Induction of Chimerism Permits Low-Dose Islet Grafts in the Liver or Pancreas to Reverse Refractory Autoimmune Diabetes

Chunyan Zhang,¹ Miao Wang,¹ Jeremy J. Racine,¹,² Hongjun Liu,¹ Chia-Lei Lin,¹ Indu Nair,¹ Joyce Lau,³ Yu-An Cao,⁴ Ivan Todorov,¹,² Mark Atkinson,⁵ and Defu Zeng¹,²

OBJECTIVE—To test whether induction of chimerism lowers the amount of donor islets required for reversal of diabetes and renders the pancreas a suitable site for islet grafts in autoimmune diabetic mice.

RESEARCH DESIGN AND METHODS—The required donor islet dose for reversal of diabetes in late-stage diabetic NOD mice after transplantation into the liver or pancreas was compared under immunosuppression or after induction of chimerism. Recipient mice were monitored for blood glucose levels and measured for insulin-secreting capacity. Islet grafts were evaluated for β-cell proliferation, β-cell functional gene expression, and revascularization.

RESULTS—With immunosuppression, transplantation of 1,000, but not 600, donor islets was able to reverse diabetes when transplanted into the liver, but transplantation of 1,000 islets was not able to reverse diabetes when transplanted into the pancreas. In contrast, after induction of chimerism, transplantation of as few as 100 donor islets was able to reverse diabetes when transplanted into either the liver or pancreas. Interestingly, when lower doses (50 or 25) of islets were transplanted, donor islets in the pancreas were much more effective in reversal of diabetes than in the liver, which was associated with higher β-cell replication rate, better β-cell functional gene expression, and higher vascular density of graft islets in the pancreas.

CONCLUSIONS—Induction of chimerism not only provides immune tolerance to donor islets, but also markedly reduces the required amount of donor islets for reversal of diabetes. In addition, this process renders the pancreas a more superior site than the liver for donor islets in autoimmune mice. Diabetes 59: 2228–2236, 2010
autoimmune NOD mice (2,23–26). Furthermore, we recently reported that induction of chimerism under a radiation-free and graft versus host disease (GVHD) preventive anti-CD3-based conditioning regimen led to reversal of autoimmunity and elimination of insulitis (2,27). Beyond this, it has been reported that adult β-cells can replicate to reverse hyperglycemia in nonautoimmune mice (12). In addition, we have observed that elimination of insulitis by induction of chimerism, residual islet β-cells in the pancreas proliferated to reverse diabetes in new-onset, although not in late-stage, diabetic NOD mice (2).

In the current study, we tested whether induction of chimerism under the radiation-free anti-CD3-based conditioning regimen lowers the amount of donor islets required for reversal of diabetes and whether the pancreas would provide a suitable site for islet grafts in late-stage diabetic NOD mice. Herein, we have demonstrated that, after induction of chimerism, the required amount of donor islets for reversal of diabetes was markedly reduced when transplanted into the liver or pancreas. In addition, our studies suggest that after induction of chimerism, the pancreas may provide a superior site for donor islets in comparison with the liver, as assessed by enhanced β-cell replication, better long-term β-cell function, and improved revascularization of the graft islets in the pancreas.

RESEARCH DESIGN AND METHODS

Female NOD/LtJ (H-2b) and FVB/N (H-2d) mice were purchased from Jackson Laboratory (Bar Harbor, ME). The luciferase transgenic (Luc+) FVB/N line was generated as previously described (28). All animals were maintained in a pathogen-free room at City of Hope Research Animal Facilities (Duarte, CA). The animal-use procedures were approved by the Institutional Committee of City of Hope.

Immunosuppressant therapy. Late-stage (>3 weeks after onset) diabetic NOD mice were given Edmonton protocol of chemotherapy before islet transplantation, including administration of rapamycin (i.p. 2 mg/kg daily, LC Laboratories, MA), tacrolimus (i.p. 0.6 mg/kg daily, LC Laboratories, Woburn, MA), and anti-mouse-IL-2 mAb (i.p. 0.5 mg/mouse weekly, S4B6–1).

Bone marrow transplantation. These procedures were described in our previous publications (2,29) and in the supplementary materials and methods, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db10-0450/DC1.

