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ARTICLE

# A new immunodeficient hyperglycaemic mouse model based on the *Ins2*$^{Akita}$ mutation for analyses of human islet and beta stem and progenitor cell function

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**Abstract**
*Aims/hypothesis* To develop and validate a new immunodeficient mouse strain that spontaneously develops a non-autoimmune hyperglycaemia to serve as a diabetic host for human islets and human beta stem and progenitor cells without the need for induction of hyperglycaemia by toxic chemicals with their associated side effects.
*Methods* We generated and characterised a new strain of immunodeficient spontaneously hyperglycaemic mice, the NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ strain and compared this strain with the NOD-*scid Il2rγ*$^{null}$ (also known as *Il2rg*) immunodeficient strain rendered hyperglycaemic by administration of a single dose of streptozotocin. Hyperglycaemic mice were transplanted with human islets ranging from 1,000 to 4,000 islet equivalents (IEQ) and were monitored for normalisation of blood glucose levels.
*Results* NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice developed spontaneous hyperglycaemia, similar to *Ins2*$^{Akita}$-harbouring strains of immunocompetent mice. Histological examination of islets in the host pancreas validated the spontaneous loss of beta cell mass in the absence of mononuclear cell infiltration. Human islets transplanted into spontaneously diabetic NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ and chemically diabetic NOD-*scid Il2rγ*$^{null}$ mice resulted in a return to euglycaemia that occurred with transplantation of similar beta cell masses.
*Conclusions/interpretation* The NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mouse is the first immunodeficient, spontaneously hyperglycaemic mouse strain described that is based on the *Ins2*$^{Akita}$ mutation. This strain is suitable as hosts for human islet and human beta stem and progenitor cell transplantation in the absence of the need for pharmacological induction of diabetes. This strain of mice also has low levels of innate immunity and can be engrafted with a human immune system for the study of human islet allograft rejection.

**Keywords** Akita · Humanised mice · Insulin · Islet transplantation · NOD mice

**Abbreviations**

| | |
|---|---|
| IEQ | islet equivalent |
| JDRF | Juvenile Diabetes Research Foundation |
| mAb | monoclonal antibody |
| NK | natural killer |

## Introduction

Immunodeficient mice hold great promise as hosts to study in vivo the transplantation of human tissues and have the potential to allow investigations of tissue preparation, surgical procedures, transplantation immunobiology and other parameters in a preclinical setting [1, 2]. Currently, NOD strain mice, rendered immunodeficient by homozygous expression of the *Prkdc*$^{scid}$ (*scid*) or *Rag1*$^{null}$ mutant alleles are considered

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the 'gold standard' hosts for human xenografts [2]. While immunodeficient NOD strain mice have multiple deficiencies in innate immunity, they retain some natural killer (NK) cell activity [3]. The residual innate immune and NK cell activity limits efficient human xenograft acceptance, particularly with respect to engraftment of embryonic, mesenchymal and haematopoietic stem cells and their progeny [4–6]. Moreover, islets are exquisitely sensitive to killing by NK cells [7–9]. To diminish innate immunity and NK cell function, targeted mutations in genes such as the cytotoxic effector molecule perforin (*Prf1*) and the 'common' $\gamma$ chain subunit of the IL-2 receptor (*Il2r*$\gamma$, also known as *Il2rg*) have been introduced onto *scid* or *Rag1*$^{null}$ strains of NOD mice [10–12].

In the setting of human islet or beta cell transplantation in mice, chemical ablation of host insulin-producing beta cells is often a necessary first step to establish a hyperglycaemic immunodeficient host suitable for evaluation of the function of transplanted insulin-producing cells. This is usually achieved with beta cell toxic agents, such as streptozotocin or alloxan [13, 14]. However, streptozotocin and alloxan are not neutral with regards to their effects on the immune system and have been reported to have deleterious effects on host immunity [15]. These agents also have detrimental effects on multiple other organs, including the kidney, the preferred site for islet and beta stem and progenitor cell transplantation [14, 16, 17]. Moreover, streptozotocin is highly genotoxic, leading to the production of DNA strand breaks. A single administration can also induce tumours in rat kidney, liver and pancreas [18], precluding long-term experiments required for analyses of beta stem cells. Therefore, alternative hyperglycaemic mouse models that do not require chemical beta cell destruction to render the immunodeficient host hyperglycaemic are of great interest as recipients of human islets and human beta cell stem and progenitor cells.

Recently, a dominant mutation in the murine insulin 2 gene, termed *Ins2*$^{Akita}$ that results in spontaneous non-immune mediated hyperglycaemia has been described [19, 20]. This spontaneously arising mutation replaces a cysteine at position 96 with tyrosine, and disrupts a disulphide linkage required for proper folding. This mutation leads to improper folding of the nascent insulin 2 molecule, induction of the unfolded protein response and finally apoptosis of beta cells that leads to hyperglycaemia [21, 22].

We now report on the first description of an immunodeficient diabetic mouse based on the presence of a mutated *Ins2*$^{Akita}$ gene that develops spontaneous hyperglycaemia and is amenable as a recipient of human islets and human beta stem and progenitor cells. The hyperglycaemic NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ strain is based on the previously described NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ strain that has been shown to accept human islet transplants and allow for allograft rejection with human peripheral blood mononuclear cells [23]. Immunodeficient NOD mice harbouring the *Ins2*$^{Akita}$ mutation are an appealing host for human islet transplants and for human beta stem and progenitor cells when concerns about drug-induced hyperglycaemia are encountered.

