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Minireview

Best practices in islet transplantation in mice

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ABSTRACT

Islet transplantation in mice serves as a crucial preclinical model for understanding alloimmune and autoimmune mechanisms, optimizing immunosuppressive strategies, and developing novel therapies for diabetes. This review provides a comprehensive overview of best practices in murine islet transplantation, including diabetes induction models, technical aspects of islet transplantation, and criteria for transplant graft and rejection. We discuss the immunological challenges posed by major histocompatibility complex disparities, the impact of various transplantation sites, and the limitations of murine models in translating findings to clinical settings. Special emphasis is placed on emerging strategies such as stem cell-derived insulin-producing cells, immune tolerance induction, and alternative transplantation sites. Although mouse models have significantly advanced our understanding of diabetes and β-cell replacement, their inherent differences from human physiology necessitate careful interpretation of findings. The review also highlights novel imaging modalities, immunosuppressive protocols, and biomarkers for graft monitoring,

Abbreviations: ATP, adenosine triphosphate; β-cell, beta cell; CD, cluster of differentiation; CIT, cold ischemia time; CTLA4-Ig, cytotoxic t-lymphocyte-associated protein 4 immunoglobulin fusion protein; DCD, donation after circulatory death; DLIs, donor leukocyte infusions; DSA, donor-specific antibody; E2, 17 β-estradiol; GLUT2, glucose transporter type 2; IEQ, islet equivalent; IL, interleukin; IPGTT, intraperitoneal glucose tolerance test; IRI, ischemia-reperfusion injury; LDLT, living donor liver transplantation; LFA-1, lymphocyte function-associated antigen-1; MHC, major histocompatibility complex; MMF, mycophenolate mofetil; MRI, magnetic resonance imaging; mTOR, mechanistic target of rapamycin; MP, machine perfusion; mIL-2/Fc+/+, murine interleukin-2 fusion protein; mIL-15/Fc+/+, murine interleukin-15 fusion protein; mIL-21R.Fc, murine interleukin-21 receptor fusion protein; mATG, murine anti-thymocyte globulin; NAD+, nicotinamide adenine dinucleotide; NHP, non-human primate; NOD, non-obese diabetic (mouse strain); PARP, poly(adp-ribose) polymerase; PET, positron emission tomography; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SCID, severe combined immunodeficient; STZ, streptozotocin; TCR, T cell receptor; Treg, regulatory T cell; UW, University of Wisconsin (preservation solution); WIT, warm ischemia time.

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underscoring the need for further refinement of these models to bridge the gap between experimental research and clinical application. By standardizing methodologies and addressing translational limitations, murine islet transplantation studies remain a key model in transplantation and can continue to shape the future of β-cell replacement therapies for insulin-dependent diabetes.

1. Technical details

1.1. Description of the model

The 2 most widely used preclinical models of islet transplantation recipients are autoimmune spontaneously diabetic nonobese diabetic (NOD) mice and mice that are rendered diabetic by the injection of alloxan or streptozotocin (STZ). These 2 chemical agents induce diabetes by the selective destruction of β-cells.[1] In spontaneous diabetic NOD mice, T cell–mediated autoimmunity is responsible for the loss of β-cells and a rapid occurence of hyperglycemia.[2] For the chemically-induced model, a single dose of STZ (225 mg/kg)[3] or alloxan (200 mg/kg)[4] usually achieves diabetes induction. STZ is a β-cell toxic glucose analog that is internalized into β-cells via GLUT2 and induces necrosis and insulin deficiency. The mechanism of STZ-β-cell cytotoxicity is through mitochondrial DNA and protein alkylation and glycosylation, which induce depletion of NAD^+ and ATP^+ through PARP activation, inducing reactive oxygen species production and ultimately leading to β-cell necrosis.[1] After diabetes induction, the transplantation of 400 to 500 pancreatic islets from fully major histocompatibility complex (MHC)-mismatched donors[5] under the kidney capsule is conducted, aiming for diabetes reversal. Normalization of glycemia delineates successful islet transplantation, while rejection is defined as blood glucose levels >250 mg/dL for at least 2 consecutive days.[3] Another known diabetogenic agent, alloxan, a cytotoxic glucose analog that is internalized into β-cells via GLUT2 contributing to their necrosis and therefore to insulin deficiency.[1] Unlike STZ, the cytotoxicity of alloxan is mediated through induction of reactive oxygen species and also triggers inhibition of glucokinase, interfering with glucose oxidation and ATP production and consequently glucose-mediated insulin production and insulin biosynthesis.[1] The instability, short half life (<2 min), poor diabetogenicity, and age-dependent response to alloxan are limiting factors compared to STZ.[4] Although it lacks autoimmunity, the STZ model offers multiple advantages, including consistent induction of diabetes, simplified experimental design, and reduced variability, making it a practical and widely used tool for studying alloimmune mechanisms in islet transplantation. Moreover, autoimmunity recurrence is rare in clinical islet transplantation due to effective immunosuppression, making its absence in models such as STZ injection less of a limitation.[6]