Isolation of islets, islet transplantation, in vivo and ex vivo bioluminescent imaging, insulin level, and glucose tolerance test. These procedures have been described in publications of ours and others (2,29,30) and in the supplementary materials and methods.

Histopathology of pancreatic islets, bromodeoxyuridine labeling and sequential chlorodeoxyuridine (CLDU) and iododeoxyuridine (IDU) labeling of proliferating β-cells and immunofluorescent staining. These procedures have been described in publications of ours and others (2,27,31) and in the supplementary materials and methods.

Measurement of gene expression levels of the retrieved islet grafts. Retrieving graft islets from the liver and pancreas were performed as described previously (32,33). The primers used for real-time PCR measurement of gene expression levels of luciferase, insulin, pancreatic duodenal homeobox-1 (PDX-1), GLUT2, glucokinase (GCK), and glucagon were described by others (34,35), and real-time PCR was performed as described in our previous publications (36) and in the supplementary materials and methods.

Evaluation of vascular density. Sections were stained for lectin from Bandeiraea simplicifolia (Sigma) followed by Texas Red conjugated Streptavidin (Jackson Immunoresearch). Vascular density was identified and evaluated as previously described (37,38) and in the supplementary materials and methods.

Amylase test. Amylase activity was measured by EnzChek Ultra Amylase Assay Kit (E30651; Molecular Probes) following the manufacturer’s protocol.

Statistical Analysis. Comparison of changes of serum blood glucose levels after hematopoietic cell transplantation (HCT) was evaluated by the log-rank test (Prism, version 4.0, GraphPad, San Diego, CA). Comparison of kinetic blood glucose change in glucose tolerance test (GTT) test was evaluated with two-way ANOVA. Comparison of means among multiple groups was evaluated with one-way ANOVA; comparison of two means was performed with two-tailed Student t test.

RESULTS

A large amount of donor islets that could reverse diabetes when implanted in the liver did not reverse diabetes when implanted in the pancreas in late-stage diabetic NOD mice under immunosuppressant therapy. It was reported that islet grafts functioned better in the pancreas than in the liver in nonautoimmune recipients (15,16). However, it is not yet clear whether the pancreas of the autoimmune recipients can be used as a site of donor islets. Under immunosuppressant therapy, 600 or 1,000 donor islets were required to reverse diabetes in diabetic NOD mice (39,40). Therefore, we transplanted 600 or 1,000 donor islets into the liver or pancreas of the late-stage diabetic NOD mice, while immunosuppressant therapy of Edmonton protocol was used to prevent graft rejection. Harvesting 1,000 islets required ~5 donors because the average yield in our studies was 209 ± 8 per donor (mean ± SE, N = 20), which was similar to a previous report (41). We observed that although 1,000 donor islets implanted in the liver were able to reverse diabetes in all (6 of 6) of the recipients, the same amount of donor islets implanted in the pancreas did not reverse diabetes (0 of 6) (P < 0.001, Fig. 1A and B). Lowering the donor islet dose to 600 in the liver also resulted in an inability to stably reverse diabetes (Fig. 1A). These results indicate that under this form of immunosuppressant therapy, the pancreas of the autoimmune NOD mice is not a suitable site for donor islets.

Induction of chimerism not only markedly reduced the required donor islet dose for reversal of late-stage diabetes, but also rendered the pancreas of the autoimmune NOD mice a suitable site for islet grafts. We previously reported that induction of chimerism resulted in reversal of autoimmunity, elimination of insulitis, and immune tolerance to donor islets when implanted under the kidney capsule (2). Therefore, we tested whether the pancreas of the chimeric diabetic NOD recipients could be a suitable site for islet grafts. Accordingly, late-stage diabetic NOD mice were induced to develop chimerism by conditioning with anti-CD3/CD8 along with...
one injection of donor CD4<sup>+</sup> T-depleted spleen cells and bone marrow cells (50 × 10<sup>6</sup> each), as described in RESEARCH DESIGN AND METHODS and in our previous publication (2). Next, 600 donor islets, which did not reverse diabetes in the late-stage diabetic NOD mice under the above mentioned immunosuppressant therapy, were transplanted into the pancreas or liver of the chimeric recipients on the next day after injection of donor bone marrow cells. We found that 100% (6 of 6) of the chimeric recipients with 600 donor islets in the pancreas or liver showed normal glycemia for >120 days, although they all had hyperglycemia (>500 mg/dl) before islet transplantation (Fig. 2A). The chimeric late-stage diabetic NOD mice without islet transplantation continued to have hyperglycemia, and the chimeric recipients with islet grafts under kidney capsule showed hyperglycemia again after nephrectomy (supplementary Fig. 1). Next, we titrated down the amount of donor islets and transplanted 300, 200, and 100 donor islets into the pancreas or liver of the chimeric recipients. We found that transplantation of 100 or more donor islets was able to reverse hyperglycemia in all the recipients (Fig. 2A–D).