## Methods

*Mice* NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/Sz (abbreviated as NOD-*scid Il2r*$\gamma^{null}$) mice have been described [12]. NOD.Cg-*Rag1*$^{tm1Mom}$ *Prf1*$^{tm1Sdz}$ *Ins2*$^{Akita}$ (abbreviated as NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$) mice were generated at The Jackson Laboratory. We first backcrossed the *Ins2*$^{Akita}$ mutation from the C57BL/6J strain ten generations onto the NOD-*Rag1*$^{null}$ strain background. The NOD.Cg-*Rag1*$^{tm1Mom}$ *Ins2*$^{Akita}$ mice were then crossed with our NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ [11] strain. Additional crosses were carried out to fix the *Prf1* mutation to homozygosity while maintaining the *Ins2*$^{Akita}$ mutation in the heterozygous state. This stock is maintained by mating NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ males with NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ females.

Development of spontaneous non-autoimmune type 1 diabetes was evaluated in cohorts of NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice by weekly glycosuria measurements with an Ames Diastix (Bayer, Elkhart, NJ, USA). Confirmation of hyperglycaemia was determined by blood glucose monitoring with an Accu-Check active glucometer (Hoffman-LaRoche, Basel, Switzerland). Mice were housed in a specific pathogen-free facility in microisolator cages, and given autoclaved food and maintained on acidified autoclaved water and sulfamethoxazole–trimethoprim medicated water (Goldline Laboratories, Ft Lauderdale, FL, USA), provided on alternate weeks. All animal use was in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts Medical School and The Jackson Laboratory and conformed to the recommendations in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 1996).

*Streptozotocin dose response* Cohorts of NOD-*scid Il2r*$\gamma^{null}$ mice were treated with a single i.p. injection of streptozotocin at the following doses: 120, 130, 140, 150 and 160 mg/kg body weight. Chemical ablation of beta cells was monitored twice weekly by glycosuria measurement and confirmed by measuring blood glucose.

*Human islet transplantation* The procurement and use of all human tissues were performed under protocols approved by the Institutional Review Board of the University of Massachusetts Medical School. Human islets designated for research were obtained from the Juvenile Diabetes Research

Foundation (JDRF) Islet Isolation Center at the University of Pittsburgh (Pittsburgh, PA, USA) or the Islet Cell Resource Consortium. Islet equivalents (IEQ) were enumerated and transplanted into recipient mice as described [23, 24]. Human IEQs at doses between 1,000 and 4,000, suspended in RPMI were transplanted into the renal subcapsular space of spontaneously diabetic NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice or NOD-*scid Il2rγ*$^{null}$ mice that had been rendered chemically diabetic (blood glucose >25 mmol/l) by a single i.p. injection of streptozotocin (150 mg/kg). Non-fasting plasma glucose levels were determined using an Accu-Check Active glucometer at twice-weekly intervals following transplantation to monitor islet graft function.

*Antibodies and flow cytometry* A monoclonal antibody (mAb) against Igκ light chain mouse leucocyte markers was purchased from BD Biosciences (San Jose, CA, USA) as an FITC conjugate. Additional mAbs were generated as hybridoma tissue culture supernatants, precipitated with ammonium sulphate, purified by size exclusion chromatography, and labelled with phycoerythrin, FITC, allophycocyanin or allophycocyanin–cyanine 7. These included: anti-mouse CD3, CD4, anti-CD8α, Ly-6c (Gr-1), anti-mouse CD122 (IL-2Rβ), B220, CD11b (Mac-1) Ly76 (Ter-119), Ly49G2 (LGL-1), MHC class I (H-2K$^{d}$) and MHC class II (IA$^{k,g7}$).

Single-cell suspensions of bone marrow and spleen were prepared from otherwise unmanipulated mice. Erythrocytes in bone marrow and spleen were removed by lysis with a hypotonic solution and cell counts were determined using a Coulter Counter (Beckman, Miami, FL, USA). Single-cell suspensions of $1\times10^{6}$ cells in a 50 μl volume of FACS buffer (PBS supplemented with 1% [vol./vol.] fetal bovine serum and 0.01% [wt/vol.] $NaN_3$) were pre-incubated with 1 mg/ml rabbit Ig (Sigma, St Louis, MO, USA). Cells were then washed and incubated for 30 min at 4°C in appropriate dilutions of antibodies as determined in preliminary experiments. All single-cell suspensions of labelled cells were washed in FACS buffer. At least 10,000–50,000 events for each sample were acquired on a BD Biosciences FACScan$^{+}$ instrument. Data analysis was performed with FlowJo (Tree Star, Ashland, OR, USA) software.

*Immunohistochemistry* Samples were prepared for immunohistochemical analysis as described [24]. Briefly, tissues were recovered from mice at necropsy, fixed in 10% (vol./vol.) buffered formalin, and embedded in paraffin. Sections 5 μm thick were cut. For routine histology, sections were stained with haematoxylin and eosin. Immunohistochemical staining was performed with mAbs specific for human insulin and glucagon using a DakoCytomation EnVision Dual Link system implemented on a Dako Autostainer Universal Staining System (Dako, Glostrup, Denmark). The sections were counterstained with haematoxylin.