1.2. Degree of MHC mismatch and autoreactivity

Allograft rejection is alloantigen recognition by recipient $CD4^+$/$CD8^+$ T cells.[7-9] C57BL/6 and BALB/c mice are fully mismatched for both MHC class I and class II.[10,11] C57BL/6 mice generate a vigorous alloreactive immune responses compared to other strains[12,13] and reject BALB/c islets with a median survival time of 12 days.[3,14] Both direct and indirect pathways of islet rejection have been demonstrated.[15] Also, in NOD mice, when used as recipients of either complete or MHC class II-matched islets, recurrent autoimmunity driven by autoreactive CD4/CD8 T cells can lead to the recurrent loss of β-cell mass.[16]

We provide details on differences in terms of graft survival within the major strain combinations and immunosuppression regimen in Table 1.[17-28] We summarize the effect of modulating important factors such as sex, cold ischemia time, diet, and site of transplantation in Table 2.[29-38]

2. Terminology/classification

After recipients are rendered hyperglycemic by STZ injection, islet transplantation restores normoglycemia immediately, except in marginal islet mass studies.[39] A return to hyperglycemia usually indicates graft rejection. Hyperglycemia (>250 mg/dL for 2 consecutive days) is commonly used to define rejection, although near-complete islet destruction is necessary for this manifestation. The intraperitoneal glucose tolerance test offers a more accurate measure of functional islet mass.[40] An increasing area under the curve in the intraperitoneal glucose tolerance test could indicate ongoing islet loss before hyperglycemia develops.

In mouse models, islets are typically transplanted under the kidney capsule or intraportally. Various methods are used to assess the graft, including blood sugar, glucose tolerance tests, C-peptide, histological and immunohistochemical analysis, bioluminescence imaging, metabolic parameters, functional tests, vascularization assessment, graft recovery with ex vivo analysis, and hypoglycemia susceptibility testing. Of note, pretermination graft biopsy is usually not performed. Histological examination focuses on immune cell infiltration, islet architecture, and α- and β-cell staining. However, findings can sometimes be inconclusive. Different immune cells (eg, lymphocytes vs myeloid cells, effector vs regulatory T cells) can indicate varied immune responses. Additionally, insulin production varies with β-cell function, not just viability; thus, lack of insulin/C-peptide staining does not necessarily indicate cell death.[41] Accurate estimation of graft size/mass from immunohistochemistry remains challenging due to the 2-dimensional nature of the analysis of a 3-dimensional structure. Islets are in fact organized into trilaminar epithelial plates, folded with different degrees of complexity.[42] Islet area can be determined using macros that calculate the total insulin and glucagon area and determine the area of the islet occupied by immune cell infiltrate.[43] Techniques such as

Table 1
An overview of the main immunosuppressive therapies tested in murine islet transplantation.