In addition, we found that transplantation of 100 islets from syngeneic NOD mice was also able to reverse late-stage diabetes in the chimeric NOD mice in which pre-existing insulitis cleared up after transplantation. Furthermore, the chimeric recipients rapidly rejected the islets from third-party donors when implanted in the pancreas (supplementary Fig. 2). These results indicate that the chimeric recipients are tolerant to both donor- and host-type graft islets in the pancreas. Taken together, induction of chimerism not only reverses autoimmunity and provides immune tolerance to donor islets, but also markedly reduces the required amount of donor islets for reversal of diabetes and renders the pancreas a suitable site for islet grafts.

**Small amounts of donor islets implanted in the pancreas were more effective in reversing diabetes than in the liver after induction of chimerism.** Next, we further titrated down the dose of donor islets to 50 and 25 islets per chimeric recipient. We observed that although 50 islets implanted in the pancreas resulted in long-term reversal of diabetes in 70% (7 of 10) chimeric recipients, 50 islets implanted in the liver resulted in long-term reversal in only 20% (2 of 10) of the chimeric recipients (P < 0.01, Fig. 2E). We should point out that some of the chimeric recipients with 50 donor islets in the liver showed transient normal glycemia and relapse of hyperglycemia. This relapse of hyperglycemia was not caused by graft rejection, because there was still strong bioluminescent imaging (BLI) signaling of the islet grafts, and no infiltration observed in the grafts (supplementary Fig. 3). In addition, 40% (4 of 10) of the chimeric recipients with 25 donor islets in the pancreas reached normal glycemia, although it took >2 months for some recipients. In contrast, none (0 of 10) of the chimeric recipients with 25 donor islets in the liver reached normal glycemia (P < 0.01, Fig. 2F). These results indicate that when small amounts of donor islets are transplanted, the pancreas is a better site than the liver for islet grafts.

**Graft islet β-cells in the pancreas had better long-term function than those in the liver.** It has been reported that, in a syngeneic transplantation, islet grafts in the liver showed reduced capacity of insulin production compared with grafts in the pancreas (15). Thus, 120 days after islet transplantation, we compared the insulin secre-
islets in the pancreas, especially at 15–90 min after glucose was significantly slower than that of recipients with 100 blood glucose in recipients with 100 graft islets in the liver was no significant difference in changes of serum blood and pancreas. Blood glucose recovery curve and serum insulin levels before 120 days after islet transplantation, a GTT test was performed with chimeric recipients by GTT. We found that there islet transplantation. The islet grafts were retrieved from the pancreas and liver, and the donor-type islets were identified with ex vivo BLI. The expression levels of metabolic genes of β-cells were compared using donor islet-specific luciferase mRNA as an internal control to avoid the influence of differences in the purity of the retrieved islets. The relative expression levels of insulin, PDX-1, GLUT2, GCK, and glucagon were calculated by the expression levels of retrieved grafts versus islets before transplantation. Mean ± SE of four replicate experiments is shown.

FIG. 3. Comparison of insulin secretion capacity of long-term islet grafts in the pancreas and liver of the long-term chimeric recipients by GTT test. A. Blood glucose and serum insulin levels before and after glucose injection are shown. Mean ± SE of 6 mice in each group is shown.