*Statistical analyses* Data are presented as means±1 SD. Differences in the means of data were compared by Student's *t* test. Significant differences were assumed for $p$ values <0.05. All statistical analyses were performed using GraphPad Prism software (version 4.0c; GraphPad, San Diego, CA, USA).

## Results

*Phenotype of the adaptive and innate immune system in NOD*-Rag1$^{null}$ Prf1$^{null}$ Ins2$^{Akita}$ *mice* We first bred NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice bearing a single copy of the *Ins2*$^{Akita}$ mutant gene to generate an *Ins2*$^{Akita}$ homozygous strain of mice. However, we observed, as previously reported for immunocompetent mice homozygous at the *Ins2*$^{Akita}$ mutation [25], that all NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ mice bearing a homozygous mutation at the *Ins2*$^{Akita}$ gene died prior to weaning. Therefore, all further studies were performed on NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ mice bearing a single *Ins2*$^{Akita}$ mutant allele.

To determine if the introduction of the *Ins2*$^{Akita}$ mutant gene altered the immune system of immunodeficient NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ mice, we first performed a phenotypic analysis of cells in the spleen and bone marrow compartments (Table 1). Overall cellularity in the bone marrow and spleen of NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice was equivalent to NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ mice ($p$=0.858 for bone marrow; $p$=0.269 for spleen). We observed, as expected, that NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice lacked mature T (CD3$^{+}$ CD4$^{+}$and CD3$^{+}$ CD8$^{+}$) and B (B220$^{+}$ Igκ light chain$^{+}$) lymphocytes, but retained cells with an NK cell (DX5$^{+}$ CD122$^{+}$) phenotype. We have previously shown that these NK cells, although present, lack cytotoxic activity because of the effect of the null allele at the perforin locus [23]. Compared with immunocompetent NOD/Lt mice, we observed the expected elevated percentages of granulocytes (Gr-1$^{+}$ Mac-1$^{+}$), monocytes/macrophages (Gr-1$^{-}$ Mac-1$^{+}$) and erythroid lineages (Ter-119$^{+}$), as a result of decreased overall cellularity because of the absence of mature B and T cells. These data document that addition of a single copy of the *Ins2*$^{Akita}$ mutant allele does not alter the phenotype of the immune system in NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ mice.

*Spontaneous type 1 diabetes in NOD*-Rag1$^{null}$ Prf1$^{null}$ Ins2$^{Akita}$ *mice* We next followed NOD-*Rag1*$^{null}$*Prf1*$^{null}$ bearing a single *Ins2*$^{Akita}$ mutant allele for the spontaneous development of non-autoimmune hyperglycaemia. Introduction of a single copy of the *Ins2*$^{Akita}$ allele resulted in impaired glucose regulation in nearly all mice beginning as early as 3 weeks of age (Fig. 1). Susceptibility to frank hyperglycaemia showed a marked sex bias, with male mice being

**Table 1** Flow cytometric profiling of NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice

| Parameter | NOD/Lt ($n$=1) | NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ ($n$=3) | NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ ($n$=6) |
|---|---|---|---|
| Cell counts×10$^6$ | | | |
| Spleen | 50.3 | 18.5±3.8 | 32.86±7.9 |
| Bone marrow | 65.4 | 38.7±4.1 | 40.36±5.5 |
| Surface marker (%) | | | |
| Spleen | | | |
| CD3$^+$ CD4$^+$ | 23.4 | 0.1±0.0 | 0.0±0.0 |
| CD3$^+$ CD8$^+$ | 11.9 | 0.1±0.0 | 0.0±0.0 |
| B220$^+$ Igκ light chain$^+$ | 39.1 | 0.1±0.0 | 0.3±0.3 |
| B220$^+$ Igκ light chain$^-$ | 2.1 | 7.9±1.4 | 10.7±4.7 |
| GR-1$^+$ Mac-1$^+$ | 5.3 | 20.2±0.5 | 23.3±5.7 |
| GR-1$^-$ Mac-1$^+$ | 4.3 | 17.5±3.2 | 23.0±4.5 |
| Ter 119$^+$ | 6.7 | 32.6±2.7 | 31.0±4.6 |
| DX5$^+$ CD122$^+$ | 1.7 | 8.6±2.1 | 7.5±2.3 |
| LGL$^+$ CD122$^+$ | 0.3 | 3.0±0.5 | 2.1±0.6 |
| H-2K$^{d+}$ I-A$^{g7-}$ | 46.4 | 67.0±1.8 | 69.5±6.5 |
| H-2K$^{d+}$ I-A$^{g7+}$ | 52.0 | 31.0±2.0 | 25.6±5.6 |
| Bone marrow | | | |
| CD3$^+$ CD4$^+$ | 0.9 | 0.3±0.1 | 0.2±0.0 |
| CD3$^+$ CD8$^+$ | 0.6 | 0.0±0.0 | 0.0±0.0 |
| B220$^+$ Igκ light chain$^+$ | 7.4 | 0.0±0.0 | 0.0±0.0 |
| B220$^+$ Igκ light chain$^-$ | 12.4 | 13.4±2.5 | 12.6±5.2 |
| GR-1$^+$ Mac-1$^+$ | 58.2 | 53.4±3.2 | 57.7±5.3 |
| GR-1$^-$ Mac-1$^+$ | 6.0 | 10.5±0.8 | 10.4±0.8 |
| Ter 119$^+$ | 9.2 | 13.9±0.9 | 10.2±1.7 |
| DX5$^+$ CD122$^+$ | 0.9 | 2.6±0.2 | 1.9±0.3 |
| LGL$^+$ CD122$^+$ | 0.2 | 1.0±0.1 | 0.5±0.1 |
| H-2K$^{d+}$ I-A$^{g7-}$ | 63.7 | 71.2±2.1 | 70.3±5.4 |
| H-2K$^{d+}$ I-A$^{g7+}$ | 27.5 | 26.5±3.0 | 26.6±6.2 |