Immunosuppressive drugs	Dose	Median survival time	Donor strain	Recipient strain	References
CTLA4-Ig + mATG	mATG (500 μg on days 0 and 4) + CTLA4-Ig (500 μg on day 0; 250 μg on days 2, 4, 6, 8, and 10)	54 d	BALB/c	NOD hyperglycemic	Vergani et al[17]
Etanercept + anakinra	etanercept (5 mg/kg) anakinra (100 mg/kg)	26 d	human islet (1500 IEQs)	B6-Rag$^{-/-}$	McCall et al[18]
Anti-CD154 + anti-LFA-1	anti-CD154 alone (250 μg/d) anti-LFA-1 (CD11a) (200 μg/d)	100 d	BALB/c	diabetic C57BL/6 mice	Nicolls et al[19]
CD3 antibody F(ab')$_2$ fragments	5 injections of 50 μg each per day	42.5 ± 2.3 d (if treatment initiated at day −1) 80 d (if treatment initiated at d +7 or +11)	BALB/c	diabetic C57BL/6 mice	You et al[20]
mIL-21R.Fc + CTLA4-Ig	mIL-21R.Fc (400 μg for 10 d) CTLA4-Ig (total of 6 injections of 500 μg every 2 d)	100 d	BALB/c	diabetic C57BL/6 mice	Petrelli et al[21]
IL-2/Fc$^{+/+}$ + mIL-15/Fc$^{+/+}$ + rapamycin	IL-2/Fc$^{+/+}$ (5 μg daily for 2 wk) + mIL-15/Fc$^{+/+}$ (5 μg daily for 2 wk) + rapamycin (3 mg/kg for 2 wk)	>110 d	DBA/2	IL-2 KO C57BL/6	Zheng et al[22]
Anti-CD154 + CTLA4-Ig + rapamycin	rapamycin (3 mg/kg for 2 wk) anti-CD154 (a dose of 0.25 mg every other day for 2 wk) + CTLA4-Ig (a dose of 0.2 mg every other day for 2 wk)	30 d	DBA/2	IL-2 KO C57BL/6	Li et al[23]
Anti-CD154 + anti-LFA-1 + memory CD4$^+$ T cells	anti-CD154 (0.25 mg) + anti-LFA-1 (4 doses of 0.1 mg) + memory CD4$^+$ T cells	14 d	BALB/c	diabetic C57BL/6 mice	Xia et al[24]
Anti-CD154 + anti-OX40L + anti-LFA-1 + memory CD4$^+$ T cells	anti-CD154 (0.25 mg) + anti-OX40L + anti-LFA-1 (4 doses of 0.1 mg) + memory CD4$^+$ T cells	>60 d	BALB/c	diabetic C57BL/6 mice	Xia et al[24]
Anti-CD154 + anti-CD122 + anti-LFA-1 + memory CD8$^+$ T cells	anti-CD154 (0.25 mg) + anti-CD122 + anti-LFA-1 (4 doses of 0.1 mg) + memory CD8$^+$ T cells	>60 d	BALB/c	diabetic C57BL/6 mice	Xia J et al[24]
Rapamycin + anti-CD154 + anti-OX40L	rapamycin (administered as a dose of 3 mg/kg for 8 d), anti-CD154 (administered as 4 doses of 0.25 mg) and anti-OX40L (administered as 4 doses of 0.25 mg)	11 d	BALB/c	sensitized diabetic C57BL/6	Hong et al[25]

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Table 1 (*continued*)

Immunosuppressive drugs	Dose	Median survival time	Donor strain	Recipient strain	References
Anti-CD154 + anti-OX40L	anti-CD154 (administered as 4 doses of 0.25 mg) and anti-OX40L (administered as 4 doses of 0.25 mg)	9 d	BALB/c	sensitized diabetic C57BL/6	Hong et al[25]
Anti-ICOS	0.1 mg/d for 2 wk	13 d	BALB/c	diabetic C57BL/6	Harada et al[26]
Anti-ICOS + Cyclosporin	anti-ICOS (0.1 mg/d for 2 wk) cyclosporin (10 mg/kg/d for 2 wk)	16 d	BALB/c	diabetic C57BL/6	Harada et al[26]
Anti-ICOS + rapamycin	anti-ICOS (0.1 mg/d for 2 wk) + rapamycin (0.2 mg/kg/d for 2 wk)	47 d	BALB/c	diabetic C57BL/6	Nanji et al[27]
Low dose exenatide	0.2μg twice/d for 2 wk	41 d	BALB/c	diabetic C57BL/6	Ben Nasr et al[28]
Low dose exenatide	2 μg twice/d for 2 wk	67 d	BALB/c	diabetic C57BL/6	Ben Nasr et al[28]
Exenatide + rapamycin	Exenatide (2 μg twice/d for 2 wk) and rapamycin (0.1 mg/d for 2 wk)	100 d	BALB/c	diabetic C57BL/6	Ben Nasr et al[28]