Graft islet β-cells replicated better in the pancreas than in the liver. We previously reported that residual islets in new-onset diabetic NOD mice could proliferate to reverse diabetes after induction of chimerism (2). We tested whether graft islet β-cells could also proliferate in the pancreas of the chimeric recipients. Accordingly, the chimeric recipients with donor islet grafts in the pancreas or liver were injected intraperitoneally with bromodeoxyuridine (BrdU) (50 μg/g body weight) for 2 weeks immediately after islet transplantation. The islet grafts were identified by in vivo and ex vivo BLI (Fig. 5A). We found that β-cells in grafts from both pancreas and liver proliferated, as determined by incorporation of BrdU, but the graft islet β-cell proliferation in pancreas was twofold more vigorous in the pancreas than in the liver, as judged by the percentage of BrdU+Insulin+ β-cells in the graft (P < 0.01, Fig. 5B and D). Furthermore, we used sequential CldU and IdU labeling to test whether the proliferation of
β-cells were from β-cell replication or neogenesis as described by Teta et al. (31). We found that the proliferating β-cells in islet grafts were either CldU+ or IdU+, and few were CldU+IdU+ (Fig. 5C and E). The percentage of CldU+ or IdU+ insulin-secreting β-cells in islet grafts from the pancreas was nearly twofold higher than those from the liver ($P < 0.01$, Fig. 5C and E). These results indicate that the proliferating β-cells in islet grafts are from β-cell replication, but not neogenesis, and donor islet β-cells replicate better in the pancreas than in the liver.

Graft islets in the pancreas had higher vascular density than in the liver. It has been reported that revascularization of islets was important for graft islet β-cell proliferation and function (42–44). It was also
reported that revascularization of graft islets was initiated within 2–4 days and was completed by 14 days after islet transplantation (45,46). Therefore, we compared the revascularization of graft islets in the pancreas and in the liver 3 weeks after transplantation. Accordingly, 3 weeks after islet transplantation, graft islets were identified by in vivo and ex vivo BLI and harvested as described in Fig. 5. The sections of the selected pancreas tissues were stained with the lectin *Bandeiraea simplicifolia* (BS-1) to visualize blood vessels inside the graft islets as described previously (37,38). We found that vascular density in the graft islets from the pancreas was similar to the donor islets before transplantation and was approximately fourfold higher than in the graft islets from the liver (\( P < 0.01 \), Fig. 6). These results indicate that the better proliferation and function of the graft islets in the pancreas than in the liver may result from better intraislet revascularization of graft islets in the pancreas.

**No pancreatitis is induced by islet transplantation into the pancreas.** One major concern regarding implantation of islets in the pancreas is induction of pancreatitis. Therefore, we carefully compared the body weight and serum amylase levels of the recipients with islet grafts in the pancreas and liver. We found that all the recipients with 300–600 islets in the pancreas or liver showed healthy appearance, similar body weight, and little amylase in their serum over an observation period of 120 days (Fig. 7A and B).

**DISCUSSION**

We have demonstrated that compared with immunosuppressant therapy, induction of chimerism under the anti-CD3-based conditioning regimen not only provided immune tolerance to donor islets, but also markedly reduced the required amount of donor islets for reversal of
diabetes. Furthermore, induction of chimerism rendered the pancreas of autoimmune diabetic recipients a better site than the liver for donor islets, especially when small amounts of donor islets were transplanted, which was associated with better replication and long-term function of donor islet β-cells, as well as better revascularization of the graft islets in the pancreas.

We observed that induction of chimerism markedly reduced the required amount of donor islets for reversal of severe late-stage type 1 diabetes. We found that with immunosuppressants of the Edmonton protocol for prevention of islet graft rejection, we needed to transplant >600 donor islets (>3 donors) in the liver to reverse diabetes. In contrast, as few as 100 donor islets implanted in the liver (± ½ islets from a donor) were able to reverse the disease after induction of chimerism. This is more than a sixfold reduction. This required amount of donor islets for reversal of diabetes in chimeric recipients is equivalent to the required amount in syngeneic islet transplantation in the liver as reported by others (47,48). In fact, transplantation of donor islets into the recipients with donor BM chimerism is similar to a syngeneic islet transplantation. Thus, the marked reduction of required donor islet amount for reversal of diabetes in the chimeric recipients is associated with avoidance of the immune rejection mediated by allo- and autoimmunity, as well as the avoidance of chemotoxicity to donor islets.