All data are presented as means±1 SD

Spleens and bone marrow were recovered from cohorts of NOD/Lt, NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ and NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice and prepared for flow cytometry to determine the indicated cell surface markers as described in the "Materials and methods" section

Differences between NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ and NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice were determined by Student's $t$ test and no significant differences were observed

Haematopoietic lineages examined include: CD4$^+$ T cells (CD3$^+$ CD4$^+$); CD8$^+$ T cells (CD3$^+$ CD8$^+$); B cells (B220$^+$ Igκ light chain$^+$); B cell precursors (B220$^+$ Igκ light chain$^-$); granulocytes (Gr-1$^+$ Mac-1$^+$); macrophage/myeloid cells (Gr-1$^-$ Mac-1$^+$); erythrocytes/erythroid precursors (Ter-119$^+$); NK cells (DX5$^+$ CD122$^+$ and LGL$^+$ CD122$^+$); and antigen-presenting cells (H2-K$^{d+}$ I-A$^{g7+}$). A single NOD/Lt mouse is included for comparison as a positive control for antibodies against differentiation antigens on cells of the adaptive immune system

more likely to develop hyperglycaemia than female mice (Fig. 1), which is in agreement with effects of the *Ins2*$^{Akita}$ mutation observed on other strain backgrounds and in immunocompetent mice [19].

The presence of the *Ins2*$^{Akita}$ mutant allele has been reported to induce beta cell apoptosis in the absence of inflammation [19]. To confirm this on the new strain of immunodeficient *Ins2*$^{Akita}$ mice, we performed immunohistochemical analysis on the pancreases of NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice at 50, 89 and 234 days of age and a single NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ mouse at 89 days of age with two wild-type alleles at the *Ins2* locus. Normal islet architecture was observed in *Ins2* wild-type mice at all ages examined (Fig. 2a–c and data not shown). As expected, strong insulin staining was found throughout the islet (Fig. 2b) with

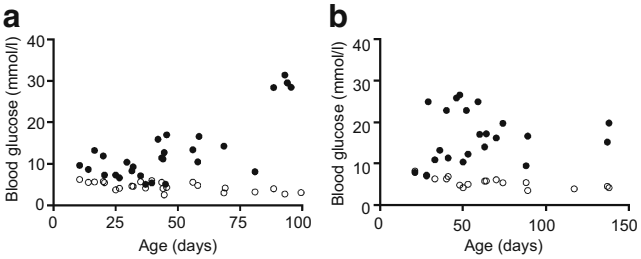


**Fig. 1** Spontaneous development of hyperglycaemia in NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice. Groups of mice were analysed for the development of hyperglycaemia over time and stratified by sex. **a** female; **b** male. Additionally, littermates of each sex were typed at *Ins2* to determine mice that were homozygous wild-type (white circles) or harboured a single *Akita* allele (black circles)