CTLA4-Ig, human cytotoxic T lymphocyte-associated antigen 4; ICOS, inducible T-cell costimulator; IEQ, islet equivalent; IL-2 KO, interleukin 2 knockout; LFA-1, lymphocyte function-associated antigen-1; mATG, murine anti-thymocyte globulin; mIL-2/Fc, murine interleukin 2 cytolytic fusion protein; mIL-15/Fc, murine interleukin 15 cytolytic fusion protein; mIL-21R.Fc, murine interleukin 21 receptor; NOD, nonobese diabetic.

stereological methods and volumetric estimations from serial sections can partially address this issue, while advanced imaging modalities, including positron emission tomography, magnetic resonance imaging, and bioluminescence imaging, offer promising noninvasive, nonpostmortem alternatives for graft size estimation.[44] Long-term islet graft survival in mice is possible[45] but does not necessarily indicate tolerance. True tolerance requires successful engraftment of a second same-donor graft without further immunological manipulation.

In mice, donor-specific antibody (DSA)-mediated rejection is not well-documented, as recipients are usually sacrificed soon after rejection. In human islet transplantation, DSAs are typically observed only after complete withdrawal of immunosuppression.[46] The presence of DSA indicates cellular immunoreactivity as CD4 help is required to develop naïve and memory DSAs.[47] Preexisting antibodies and memory T and B cells can lead to rapid graft rejection.[48]

3. Benefits of the mouse model

The mouse islet transplantation model serves multiple purposes to dissect the immunological mechanisms resulting in graft acceptance or rejection. Investigators employ it to investigate mechanisms of immune tolerance, autoimmune-mediated rejection, immune suppression, immune regulation, and the role of donor or recipient characteristics (eg, age, sex, body mass index, etc). The model facilitates studies on stem cells and islet differentiation, as well as stem cell-derived products. It allows exploration of tissue and cellular engineering approaches, including organoids and islet β-cell encapsulation. Additionally, it helps evaluate the role of immunosuppressive drugs and any drug in general in the recipient. The model is crucial in developing humanized mouse models and enables imaging experiments using mouse islets transplanted into the anterior chamber of the eye. Lastly, it is valuable for conducting preclinical studies to test new therapeutics. This versatility makes it an invaluable tool in various areas of diabetes and transplantation research.

Additional benefits include transplant ease, genetic variants of mice to track different cells, cytokines, or genes of interest involved in diabetes or islet survival. A preferred site for transplantation of mouse islets is under the kidney capsule because it is less technically demanding without the requirements of vascular anastomoses, as for heart or kidney transplantation, making it a higher throughput technique to study allo- and xenotransplantation. However, islets do require neoangiogenesis for oxygen and nutrients, and such conditions are met under the kidney capsules and at various implantation sites.[49] Many islet transplants can be accomplished in a single day, allowing investigators to achieve statistical scientific rigor. There are many genetic variations of mice available to test mechanisms of islet graft acceptance or rejection, with transgenic expression or conditional knockout of many genes and markers to track immune or β-cells following transplantation or CD4 and CD8 T cells from T-cell receptor transgenic mice useful for tracking T-cell specificity.[50-52]

Mouse models of islet transplantation yield immunologically and physiologically relevant information that can be applied, to some degree, to our understanding of human islet transplantation. Autoimmune and alloimmune targets can be evaluated by assessing common islet antigens for autoimmune (eg, insulin, insulinoma-associated protein-2, glutamate decarboxylase, and zinc transporter 8) and alloimmune (major and minor histocompatibility differences) responses. Despite some similarities between mice and humans, there are substantial differences, discussed below, that make interpretation of results not consistently generalizable to humans.

Table 2
Impact of sex, cold ischemia time, diet, and various factors on graft survival.

