x
33. Contreras JL, Eckstein C, Smyth CA, Bilbao G, Vilatoba M, Ringland SE, et al. Activated protein C preserves functional islet mass after intraportal transplantation: a novel link between endothelial cell activation, thrombosis, inflammation, and islet cell death. Diabetes 2004; 53:2804-14; PMID:15504960; http://dx.doi.org/10.2337/diabetes.53.11.2804
34. Vriens MR, Marinelli A, Harinck HI, Zwinderman KH, van de Velde CJ. The role of allopurinol in human liver ischemia/reperfusion injury: a prospective randomized clinical trial. Hepatogastroenterology 2002; 49:1069-73; PMID:12143204
35. Gustafson EK, Elgue G, Hughes RD, Mitry RR, Sanchez J, Haglund U, et al. The instant blood-mediated inflammatory reaction characterized in hepatocyte transplantation. Transplantation 2011; 91:632-8; PMID:21289595; http://dx.doi.org/10.1097/TP.0b013e31820ae459
36. Citro A, Cantarelli E, Maffi P, Nano R, Melzi R, Mercalli A, et al. CXCR1/2 inhibition enhances pancreatic islet survival after transplantation. J Clin Invest 2012; 122:3647-51; PMID:22996693; http://dx.doi.org/10.1172/JCI63089
37. van Suylichem PT, Strubbe JH, Houwing H, Wolters GH, van Schilfgaarde R. Rat islet isograft function. Effect of graft volume and transplantation site. Transplantation 1994; 57:1010-7; PMID:8165696
38. Song HJ, Xue WJ, Li Y, Tian XH, Ding XM, Feng XS, et al. Prolongation of islet graft survival using concomitant transplantation of islets and vascular endothelial cells in diabetic rats. Transplant Proc 2010; 42:2662-5; PMID:20832565; http://dx.doi.org/10.1016/j.transproceed.2010.06.003
39. Rackham CL, Chagastelles PC, Nardi NB, Hauge-Evans AC, Jones PM, King AJ. Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice. Diabetologia 2011; 54:1127-35; PMID:21267536; http://dx.doi.org/10.1007/s00125-011-2053-4
40. Sordi V, Melzi R, Mercalli A, Formicola R, Doglioni C, Tiboni F, et al. Mesenchymal cells appearing in pancreatic tissue culture are bone marrow-derived stem cells with the capacity to improve transplanted islet function. Stem Cells 2010; 28:140-51; PMID:19924826; http://dx.doi.org/10.1002/stem.314
41. Sakata N, Chan NK, Chrisler J, Obenaus A, Hathout E. Bone marrow cell cotransplantation with islets improves their vascularization and function. Transplantation 2010; 89:686-93; PMID:20101199; http://dx.doi.org/10.1097/TP.0b013e3181cb3e8d
42. Melzi R, Antonioli B, Mercalli A, Battaglia M, Valle A, Pluchino S, et al. Co-graft of allogeneic immune regulatory neural stem cells (NPC) and pancreatic islets mediates tolerance, while inducing NPC-derived tumors in mice. PLoS One 2010; 5:e10357; PMID:20436918; http://dx.doi.org/10.1371/journal.pone.0010357
43. Dufour JM, Lord SJ, Kin T, Rayat GR, Dixon DE, Bleackley RC, et al. Comparison of successful and unsuccessful islet/Sertoli cell cotransplant grafts in streptozotocin-induced diabetic mice. Cell Transplant 2008; 16:1029-38; PMID:18351019; http://dx.doi.org/10.3727/000000007783472417
44. Wood KJ, Goto R. Mechanisms of rejection: current perspectives. Transplantation 2012; 93:1-10; PMID:22138818; http://dx.doi.org/10.1097/TP.0b013e31823cab44

45. Farney AC, Xenos E, Sutherland DE, Widmer M, Stephanian E, Field MJ, et al. Inhibition of pancreatic islet beta cell function by tumor necrosis factor is blocked by a soluble tumor necrosis factor receptor. Transplant Proc 1993; 25:865-6; PMID:8382881
46. Sandberg JO, Eizirik DL, Sandler S. IL-1 receptor antagonist inhibits recurrence of disease after syngeneic pancreatic islet transplantation to spontaneously diabetic non-obese diabetic (NOD) mice. Clin Exp Immunol 1997; 108:314-7; PMID:9158104; http://dx.doi.org/10.1046/j.1365-2249.1997.3771275.x
47. McCall M, Pawlick R, Kin T, Shapiro AM. Anakinra potentiates the protective effects of etanercept in transplantation of marginal mass human islets in immunodeficient mice. Am J Transplant 2012; 12:322-9; PMID:22053751; http://dx.doi.org/10.1111/j.1600-6143.2011.03796.x
48. Cantarelli E, Melzi R, Mercalli A, Sordi V, Ferrari G, Lederer CW, et al. Bone marrow as an alternative site for islet transplantation. Blood 2009; 114:4566-74; PMID:19773545; http://dx.doi.org/10.1182/blood-2009-03-209973
49. Williams MA, Trambley J, Ha J, Adams AB, Durham MM, Rees P, et al. Genetic characterization of strain differences in the ability to mediate CD40/CD28-independent rejection of skin allografts. J Immunol 2000; 165:6849-57; PMID:11120808
50. Mysliwietz J, Thierfelder S. Analysis of peripheral immune tolerance uncovers a mouse strain-dependent in situ type of graft tolerance. Eur J Immunol 1999; 29:150-5; PMID:9933096; http://dx.doi.org/10.1002/(SICI)1521-4141(199901)29:01<150::AID-IMMU150>3.0.CO;2-7
51. van Maurik A, Wood KJ, Jones ND. Impact of both donor and recipient strains on cardiac allograft survival after blockade of the CD40-CD154 costimulatory pathway. Transplantation 2002; 74:740-3; PMID:12352896; http://dx.doi.org/10.1097/00007890-200209150-00026
52. Melzi R, Sanvito F, Mercalli A, Andralojc K, Bonifacio E, Piemonti L. Intrahepatic islet transplant in the mouse: functional and morphological characterization. Cell Transplant 2008; 17:1361-70; PMID:19364073; http://dx.doi.org/10.3727/096368908787648146
53. Yoon IH, Choi SE, Kim YH, Yang SH, Park JH, Park CS, et al. Pancreatic islets induce CD4(+) [corrected] CD25(–)Foxp3(+) [corrected] T-cell regulated tolerance to HY-mismatched skin grafts. Transplantation 2008; 86:1352-60; PMID:19034003; http://dx.doi.org/10.1097/TP.0b013e31818aa43c
54. Millrain M, Chandler P, Dazzi F, Scott D, Simpson E, Dyson PJ. Examination of HY response: T cell expansion, immunodominance, and cross-priming revealed by HY tetramer analysis. J Immunol 2001; 167:3756-64; PMID:11564792