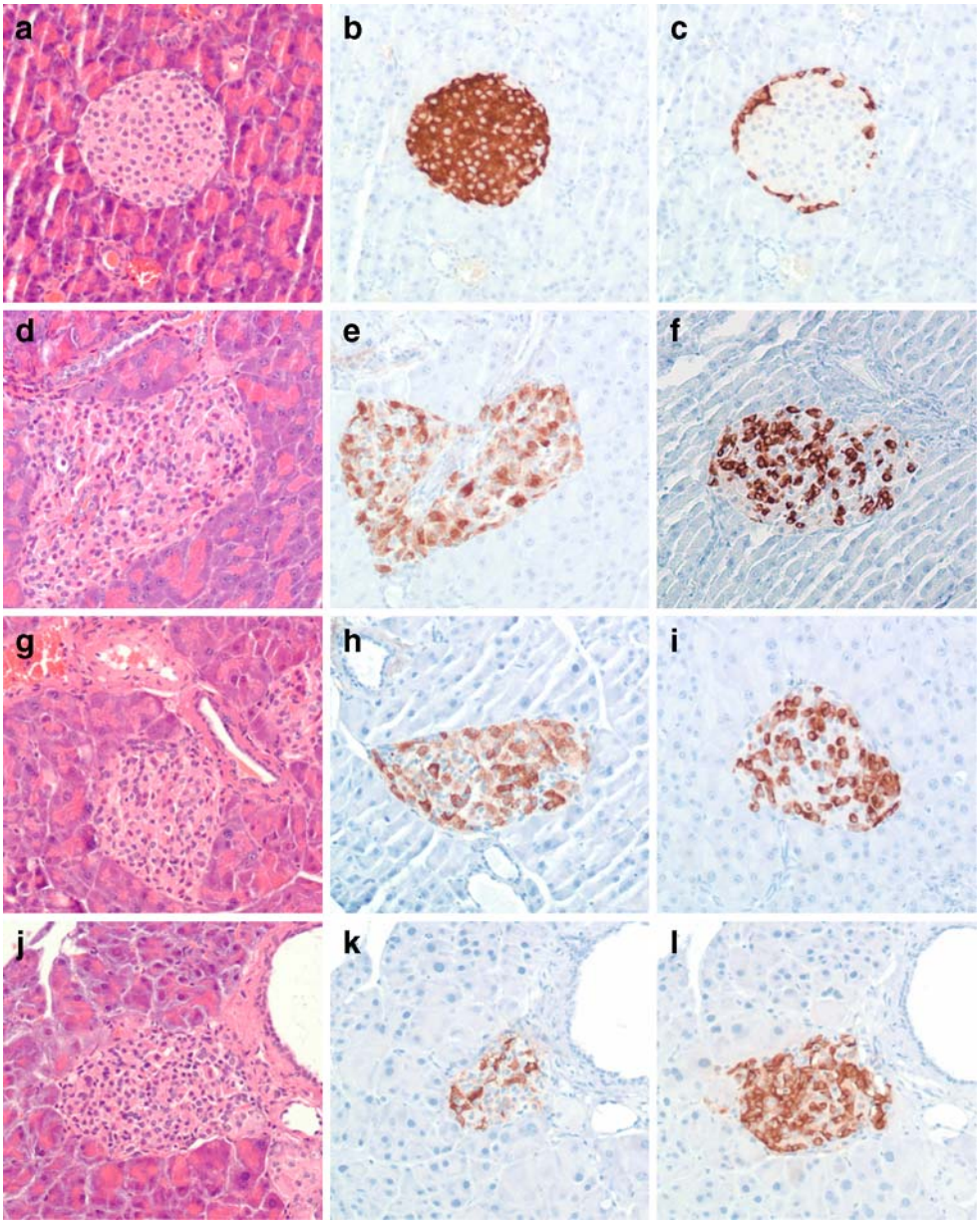


**Fig. 2** Immunohistochemical analysis of pancreatic islets in NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ and NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/+}$ control mice. Pancreas sections were prepared for immunohistochemical analysis from formalin-fixed, paraffin-embedded, tissues as described in the Methods. **a**, **d**, **g**, **j** Haematoxylin and eosin staining of pancreases from control NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/+}$mice (**a**) and hyperglycaemic NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice (**d**, **g**, **j**). **b**, **e**, **h**, **k** Anti-insulin staining of pancreases from control NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/+}$mice (**b**) and hyperglycaemic NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice (**e**, **h**, **k**). **c**, **f**, **i**, **l** Anti-glucagon staining of pancreases from control NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/+}$ mice (**c**) and hyperglycaemic NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice (**f**, **i**, **l**). All mice were males. Ages and fasting blood glucose levels of mice were: **a**–**c**, 89 days, 4.1 mmol/l; **d**–**f**, 50 days, 9.5 mmol/l; **g**–**i**, 89 days, 27.4 mmol/l; **j**–**l**; 234 days, 24.8 mmol/l. All images shown at ×200 magnification

glucagon staining localising to the periphery of the islet (Fig. 2c) [26]. Islets were still present in the pancreases of NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice. However, they exhibited a progressively altered morphology with age (Fig. 2d–l). Insulin staining was lower compared with *Ins2* wild-type mice at 50 days of age and continued to diminish as mice aged (Fig. 2e,h,k). Glucagon staining was present throughout the islet at all time points (Fig. 2f,i,l), consistent with the phenomenon of a 'collapsed' islet that is largely devoid of beta cells [26]. These findings correlated with elevated fasting blood glucose levels in *Ins2*$^{Akita}$ mice taken immediately before killing for immunohistological analysis.

*Streptozotocin-induced type 1 diabetes in NOD*-scid Il2rγ$^{null}$ *mice* In a previous study, we documented that streptozotocin is effective at inducing hyperglycaemia in NOD-*scid* *Il2rγ*$^{null}$ mice, but did not determine if sex-specific differences exist in their susceptibility to streptozotocin, nor was an optimal dose determined [24]. Therefore, we delivered several different doses of a single administration of streptozotocin to cohorts of male and female NOD-*scid* *Il2rγ*$^{null}$ mice. Hyperglycaemia was induced in both male and female mice at doses ranging from 120 to 160 mg/kg (Fig. 3 and data not shown). At all doses tested, some mice failed to become hyperglycaemic, but the proportion of mice remaining normoglycaemic did not correlate with the dose of streptozotocin administered or their sex.

*Restoration of euglycaemia by transplantation of human islets in diabetic NOD*-Rag1$^{null}$Prf1$^{null}$Ins2$^{Akita}$ *and NOD-*