We also observed that induction of chimerism rendered the pancreas of an autoimmune diabetic recipient a suitable site for donor islet grafts. It was shown that the pancreas appears to be a better site than the liver for donor islets in the syngeneic islet transplantation (15,16). However, we found that under immunosuppressant therapy for prevention of rejection, a large dose (1,000) of donor islets that could reverse diabetes when implanted in the liver failed to reverse diabetes when implanted in the pancreas of the late-stage diabetic NOD mice. In contrast, after induction of chimerism, a transplantation of as few as 25 islets into the pancreas was able to reverse the disease in 40% of the recipients. This is a more than a 40-fold reduction in the required amount of donor islets for reversal of diabetes, as compared with immunosuppressant therapy for prevention of rejection. One important factor for induction of chimerism to render the pancreas a suitable site for donor islets is the elimination of pre-existing insulitis. Our previous studies have shown that induction of chimerism not only prevented the recurrence of autoimmunity, but also eliminated insulitis in the host pancreas (2,27). This graft versus autoimmunity (GVA) effect was mediated by donor CD8+ T-cells in the bone marrow transplant, and this GVA effect was not associated with GVHD in the recipients conditioned with anti-CD3–based regimen as shown in our previous reports (27,29). We observed that when small numbers of islets (i.e., less than 100) were transplanted, islet grafts in the pancreas were much more effective in reversing diabetes than in the liver of the chimeric recipients. Furthermore, this difference resulted from better replication of graft islet β-cells early after transplantation and better long-term β-cell function in the pancreas than in the liver, which was associated with improved revascularization of the graft islets in the pancreas. It is not yet clear how graft islets in the pancreas can have better revascularization and proliferation than in the liver. It was reported that during pregnancy in adult rats, insulin from islet β-cells augmented islet vascular endothelial cell proliferation, and in turn, endothelial growth factor from the vascular endothelial cells augmented β-cell proliferation (44). Therefore, it is possible that the existence of an endothelial-endocrine axis in the pancreas favors β-cell proliferation and islet revascularization. In addition, the defective expression of β-cell functional genes of the graft islets in the liver of long-term recipients may be caused by graft islets in the liver (but not in the pancreas) that are chronically exposed to high levels of glucose absorbed from the intestine and produced by the hepatocytes that are toxic to islet β-cells (49).

There have been concerns that the differences in gene expression profiles of islet grafts from the liver and pancreas could be caused by the difference in purity of the retrieved islets, as noted in a previous publication (15). In the current study, this concern has been markedly reduced, if not eliminated. We used luciferase transgenic donor islets for transplantation and we used luciferase mRNA as baseline to avoid the impact of purity difference in retrieved islets when we compared the gene expression levels of the retrieved graft islets.

We are aware that it is still a concern that injection of donor islets into the pancreas could potentially induce pancreatitis in humans. However, we did not observe any clinical signs of pancreatitis after injection of donor islets into the mouse pancreas, because the weight changes and serum levels of amylase in the mouse recipients with islet grafts in the pancreas or liver were similar. Reports by others also showed that intrapancreatic injection of donor islets in both rats and dogs did not induce pancreatitis (16), and intrapancreatic injection of marrow stem cells in humans did not induce pancreatitis either (50). Therefore, it is possible that intrapancreatic injection of donor islets in humans will not cause pancreatitis, and a nonhuman primate trial is warranted to further test the feasibility.

In summary, we have demonstrated that induction of chimerism under the radiation-free anti-CD3–based conditioning regimen markedly reduces the required amount of donor islets for reversal of late-stage diabetes and also renders the pancreas of autoimmune diabetic recipients a more suitable site for graft islets than the liver. Thus, induction of chimerism under a nontoxic conditioning regimen (i.e., anti-CD3–based conditioning) and implantation of donor islets into the pancreas of the chimeric recipient may be a curative therapy in the future for refractory late-stage type 1 diabetes.

ACKNOWLEDGMENTS
This work was supported by funds from the National Institutes of Health (R21 DK-71007 to D.Z.) and Juvenile Diabetes Research Foundation (Research Grant 1-2006-136 to D.Z.). No potential conflicts of interest relevant to this article were reported.

C.Z., M.W., and D.Z. researched data and wrote the manuscript. J.J.R., H.L., C.-L.L., I.N., J.L., Y.-A.C., and I.T. researched data and discussed, reviewed, and edited the manuscript.