Variable tested	Dose	Median survival time	Donor strain	Recipient strain	References
Female sex hormone 17 β-estradiol + sirolimus + tacrolimus	E2 (0.18 mg) sirolimus (0.1 mg/kg for 4 wk)/ tacrolimus (1 mg/kg)	100 d	1000 of human IEQ	Diabetic male immunodeficient nude	Contreras et al[29]
Cold ischemia	Islets stored at 4 °C for 24 h	8 d	BALB/c mice	Diabetic BALB/c mice	Wassmer et al[30]
Cold ischemia + liraglutide	Islets stored at 4 °C for 24 h +/− liraglutide (12 μg/mL)	25 d	BALB/c mice	Diabetic BALB/c mice	Wassmer et al[30]
Cold ischemia + liraglutide	Islets stored at 4 °C for 24 h +/− liraglutide (12 μg/mL)	51 d	1500 human IEQ	Diabetic immunodeficient nude mice	Wassmer et al[30]
Cold ischemia	Islets stored at 4 °C for 24 h	19 d	1500 human IEQ	Diabetic immunodeficient nude mice	Wassmer et al[30]
IRI and ROS production under UW + AP39	UW media + AP39 (400 nM) under CIT 18 h and WIT 27 min	20 d	1500 IEQ of porcine islets	Diabetic-SCID mice	Nishime et al[31]
IRI and ROS production under UW	DMSO + UW media under Cold ischemic time (CIT) = 18 h and WIT = 27 min	0 d	1500 IEQ of porcine islets	Diabetic-SCID mice	Nishime et al[31]
IRI in presence of redox modulator CA (FBC007)	CA-treated (68 μmol/L) islets for 24 h	31 d	BALB/c	Diabetic-C57BL/6 mice	Sklavos et al[32]
A_{2A} receptor agonists (ATL146e) treatment on IRI	50 Islets + ATL146e (60 ng/kg/min for 7 d)	10 d vs 16 d in untreated	C57BL/6 mice	Diabetic-C57BL/6 mice	Chhabra et al[33]
A_{2A} receptor agonists (ATL146e and ATL313) treatment on IRI	50 Islets +ATL146e (60 ng/kg/min for 7 d)	28.5 d vs 26 d in untreated	BL/6-A_{2A}AR-KO mice	Diabetic-C57BL/6 mice	Chhabra et al[33]
Calcitrol (vitamin d3 analog) + mmf	calcitrol (5 μg/kg 3 times/wk); MMF (100 mg/kg/d)	85	C57BL/6 mice	Diabetic BALB/c mice	Infante et al[34]
Calcitrol (vitamin d3 analog)	5 μg/kg 3 times/wk	52	C57BL/6 mice	Diabetic BALB/c mice	Infante et al[34]
Mmf	MMF (100 mg/kg/day)	48	C57BL/6 mice	Diabetic BALB/c mice	Gregori et al[35]
Calcitrol/MMF	Calcitrol (5 μg/kg 3 times/wk) MMF (100 mg/kg/d)	85	C57BL/6 mice	Diabetic BALB/c mice	Gregori et al[35]
ω-3 PUFAs (EPA and DHA) + Vitamin D3 + daclizumab	ω-3 PUFAs (EPA and DHA) (7 mg/kg) + vitamin D3 (5 μg/kg) + daclizumab (0.05 mg/kg)	18 d Vs 14.8 d	Sprague-Dawley rats	Diabetic Sprague-Dawley rats	Gurol et al[36]
High Zn diet	$ZnSO_4$ at 1000 ppm (high Zn diet group) vs $ZnSO_4$ at 50 ppm (control group)	6.7% Showed early graft failure at day 3 vs 33.3% in control	Male Wistar rats	Male Wistar rats	Okamoto et al[37]

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Table 2 (*continued*)

Variable tested	Dose	Median survival time	Donor strain	Recipient strain	References
Kidney subcapsular space	220-250 Islets per recipient	100%	C57BL/6 mice	Diabetic C57BL/6 mice	Stokes et al[38]
Spleen subcapsular space	220-250 Islets per recipient	29%	C57BL/6 mice	Diabetic C57BL/6 mice	Stokes et al[38]
Liver subcapsular space	220-250 islets per recipient	0	C57BL/6 mice	Diabetic C57BL/6 mice	Stokes et al[38]
Muscle		70%	C57BL/6 mice	Diabetic C57BL/6 mice	Stokes et al[38]
Portal vein	220–250 Islets per recipient vs 440–500 islets per recipient	60% Vs 78% with double dose of islets	C57BL/6 mice	Diabetic C57BL/6 mice	Stokes et al[38]
Kidney subcapsular space	2000 IEQ per recipient	78%	Human islets	Diabetic BALB/c SCID mice	Stokes et al[38]
Spleen subcapsular space		0	Human islets	Diabetic BALB/c SCID mice	Stokes et al[38]
Liver subcapsular space	2000 IEQ per recipient	0	Human islets	Diabetic BALB/c SCID mice	Stokes et al[38]
Muscle	2000 IEQ per recipient vs 4000 IEQ	29% Vs 56% with double dose of IEQ	Human islets	Diabetic BALB/c SCID mice	Stokes et al[38]
Portal vein	2000 IEQ per recipient	29%	Human islets	Diabetic BALB/c SCID mice	Stokes et al[38]