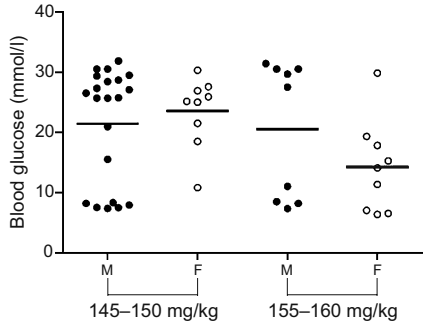


**Fig. 3** Induction of diabetes in NOD-*scid Il2rγ*$^{null}$ mice. Cohorts of male (black circles) and female (white circles) NOD-*scid Il2rγ*$^{null}$ mice were randomised to receive a single i.p. injection of streptozotocin at doses of 145, 150, 155 or 160 mg/kg body weight and the results are stratified into lower dose (145 and 150 mg/kg) and higher dose (155 and 160 mg/kg). Maximum blood glucose readings for each recipient at up to 10 days after injection are shown. Horizontal bar, mean value

scid Il2rγ$^{null}$ *mice* The ability to quantify the beta cell mass needed to restore euglycaemia is an important goal in regenerative medicine treatments for type 1 diabetes. To test human beta cell function in vivo, researchers need standardised hyperglycaemic translational models that are amenable hosts for human cells and tissues. Therefore, we evaluated the number of transplanted human islets required to restore euglycaemia in spontaneously hyperglycaemic NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ and streptozotocin-diabetic NOD-*scid Il2rγ*$^{null}$ mice. Mice of each strain were verified to be hyperglycaemic before receiving islet transplants into the renal subcapsular space.

In NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice, all mice demonstrated an initial drop in blood sugar, indicative of graft function (Fig. 4). However, at 2,000 and 3,000 IEQ per recipient, hyperglycaemia eventually returned in a majority of the transplanted mice. At a transplanted islet dose of 4,000 IEQ, all recipient mice were restored to euglycaemia for the entire period of observation (Figs 4 and 6). Similarly, in streptozotocin-diabetic NOD-*scid Il2rγ*$^{null}$ mice, human islet transplants of 1,000 to 3,000 IEQ per recipient failed to reliably restore euglycaemia, while 4,000 IEQ per recipient was successful in two long-term surviving mice receiving this islet dose (Figs 5 and 6), similarly to what we have observed in other studies using this recipient [24].

## Discussion

In this study we describe the development and characterisation of a spontaneously diabetic immunodeficient strain of NOD mice that can be transplanted with human islets to test their ability to regulate glucose homeostasis and maintain normoglycaemia. This is the first immunodeficient hyperglycaemic mouse model that has been described based on the *Ins2*$^{Akita}$ mutation that can be used to investigate in vivo the function of human islets or human beta stem and progenitor cells without the need for administration of toxic agents such as streptozotocin or alloxan. This model system will now permit human islets or beta stem and progenitor cells to be transplanted into normoglycaemic mice that will subsequently lose endogenous beta cell function over time without the need for administration of toxic agents. This model can also be used to investigate the function of human islet and beta stem or progenitor cells in the presence of a human immune system following engraftment with allogeneic human peripheral blood mononuclear cells [23].

We first observed that hyperglycaemia developed by 3–5 weeks of age in male NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice, but developed at a slower rate with lower levels of hyperglycaemia in female mice. The basis for this sex bias in the ability of the *Ins2*$^{Akita}$ mutation to induce hyperglycaemia is not known, but may result from sex differences in steroid hormones. Natural oestrogens are known to improve glucose tolerance through a beta cytotrophic effect and enhanced insulin sensitivity [27]. The sex bias and the role of sex-steroid hormones in the hyperglycaemic-

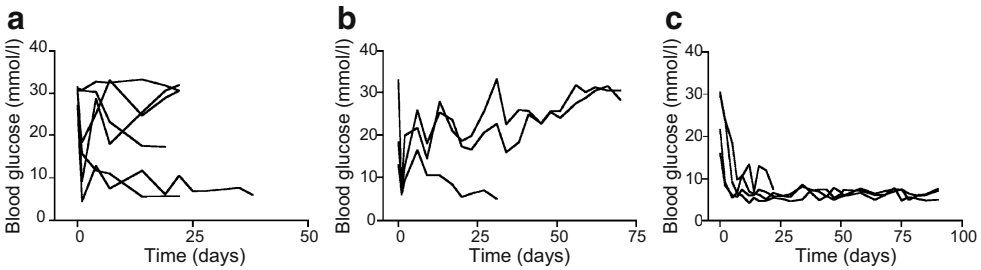


**Fig. 4** Reversal of diabetes in NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice following human islet transplantation. Hyperglycaemic male NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ mice were randomised into three groups and received either 2,000 (**a**, $n$=6), 3,000 (**b**, $n$=3) or 4,000 (**c**, $n$=4) IEQ of human islets into the renal subcapsular space. Blood glucose levels in individual mice at each human islet dose are shown over the follow-up period

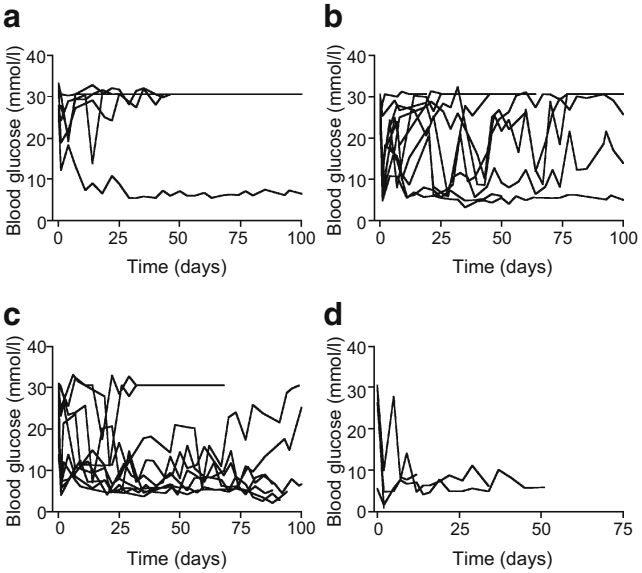


**Fig. 5** Reversal of diabetes in *NOD-scid Il2rγ*$^{null}$ mice following human islet transplantation. NOD-*scid Il2rγ*$^{null}$ mice were rendered hyperglycaemic by a single i.p. injection of 140 mg/kg streptozotocin, randomised into four different groups and received either 1,000 (**a**, $n$=6), 2,000 (**b**, $n$=10), 3,000 (**c**, $n$=11) or 4,000 (**d**, $n$=4) IEQ of human islets into the renal subcapsular space. Blood glucose levels in individual mice at each human islet dose are shown over the follow-up period

inducing effects of the *Ins2*$^{Akita}$ mutation is an important next step of investigation.

