Published in final edited form as:

Xenotransplantation. 2014 November ; 21(6): 567–573. doi:10.1111/xen.12126.

An Immuno-Sufficient Murine Model for the Study of Human Islets

Gaoping Zhao^{1,2,3}, Daniel J. Moore⁴, James I. Kim¹, Kang Mi Lee¹, Matthew O'Connor¹, Maozhu Yang^{1,2,3}, Andrew F. Marshall⁴, Ji Lei¹, Christian Schuetz¹, James F. Markmann¹, and Shaoping Deng^{1,2,3}

¹Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02114

²Sichuan Provincial Key Laboratory for Translational Medicine of Organ Transplantation, Department of Surgery, Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, Chengdu 610072, Sichuan Province, P. R. of China

³School of Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan Province, P. R. of China

⁴Department of Pediatrics and Pathology, Microbiology, and Immunology, Ian Burr Division of Endocrinology and Diabetes, Vanderbilt University, 2200 Children's Way, 11136 Doctor's Office Tower, Nashville, TN 37232-9170

Abstract

Background—For the sake of therapy of diabetes, it is critical to understand human beta cell function in detail in health and disease. Current studies of human beta cell physiology in vivo are mostly limited to immunodeficient mouse models, which possess significant technical limitations. This study aimed to create a new model for the study of human islets through induction of transplant tolerance in immunosufficient mice.

Methods—B6 diabetic mice were transplanted with human islets and treated with anti-CD45RB. To assess whether anti-CD45RB-induced transplant tolerance requires B cells, B6 recipients received additional anti-CD20 or B6 μ MT^{-/-} mice were used. For some anti-CD45RB-treated B6 μ MT^{-/-} mice, additional anti-CD25 mAb was applied at the early or late stage post-transplant. Immunohistology was performed to show the Foxp3 cells in grafted anti-CD45RB/anti-CD20-treated Foxp3-GFP B6 mice.

Address Correspondence to: Shaoping Deng, MD, PhD, Transplant Center, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street Boston, MA 02114, Phone: 617-643-0373, Fax: 617-724-7165.

Current address: 32 West Second Section, First Ring Road, Chengdu 610072, Sichuan province, P. R. of China. Tel.: +86-28-8739-3987. sdeng10@yahoo.com

Author contributions

G.Z. and D.J.M. designed the study, researched data, and wrote the manuscript; J.I.K., K.M.L., M.O. and A.F.M. researched data; M.Y., J.L. and C.S. isolated human islets and provided data; J.F.M. designed the study. S.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

No potential conflicts of interest relevant to this article were reported.

Results—Anti-CD45RB alone allowed indefinite graft survival in 26.6% of B6 mice, however 100% of xenografts were accepted in mice treated simultaneously with anti-CD20, and 88.9% of xenografts accepted in anti-CD45RB-treated μ MT $^{-/-}$ mice. These μ MT $^{-/-}$ mice accepted the islets from another human donor but rejected the islets from baboon. Additional administration of anti-CD25 mAb at the time of transplantation resulted in 100% rejection, whereas 40% of grafts were rejected while the antibody was administered at days 60 post-transplant.

Immunohistological examination showed Foxp3 $^{+}$ cells accumulated around grafts.

Conclusion—Induction of tolerance to human islets in an immunosufficient mouse model could be generated by targeting murine CD45RB and CD20. This new system will facilitate study of human islets and accelerate the dissection of the critical mechanisms underlying islet health in human disease.