CIT, cold ischemic time; DHA, docosahexaenoic acid; DMSO, dimethylsulfoxide; EPA, eicosapentaenoic acid; IEQ, islet equivalent; IRI, ischemia-reperfusion injury; KO, knockout; MMF, mycophenolate mofetil; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SCID, severe combined immunodeficient; UW, University of Wisconsin solution; WIT, warm ischemic time.

Several approaches for developing immune tolerance using the mouse model have formed the basis for antigen-specific immunotherapies in human clinical trials. Examples include prevention of islet xenograft rejection by masking donor MHC class I antigens.[53] CTLA4-Ig, a soluble fusion protein that binds to CD80 and CD86 and blocks T cell activation in vitro, blocks human islet rejection in mice, inducing long-term, donor-specific tolerance.[54] These studies led to clinical trials for CTLA4-Ig. Bluestone and colleagues[55] showed anti-ICAM-1 antibody prolonged islet transplants, as did targeting costimulation using anti-CD80 and anti-CD86.[56] Others[57] identified donor-specific therapy using antibodies targeting CD40 ligand. CD40-CD40L interactions play a critical role during allograft rejection in mouse islets.[58] Anti-CD3 antibody was shown to induce long-term remission of overt autoimmunity in NOD mice,[59] which formed the basis of the current therapy for new onset type 1 diabetics, teplizumab, currently approved for human therapy.[60] These initial studies paved the way for current costimulatory blockade therapies in islet transplantation, kidney transplant, and continued investigation in multiple transplant studies in humans and nonhuman primates (NHPs).

The mouse model[61,62] has been extensively used to develop donor-specific regulatory T cells tested in multiple NHP systems[63] and in human clinical trials.[64,65] Tolerance via infusion of apoptotic donor leukocytes was developed using mouse islet transplant models[66] and has since been tested in NHPs[63] and human trials[67] for allo- and autoimmunity.

As new tolerance strategies enter clinical trials, mouse models may help predict whether specific immunosuppressive mediations may be beneficial or harmful in the induction of donor-specific tolerance. Eg, calcineurin inhibitors inhibit tolerance induction, whereas mTOR inhibitors are favorable.[68]

4. Limitations

One significant limitation is the difference in immunologic responses between mice and humans.[69,70] Mice have a different

repertoire of immune cells and cytokine responses, which make rejection mechanisms, timing, and intensity significantly different between various mouse strains and between mice and humans. A classic example is that BALB/c islets transplanted in C57BL/6 mice will be rejected, while C57BL/6 islets transplanted in BALB/c mice will not, owing to the differences in Th1/Th2 responses in these 2 strains.[71] The underlying autoimmune component in type 1 diabetic patients is absent in mice unless the NOD mouse model is used (which is the case in <25% of studies[72]). Moreover, the genetic homogeneity of inbred mouse strains does not capture the genetic diversity seen in the human population. This lack of genetic variability can lead to an oversimplification of the complex interactions between the immune system and transplanted islets. These issues could be partially addressed by using mice with fully humanized immune systems (eg, TruHuX mice).[73] The use of humanized immune system models is attractive but comes with limitations, including incomplete replication of human immune responses and challenges in engraftment efficiency. Inducing tolerance to allogeneic islets is facilitated by recipient factors such as genetic strain, absence of autoimmunity, and lack of preexisting allogeneic memory, which may not fully reflect clinical scenarios. Another concern is the efficacy and potency of immunosuppressive drugs in murine models, which are notably different from humans. Eg, a single dose of costimulation blockade can lead to indefinite allogeneic islet graft survival in mice.[74] In addition, steroids, mTOR, and calcineurin inhibitor-based immunosuppressive therapies can impair β-cell function and survival with potential magnitude differences between human and mice. These immune discrepancies can lead to challenges in translating findings from murine models to human clinical settings.