We next determined that permanent euglycaemia could be restored in NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice when sufficient human islet cell mass was transplanted. However, at suboptimal doses of transplanted human islets, hyperglycaemia was only transiently reversed, and these mice eventually returned to their pretransplant hyperglycaemic state. Interestingly, NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice transplanted with suboptimal human islet doses returned to hyperglycaemia more rapidly than streptozotocin-treated NOD-*scid Il2rγ*$^{null}$ mice transplanted with the same human islet dose. Thus, NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice represent a more stringent model to evaluate the efficacy of islet cell transplantation. The reasons why NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice are more stringent than NOD-*scid Il2rγ*$^{null}$ mice are not immediately clear, but we speculate that the spontaneous elimination of beta cells in NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice as a result of the *Ins2*$^{Akita}$ mutation may be more complete than the streptozotocin-mediated elimination of beta cells in NOD-*scid Il2rγ*$^{null}$ mice. This would permit a low level of endogenous insulin production in chemically diabetic mice that may relieve the stress on the transplanted islets and prolong their lifespan under conditions of suboptimal islet cell mass.

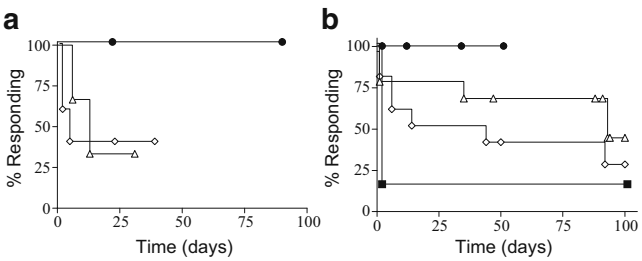


**Fig. 6** Kaplan–Meier plots of islet graft function in human islet transplanted mice. The human islet transplantation data presented in Figs 4 and 5 are displayed as Kaplan–Meier plots for NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{+/Akita}$ (**a**) and NOD-*scid Il2rγ*$^{null}$ (**b**) mice. Loss of graft function ($y$-axis) was designated as three consecutive blood glucose readings of >13.9 mmol/l. Squares, 1,000 IEQ; diamonds, 2,000 IEQ; triangles, 3,000 IEQ; circles, 4,000 IEQ

Differentiation of stem cells into glucose-responsive, insulin-producing cells is an attractive modality to restore euglycaemia in type 1 diabetes. An impediment to achieving this goal in regenerative medicine is the inability to test the ability of human beta stem and progenitor cells to differentiate into glucose-responsive, insulin-producing cells that can then regulate glucose homeostasis in vivo [1, 2]. We propose that the NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mouse represents an ideal host for this purpose. Hyperglycaemic NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ mice are excellent hosts for human islets [23], and we document that NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mice are also excellent hosts for human islets. Implantation of human beta stem and progenitor cells into young mice, prior to the development of hyperglycaemia, would provide a 'normoglycaemic environment' for the progenitor cells that will then be exposed to the inductive signals for beta cell differentiation and function as endogenous beta cell death and hyperglycaemia gradually develop. Moreover, this will avoid the potential effects of 'glucose toxicity' on the transplanted beta progenitor cells [28, 29] as they will initially encounter a normoglycaemic environment upon transplantation. At the present time, other potential models to induce hyperglycaemia after transplantation of human beta progenitor cells such as chemical toxins or approaches based on the diphtheria toxin receptor expressed on endogenous beta cells have the potential to also harm the transplanted human beta progenitor cells. In a recent model of endogenous beta cell destruction, induction of hyperglycaemia was induced by administration of doxycycline in a transgenic mouse [30]. Upon withdrawal of the drug, the endogenous beta cells undergo proliferation and restore normoglycaemia, preventing the use of this model for analyses of the function of transplanted human islets or beta progenitor cells.

This study now provides an important baseline level of the human beta cell mass needed to restore euglycaemia in the immunodeficient NOD-*Rag1*$^{null}$ *Prf1*$^{null}$ *Ins2*$^{Akita}$ mouse that should be helpful in guiding investigators when testing the efficacy of their cell-based therapy in diabetes reversal. However, this study does not address the ability of beta

cells alone (as opposed to intact islets) to reverse diabetes, an area of ongoing investigation in our laboratory.

In summary, we have described a new, spontaneously hyperglycaemic immunodeficient mouse strain based on the *Ins2*$^{Akita}$ mutation and shown that it supports curative human islet transplantation. We propose that this model will be of great utility for the in vivo study of human islets and human beta stem and progenitor cell function.