The authors thank Dr. Arthur Riggs for his continuous encouragement and support of this research; Lucy Brown at City of Hope Flow Cytometry Facility; Sofia Loera at City of Hope Anatomic Pathology Laboratory; Alina Ava- kian-Mansoorian at the Department of Diabetes The Beckman Research Institute of City of Hope; and Clive Wasserfall at University of Florida for their excellent
diabetes.diabetesjournals.org
technical assistance. The authors also thank Dr. Richard Ermel and his staff at City of Hope Research Animal Facility for providing excellent animal care.

REFERENCES

1. von Herrath M, Sanda S, Herold K. Type 1 diabetes as a relapsing-remitting disease? Nat Rev Immunol 2007;7:798–804
2. Zhang C, Todvorov I, Lin CL, Atkinson M, Kandeel F, Forman S, Zeng D. Elimination of insulitis and augmentation of islet beta cell regeneration via induction of chimerism in overtly diabetic NOD mice. Proc Natl Acad Sci U S A 2007;104:2337–2342
3. Atkinson MA. ADA Outstanding Scientific Achievement Lecture 2004. Thirty years of investigating the autoimmune basis for type 1 diabetes: why can’t we prevent or reverse this disease? Diabetes 2005;54:1253–1263
4. Fiorina P, Shaprio AM, Ricordi C, Secchi A. The clinical impact of islet transplantation. Am J Transplant 2008;8:1990–1997
5. Belghith M, Bluestone JA, Barriot S, Meignan M, Atkinson M, Kandeel F, Forman S, Zeng D. Beta-cell-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. Nat Med 2005;11:1202–1208
6. Harlan DM, Kenyon NS, Korsgren O, Roep BO. Current advances and technical assistance. The authors also thank Dr. Richard
7. Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, De Block C, Seigneurin JM, De Pauw P, Pierard D, Weets I, Rebello P, Rother K, Diamond B, Harlan DM, Bluestone JA. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes 2005;54:1763–1769
8. Bresson D, von Herrath M. Limitations in immunotherapy with CD3 antibodies: comment on the article by Drs. Chatenoud and Bach. Rev Diab Stud 2005;2:187–188; discussion 190–191
9. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Aleandro R, Ryan EA, DiMurchio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kendaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preliasakitis J, Korbott GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355:1318–1330
10. Eisenbarth GS, Segall M. Islet and pancreatic transplantation-autoimmunity and allografting. J Diab 2005;6:355–369
11. Harlan DM, Kenyon NS, Korsgren O, Reep BO. Current advances and technical assistance. The authors also thank Dr. Richard
12. Nir T, Melton DA, Dor Y. Recovery from diabetes in mice by beta cell regeneration. Diabetes 2003;52:1838–1844
13. Harlan DM, Kenyon NS, Korsgren O, Roep BO. Current advances and technical assistance. The authors also thank Dr. Richard
14. Johnson JD, Ao Z, Ao P, Li H, Dai LJ, He Z, Tee M, Potter KJ, Klimek AM, Masek MA, Shizuru JA. Purified allogeneic hematopoietic stem cell transplantation blocks diabetes pathogenesis in NOD mice. Diabetes 2003;52:59–68
15. Nikolie B, Takeuchi Y, Leykin I, Fubala Y, Smith RN, Sylkes M. Mixed hematopoietic chimerism allows cure of autoimmune diabetes through allogeneic tolerance and reversal of autoimmunity. Diabetes 2004;53:376–383
16. Li H, Kaufman CL, Boggs SS, Johnson PC, Patrene KD, Illstad ST. Mixed allogeneic chimerism induced by a sublethal approach prevents autoimmune diabetes and reverses insulins in nonobese diabetic (NOD) mice. J Immunol 1996;156:380–388
17. Seung E, Iwakoshi N, Woda BA, Markee TG, Mordes JP, Rossini AA, Greiner DL. Allogeneic hematopoietic chimerism in mice treated with sublethal myeloablation and anti-CD154 antibody: absence of graft-versus-host disease, induction of skin allograft tolerance, and prevention of recurrent autoimmunity in islet-allografted NOD/Ltj mice. Blood 2000;95:2175–2182