Keywords

xenograft; transplant tolerance; Treg; human islet; transplant model

Introduction

With the waning use of human islets for transplantation, there has been an increasing pool of human islets available for study to specifically address islet beta cell dysfunction. The availability of these new resources corresponds to an increasing appreciation that in all forms of diabetes, including Type 1, Type 2, and Cystic Fibrosis-related diabetes, that there is intrinsic beta cell dysfunction in addition to the long appreciated extrinsic factors that stress and injure beta cells (1–4). The case is best established in Type 2 diabetes, wherein the majority of disease associated genetic polymorphisms relate to islet beta cell function (1; 5). However, more recent data has suggested similar intrinsic beta cell dysfunction driving disease even in the setting of type 1 diabetes (3; 6). Studies of human islet beta cells have been further augmented by efforts such as the establishment of the nPOD (Network for Pancreatic Organ Donors) consortium to provide access to tissues from patients with type 1 diabetes (T1D) (7). Pioneering studies are utilizing these new resources to understand islet cell function and to identify new strategies to promote islet cell survival and engraftment in transplantation. These investigations often rely on immunodeficient recipients as hosts. However, this reliance is a significant limitation as it is difficult to use advanced mouse genetics to study the effects of human gene products on islet engraftment and survival as the introduction of any human transgene must then be intercrossed to restore immune deficiency. We have considered that the induction of tolerance to human islet xenografts in a conventional mouse background would significantly accelerate studies of human islet function in murine hosts and would also diminish the associated costs in terms of animal generation and care.

The xenogeneic barrier has been challenging to surmount as the immune response includes both innate and adaptive barriers that must be overcome to induce tolerance (8; 9). We and others have previously demonstrated that a short course of treatment with anti-CD45RB induces tolerance in numerous models of allogeneic transplantation but has not been previously adapted for tolerance induction to human xenografts (10–14). although there has also been some success reported with xenografts from other species in mice with both anti-

CD45RB and other approaches(15–18). We now report induction of tolerance to human islets in a standard immunosufficient mouse model by therapy targeting murine CD45RB. This tolerance is species-specific, is inhibited by B lymphocytes, and is abrogated by depletion of CD4+ Tregs. Introduction of this new approach advances our understanding of xenogeneic tolerance and presents a new system in which to analyze human islet function and dysfunction in vivo.

Materials and Methods

Animals

C57BL/6J (B6) and B6 μ MT^{-/-} mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Foxp3-IRES-GFP knock-in mice which share 98–99% identity to C57BL/6J were provided by Mohamed Oukka (19). Mice were housed in a specific pathogen-free facility. An 18 kg baboon was obtained from the large animal facility at Massachusetts General Hospital. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at MGH.

Islet isolation

Islets were obtained from six human donors for transplantation (New England Organ Bank). Blood group and human leukocyte antigen (HLA) were: Donor 1, group O, HLA A 23, 74; B 7, 72; DR 15, 7. Donor 2, group O, HLA A 3, 28; B 18, 44; DR 7, 11. Donor 3, group AB, HLA A 2, 32; B 35, 62; DR 1, 9. Donor 4, group A+, HLA A 24, 32; B 7, 18; DR 11, 15. Donor 5, group B+, HLA A 1, 31; B 55, 57; DR 4, 13l. Donor 6, group O, HLA A 1, 26; B 8, 37; DR 13, 17. Pancreatic islets were isolated using a modification of the automated Ricordi method (20).

Islet transplantation

Recipient mice were made diabetic by a single intraperitoneal injection of streptozotocin (Sigma-Aldrich, St. Louise, MO) at a dose of 200mg/kg of body weight. Mice with blood glucose level of over than 350 mg/dl on two consecutive days were accepted as recipients. Roughly ~1500 islet equivalent (IEQ) were transplanted under the right kidney capsule of recipients. Blood glucose was monitored on regular intervals using a blood glucose meter (ACCU-CHEK, Roche Diagnostics, Indianapolis, IN). Rejection was defined as more than 200mg/dl of blood glucose level on two consecutive days. Long-term acceptance was confirmed in all reported mice by return of hyperglycemia following nephrectomy for removal of the transplant. Recipients demonstrating long-term acceptance of human islets were nephrectomized and 2 days later, re-transplanted under the left kidney capsule with a second human islet graft or with baboon islets.

Antibody therapies

Recipient animals were treated with i.p. injection of 100 μ g rat anti-mouse CD45RB antibody (HB220, IgG2a, BioExpress, West Lebanon, NH) on days 0, 1, 3, 5, and 7 post-transplant. For B lymphocyte depletion, 250 μ g of rat anti-mouse CD20 antibody(18B12, IgG2a, gift of Dr. Benichou, MGH) was administered i.p. on the day of transplantation.