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**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

## References

1. Shultz LD, Pearson T, King M et al (2007) Humanized NOD/LtSz-scid IL2 receptor common gamma chain knockout mice in diabetes research. Ann N Y Acad Sci 1103:77–89
2. Shultz LD, Ishikawa F, Greiner DL (2007) Humanized mice in translational biomedical research. Nat Rev Immunol 7:118–130
3. Shultz LD, Schweitzer PA, Christianson SW et al (1995) Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol 154:180–191
4. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M (2006) Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 24:74–85
5. Tian X, Woll PS, Morris JK, Linehan JL, Kaufman DS (2006) Hematopoietic engraftment of human embryonic stem cell-derived cells is regulated by recipient innate immunity. Stem Cells 24:1370–1380
6. Greiner DL, Hesselton RA, Shultz LD (1998) SCID mouse models of human stem cell engraftment. Stem Cells 16:166–177
7. Nakamura N, Woda BA, Tafuri A et al (1990) Intrinsic cytotoxicity of natural killer cells to pancreatic islets in vitro. Diabetes 39:836–843
8. Koevary SB (1988) In vitro natural killer cell activity in the spontaneously diabetic BB/Wor rat: effects of serum on lysis of insulinoma cells. Diabetes Res 8:77–84
9. MacKay P, Jacobson J, Rabinovitch A (1986) Spontaneous diabetes mellitus in the Bio-Breeding/Worcester rat. Evidence in vitro for natural killer cell lysis of islet cells. J Clinical Invest 77:916–924
10. Traggiai E, Chicha L, Mazzucchelli L et al (2004) Development of a human adaptive immune system in cord blood cell-transplanted mice. Science 304:104–107
11. Shultz LD, Banuelos S, Lyons B et al (2003) NOD/LtSz-Rag1nullPfpnull mice: a new model system with increased levels of human peripheral leukocyte and hematopoietic stem-cell engraftment. Transplantation 76:1036–1042
12. Shultz LD, Lyons BL, Burzenski LM et al (2005) Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol 174:6477–6489
13. Junod A, Lambert AE, Stauffacher W, Renold AE (1969) Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J Clin Invest 48:2129–2139
14. Lenzen S, Panten U (1988) Alloxan: history and mechanism of action. Diabetologia 31:337–342
15. Luo B, Chan WF, Lord SJ et al (2007) Diabetes induces rapid suppression of adaptive immunity followed by homeostatic T cell proliferation. Scand J Immunol 65:22–31
16. Rana SV, Rastogi N (2000) Antioxidative enzymes in the liver and kidney of alloxan induced diabetic rats and their implications in cadmium toxicity. Physiol Chem Phys Medical NMR 32:67–74
17. Levine BS, Henry MC, Port CD, Rosen E (1980) Toxicologic evaluation of streptozotocin (NSC 85998) in mice, dogs and monkeys. Drug Chem Toxicol 3:201–212
18. Bolzan AD, Bianchi MS (2002) Genotoxicity of streptozotocin. Mutat Res 512:121–134
19. Yoshioka M, Kayo T, Ikeda T, Koizumi A (1997) A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice. Diabetes 46:887–894
20. Mathews CE, Langley SH, Leiter EH (2002) New mouse model to study islet transplantation in insulin-dependent diabetes mellitus. Transplantation 73:1333–1336
21. Ron D (2002) Proteotoxicity in the endoplasmic reticulum: lessons from the Akita diabetic mouse. J Clinic Invest 109:443–445
22. Izumi T, Yokota-Hashimoto H, Zhao S, Wang J, Halban PA, Takeuchi T (2003) Dominant negative pathogenesis by mutant proinsulin in the Akita diabetic mouse. Diabetes 52:409–416
23. Banuelos SJ, Shultz LD, Greiner DL et al (2004) Rejection of human islets and human HLA-A2.1 transgenic mouse islets by alloreactive human lymphocytes in immunodeficient NOD-scid and NOD-Rag1(null)Prf1(null) mice. Clin Immunol 112:273–283
24. King M, Pearson T, Shultz LD et al (2008) A new Hu-PBL model for the study of human islet alloreactivity based on NOD-scid mice bearing a targeted mutation in the IL-2 receptor gamma chain gene. Clin Immunol 126:303–314
25. Kayo T, Koizumi A (1998) Mapping of murine diabetogenic gene mody on chromosome 7 at D7Mit258 and its involvement in pancreatic islet and beta cell development during the perinatal period. J Clin Invest 101:2112–2118
26. Reddy S, Chai RC, Rodrigues JA, Hsu TH, Robinson E (2008) Presence of residual beta cells and co-existing islet autoimmunity in the NOD mouse during longstanding diabetes: a combined histochemical and immunohistochemical study. J Mol Histol 39:25–36
27. Lenzen S, Bailey CJ (1984) Thyroid hormones, gonadal and adrenocortical steroids and the function of the islets of Langerhans. Endocr Rev 5:411–434
28. Kaneto H, Matsuoka TA, Katakami N et al (2007) Oxidative stress and the JNK pathway are involved in the development of type 1 and type 2 diabetes. Curr Mol Med 7:674–686
29. Palmeira CM, Rolo AP, Berthiaume J, Bjork JA, Wallace KB (2007) Hyperglycemia decreases mitochondrial function: the regulatory role of mitochondrial biogenesis. Toxicol Appl Pharmacol 225:214–220
30. Nir T, Melton DA, Dor Y (2007) Recovery from diabetes in mice by beta cell regeneration. J Clin Invest 117:2553–2561