Another potential limitation is the small size of mice, which modifies the number of islets and the site where they can be transplanted, acknowledging the fact that mouse and human islets are the same size, whereas organ and vessel sizes are tremendously different. Thus, most transplant sites in mice are not intravascular spaces, and, therefore, do not account for the instant blood-mediated inflammatory reaction that takes place and destroys 25% to 75% of the islets in humans.[75,76] Inflammation, vascularization, oxygen tension, rejection mechanisms, and scarring are notably different between transplant sites in mice and humans. The kidney capsule is typically not used in humans. The liver portal system, routinely used and largely preferred in humans,[76–81] is a suboptimal transplant site in mice due to the embolic risk posed by the size of the islets.[82] Other transplant sites in mice such as the peritoneal cavity, spleen, intramuscular, subcutaneous tissue, and anterior eye chamber all possess very specific microenvironments with or without immune privileges and different revascularization capacities, and scientific observations cannot be immediately translatable to humans.[49] Eg, islets transplanted in bone marrow seemed to work well in mice compared to liver[83]; however, this observation was not validated compared to the kidney capsule in mice[84] and showed poor results human trials.[85] There is a discrepancy between the ability of alginate microencapsulated xenogeneic islets to durably reverse diabetes in mice in the absence of immunosuppression[86] (up to 3 months in that study) compared to the inability to do so in large animals or humans.[87] The main reason is that the intraperitoneal space is a highly vascularized site in mice, allows rapid diffusion of oxygen and nutrients, and can easily sustain 1 mL of encapsulated islets. In contrast, 1 L of islets will have severe hypoxia and absence of nutrient diffusion in humans (especially in the center of the capsule plug).

The metabolic rate of mice is much higher than that of humans, which affects the interpretation of glucose tolerance tests and other metabolic assessments.[40] Eg, mice are mostly nocturnal feeders, and an overnight fast has a much more profound effect on mice than on humans.[40] The intraperitoneal administration of glucose leads to no incretin response in mice, which can potentiate the glucose-mediated insulin response.[40] Some of the abovementioned limitations can be addressed by using large animal models of diabetes.[88]

The induction of diabetes in mice using STZ (or alloxan) has limitations. While STZ selectively destroys β-cells, the resulting model does not fully replicate autoimmune type 1 diabetes in humans. STZ also causes severe tissue damage in other organs such as the liver and kidneys and in the immune system.[72] Eg, T regulatory cells are notably enhanced, artificially promoting tolerance.[72] Additionally, the acute onset of diabetes in STZ-treated mice does not mimic the chronic progression of the disease in humans and lacks the autoimmune components that characterize it, potentially affecting the evaluation of long-term therapeutic interventions. Finally, several studies demonstrated that diabetic mice treated with high-dose STZ showed significant restoration of endogenous β-cell function after the removal of transplanted islets.[86] This suggests that native islets can regenerate and contribute to the recovery of B cell function, which is different from type 1 diabetes in humans.[89] Some of the limitations of the STZ model can be addressed with the NOD mouse model. While the NOD mouse model provides a more accurate representation of autoimmune diabetes, its spontaneous and variable disease onset, unique immune profile, and genetic homogeneity introduce complexities that the more predictable and controlled STZ model avoids. A last limitation worth mentioning is that while bulk transplantation is more efficient, hand picking islets may enhance graft quality by selecting higher viability islets; however, this approach is labor-intensive and less feasible for large-scale clinical applications. An overview of the main limitations is displayed in Table 3.[40,69,70,72,73,75,76,82,89]

5. Future perspectives: Using mouse models to address critical gaps in knowledge

To facilitate translation of knowledge from mouse islet transplant models to human islet transplantation, it is paramount that the models be tailored to address critical gaps in knowledge in the field:

1. Identification of biomarkers of immune damage for experimental and clinical monitoring: Identifying biomarkers of immune-mediated damage before the development of hyperglycemia would allow early interventions to prevent irreversible loss of β-cell mass.[90] Global immune activation by serum cytokines has been

Table 3
Limitations and characteristics in mice vs humans.