CD25+ T cell depletion was achieved with 250 µg of anti-CD25 mAb (PC61, IgG1, BioExpress, West Lebanon, NH) on the indicated days.

Immunohistology

Engrafted kidneys were harvested by nephrectomy in anti-CD45RB and/or anti-CD20-treated Foxp3-GFP mice at day 14 or no-treated mice at day 7, or tolerant mice at day > 100 post transplantation. Kidney was cut in half along the middle of graft, one part preserved in 10 % formalin overnight and another part sliced and examined under fluorescence microscopy (Nikon Eclipse 50i, Japan). Immunostaining procedures were performed by a standard approach. In briefly, 4–6 µm tissue sections were deparaffinized and stained with a 1:75 dilution of anti-CD3 antibody (ab5690; Abcam, Cambridge, MA), followed by incubations with biotinylated rabbit anti-mouse antiserum 1:200 (Dako North American, Inc. Carpinteria, CA) and peroxidase-labeled streptavidin 1:300 (Dako North American, Inc. Carpinteria, CA). Color was developed in presence of 250 mg/ml AEC and 0.03% H₂O₂ and sections were counterstained in Harris modified hematoxylin (Sigma Aldrich, St. Louis, MO) before coverslipping. For insulin and Foxp3 double immunostaining, deparaffinized sections were exposed to 1:10 dilution of anti-insulin antibody (Dako North American, Inc. Carpinteria, CA), followed by biotinylated anti-rat IgG (Vector Labs. Burlingame, CA). Visualization of antibody for insulin was accomplished using an ABC kit (Vector Labs. Burlingame, CA) and diaminobenzidine followed by a hematoxylin counterstain. Thereafter, the insulin-staining sections were blocked by Double Stain Block (Dako North American, Inc. Carpinteria, CA) and then stained with a 1:50 dilution of anti-Foxp3 antibody (eBioscience, San Diego, CA), followed by binding to a rat probe (Biocare Medical, Concord, CA) specific to rat primary antibodies and AP-labeled micro-polymer (Biocare Medical, Concord, CA). Color was observed by addition of AP blue (Vector Labs. Burlingame, CA). As a negative control, sections were stained in the absence of the primary antibody as well as with isotype control sera.

Statistical analysis

Kaplan-Meier survival curves were compared with the log-rank test using the Prism 5 Program (Graph Pad, San Diego, CA). A p-value < 0.05 was considered significant.

Results

Anti-CD45RB mAb monotherapy induces species-specific tolerance that is enhanced in the absence of B lymphocytes

Human islets (1500 IEQ) were transplanted under the kidney capsule of chemically diabetic C57BL/6 mice. In untreated control mice, human islet xenografts were rejected rapidly with mean survival time (MST) of 10.0 ± 2.5 days. Treatment with anti-CD45RB induced long-term survival but only in a minority of recipients (4 of 15 grafts (26.6%); MST for all grafts > 77.0 ± 28.8 days, $p < 0.0001$ (Figure 1A)).

We considered that pre-formed antibody may exist that could impede the induction of tolerance in this combination and thus, transplantation into B cell deficient animals could improve graft survival. Tolerance induction was first assessed in mice genetically deficient

in B cells ($\mu\text{MT}^{-/-}$ mice) as these mice have neither extant B cells nor any pre-formed antibody. B cell deficient mice were competent to reject grafts as none of the grafts survived in untreated mice. However, eight of nine (88.9%) grafts in anti-CD45RB treated B cell deficient mice demonstrated long-term survival with $\text{MST} > 109.9 \pm 30.3$ days (Figure 1A).

To determine whether B lymphocytes in this system inhibited tolerance through antibody production, we performed transplants following B cell depletion by anti-CD20. In this combination, circulating antibody would remain available. Administration of anti-CD20 (250 μg at the day of transplantation) did not hinder graft rejection as all islet xenografts were rapidly rejected ($\text{MST} = 11.8 \pm 1.6$ days) in the absence of anti-CD45RB. However, combination of anti-CD20 and anti-CD45RB treatment resulted in long-term survival of grafts in 100% of recipients (Figure 1A).