18. Beilhack GF, Scheffold YC, Weissman IL, Taylor C, Herold K. Type 1 diabetes as a relapsing-remitting disease? Nat Rev Immunol 2007;7:988–994
19. Sykes M. Mixed chimerism and transplant tolerance. Immunity 2001;14:18–19
20. Zhou H, Zhang T, Bogdani M, Oseid E, Parazzoli S, Vantyghem MC, Harmon DH. HLA-mismatched renal transplantation without maintenance immunosuppression. N Engl J Med 2008;358:353–361
21. Scandling JD, Busque S, Dejakhis-Jones S, Benike C, Millan MT, Shizuru JA, Hoppe RT, Lowsky R, Englemann EG, Strober S. Tolerance and chimerism after renal and hematopoietic-cell transplantation. N Engl J Med 2006;358:362–369
22. Alexander SI, Smith N, Hu M, Verran D, Shun A, Dornay S, Smith A, Webster B, Shaw PJ, Lammin A, Stormon MO. Chimerism and tolerance in a recipient of a deceased-donor liver transplant. N Engl J Med 2008;358:369–374
23. Li H, Kaufman CL, Boggs SS, Johnson PC, Patrene KD, Illstad ST. Mixed allogeneic chimerism induced by a sublethal approach prevents autoimmune diabetes and reverses insulins in nonobese diabetic (NOD) mice. J Immunol 1996;156:380–388
Smith RN, Freeman G, Sayegh MH. Role of ICOS pathway in autoimmune and alloimmune responses in NOD mice. Clin Immunol 2008;126:140–147
41. Satoh M, Yamasaki M, Tani K, Nakano M, Itoh T, Nitta T, Anzai K, Ono J, Taniguchi M, Ikeda S. Successful islet transplantation to two recipients from a single donor by targeting proinflammatory cytokines in mice. Transplantation 2007;83:1085–1092
42. Nyman LR, Wells KS, Head WS, McLaughly M, Ford E, Brissova M, Piston DW, Powers AC. Real-time, multidimensional in vivo imaging used to investigate blood flow in mouse pancreatic islets. J Clin Invest 2008;118:3790–3797
43. Ollendorf J, Johansson M, Lawler J, Welsh N, Carlsson PO. Improved vascular engraftment and graft function after inhibition of the angiostatic factor thrombospondin-I in mice. Diabetes 2008;57:1870–1877
44. Johansson M, Mattsson G, Anderson A, Jansson L, Carlsson PO. Islet endothelial cells and pancreatic beta-cell proliferation: studies in vitro and during pregnancy in adult rats. Endocrinology 2006;147:2315–2324
45. Sandberg JO, Margulis B, Jansson L, Karsten R, Korsgren O. Transplantation of fetal porcine pancreas to diabetic or normoglycemic nude mice. Evidence of a rapid engraftment process demonstrated by blood flow and heat shock protein 70 measurements. Transplantation 1995;59:1665–1669
46. Mendola J, Corominola H, Gonzalez-Clemente JM, Esmatjes E, Saenz A, Fernandez-Cruz L, Genis R. Follow-up study of the revascularization process of cryopreserved islets of Langerhans. Cryobiology 1996;33:530–543
47. Toyofuku A, Yamasaki M, Nakano M, Satoh M, Matsuoka N, Ono J, Nakayama T, Taniguchi M, Tanaka M, Ikeda S. Natural killer T-cells participate in rejection of islet allografts in the liver of mice. Diabetes 2006;55:34–39
48. Yamasaki M, Kojo S, Kitamura H, Toyofuku A, Satoh M, Nakano M, Nabeyama K, Nakamura Y, Matsuoka N, Ikeda S, Tanaka M, Ono J, Nagata N, Ohara O, Taniguchi M. Valpha14 NK T cell-triggered IFN-gamma production by Gr-1+CD11b+ cells mediates early graft loss of syngeneic transplanted islets. J Exp Med 2005;202:913–918
49. Makhoul L, Duvivier-Kali VF, Bonne-Weir S, Dieperink H, Weir GC, Sayegh MH. Importance of hyperglycemia on the primary function of allogeneic islet transplants. Transplantation 2003;76:657–664
50. Fernandez Vina JFL, Rij RJ, Kraft D, Sadowsky J, Camozzi L, Fernandez Vina R, Vrsalovick F, Andrin O. Report case of using marrowminer system for cell therapy in diabetes type 1 patient. In ISCT Annual Meeting; Miami, Florida, 2008, p. 07-A-170-ISCT