Limitations	Characteristics in mice	Characteristics in humans	Reference
Immune system differences	Rejection primarily involves predictable, rapid T cell–mediated responses due to uniform genetic backgrounds	Rejection is more complex, often involving antibody-mediated and chronic rejection influenced by diverse HLA profiles	Shay et al[69] Bjornson-Hooper et al[70]
Genetic homogeneity	Limited genetic diversity; oversimplified interactions between immune system and islets	High genetic diversity; more complex immune and genetic interactions	Muller et al[72]
Small size of the organism	Alters the no. of islets and limits transplant site options; kidney capsule commonly used	Larger size dictates the liver as the preferred site; more options for transplant sites	Delaune et al[82]
Anatomical and physiological differences	Vascularization, oxygenation, and transplant microenvironments differ significantly	Human liver portal system is preferred; physiological conditions more aligned with clinical practice	Niclauss et al[76]
High metabolic rate	Impacts glucose tolerance test results; nocturnal feeding and fasting effects more profound	Lower metabolic rate; fasting and feeding impacts are less extreme	Andrikopoulos et al[40]
Streptozotocin-induced diabetes model	Effective in destroying beta cells but lacks chronic progression and autoimmune features	Autoimmune nature of Type 1 diabetes; chronic progression over time	Muller et al[89]
Humanized immune system models	Genetically homogenous strains, no preexisting allogeneic memory, no autoimmunity, facilitated tolerance induction, incomplete replicate, challenging	Genetic diversity, allogeneic memory, autoimmune recurrence	Chupp et al[73]
Transplant site and IBMIR differences	Sites in mice are nonintravascular; IBMIR not accounted for	Intravascular sites such as liver; IBMIR significantly impacts outcomes	Cabric et al[75]

HLA, human leukocyte antigen; IBMIR, instant blood-mediated inflammatory reaction.

explored as a potential biomarker, although byproducts such as exosomes released by damaged islets may provide more specific indications of β-cell injury.[91] However, these changes may result from mechanical injury during islet isolation and transplantation rather than donor-specific immunity. Although individual biomarkers have been studied, a combination of markers representing diverse processes of β-cell damage may ultimately serve as a more accurate surrogate. These biomarkers must also consider the specifics of the graft, such as allogeneic vs xenogeneic islets and whole islets vs stem cell-derived insulin-producing cells.

2. Defining mouse and human immune responses to stem cell-derived insulin-producing cells vs fully differentiated islets: As the field progresses toward renewable sources of insulin-producing cells for β-cell replacement therapies,[92] adapting mouse models to study stem cell-derived insulin-producing cells becomes critical. This includes evaluating the feasibility of intraportal transplantation for single cells compared to whole islets and integrating bioscaffolds[93] or immune isolating devices[94] to better reflect human transplantation scenarios. Furthermore, humanized mouse models are essential to mimic human immune responses accurately. Although these models have advanced significantly (reviewed by Brehm et al[95]), their ability to capture the entire spectrum of human immune reactions, particularly in β-cell replacement therapies, warrants careful assessment.
3. Investigations to identify sites of transplantation[49] and approaches to improve delivery of islets vs islet stem cells: Mouse models must address whether specific transplant sites and delivery methods are suitable for single cells, stem cell-derived insulin-producing cells, and whole islets. Sites such as the anterior chamber of the eye have emerged as promising options, allowing for longitudinal imaging.[96,97,98] Innovative encapsulation strategies, such as nanoencapsulation and microencapsulation[99] or macroencapsulation devices, show promise in immune protection and engraftment improvement.
4. Impact of preexisting autoimmunity: The NOD mouse model[100] has demonstrated the influence of preexisting β-cell autoimmunity on the destruction of syngeneic islets. Whether preexisting autoimmunity affects subsequent alloimmunity or xenoimmunity to transplanted islets remains a critical question. Studies should also explore its impact on stem cell-derived insulin-producing cells.

Recent advances in mouse models have facilitated critical insights into islet transplantation, including the development of humanized models to study immune interactions,[101] intravital microscopy for real-time visualization of islet function and vascularization,[102,103] and advanced imaging modalities such as positron emission tomography, magnetic resonance imaging, and bioluminescence imaging.[104,105] Additional innovations include genetically engineered biosensors for monitoring cellular processes,[102,106] cotransplantation with accessory cells such as mesenchymal stem cells,[107] and efforts to create "invisible" stem cell-derived islets to evade immune detection.[108] These efforts collectively contribute to a more comprehensive understanding of islet biology and improved diabetes treatment strategies.

Declaration of competing interest

The authors of this manuscript have no conflicts of interest to disclose as described by *American Journal of Transplantation*.

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