To determine whether anti-CD45RB treated B6 $\mu\text{MT}^{-/-}$ mice had specific tolerance to grafts, animals ($n=8$) with long-term surviving human islet grafts (>120 days) underwent nephrectomy to remove the islets. Following graft removal, hyperglycemia was restored (blood glucose level > 300 mg/dl), showing that the human islets were responsible for the euglycemia. These eight mice were then divided into two groups, one receiving islets from a second, mismatched human donor ($n = 5$) and the other group receiving islets from a baboon ($n=3$). The five recipients tolerant to the first human islets accepted their second human grafts for an indefinite period of time whereas baboon islet graft recipients rejected their grafts by day 7 (Figure 1B).

Foxp3⁺ cells accumulate within grafts of treated mice

We have previously established that regulatory CD4⁺ T and B cells participate in the induction of allogeneic tolerance by anti-CD45RB (10; 11; 21; 22). Since B cell mediated regulation did not appear required for tolerance induction in the xenogeneic setting, we considered whether Tregs were involved. To examine the presence of Tregs within grafts by either immuno-histochemistry or immunofluorescence, Foxp3-IRES-GFP knock-in mice, which share 98–99% identity to C57BL/6J, were used in this study. As shown in Figure 2, lymphocyte infiltration into the graft is associated with both rejection in the absence of treatment and in the establishment of long-term acceptance. A significant proportion of graft-infiltrating cells are CD3⁺. In the presence of therapy, these cells are predominantly Foxp3⁺ as shown. In untreated control mice, Foxp3⁺ cells are infrequent, suggesting that the accumulation of these cells may play an important role in graft protection. This is further supported by their presence in “nests” around the islet graft in the setting of long-term survival of over 100 days. Moreover, we analyzed the role of Tregs by Foxp3 expression tracking in the anti-CD20/anti-CD45RB treated Foxp3-GFP reporter mice. Disperse green dots indicating Foxp3⁺ GFP cells were found within grafts by immunofluorescence, which were largely absent in anti-CD20 treated alone mice and were most expanded in the anti-CD20/anti-CD45RB group (Figure 2J–L). However, Foxp3 can also be expressed by activated effector cells (23), and Foxp3⁺ cells exhibit significant plasticity in vivo (24). Therefore, we sought to confirm the physiologic role of Foxp3⁺ Tregs.

Tregs promote establishment and maintenance of human islet xenografts

To confirm the role of CD4⁺ Tregs in prolonged xenograft survival, we depleted CD25⁺ cells with 250 µg of anti-CD25 mAb (PC61, IgG1) on days 6 and 1 before transplantation. This resulted in rejection of all grafts in anti-CD45RB-treated recipients within 40 days post-transplant (Figure 3A) indicating that Tregs were required for graft protection during engraftment. Tregs also played some role in long-term graft maintenance. When anti-CD25 was given to the tolerant mice at days 60 post-transplant, rejection occurred in 2 of 5 grafts (Figure 3B), suggesting Tregs play a role in the tolerance maintenance after islet xenotransplantation but that this role may not be absolutely required. Alternatively, given the substantial accumulation of Tregs in the graft, there may not have been complete Treg depletion in all treated animals.

Discussion

A readily-available, short-course combined therapy to deplete B lymphocytes with anti-CD20 and simultaneously target CD45RB induced long-term survival of human islet xenografts in otherwise immune-sufficient recipients. This model required no additional genetic manipulations of the recipient rendering it widely applicable for the study of human islet function in vivo. Remarkably, the induced tolerance appears to be species-specific as a second human islet graft is accepted without therapy while baboon islet grafts were rapidly rejected. This feature may allow study of human islet function without exposure to the induction therapy, by transplanting the tolerant recipient with a second human graft at a later date. Although the two human islet grafts were largely MHC mismatched, there was some sharing of MHC loci in some cases. This sharing may permit infectious tolerance, whereby Tregs can extend protection to tissues bearing shared antigens with those tissues to which tolerance was originally induced, as our data indicate some dependence on CD4 Treg action (25; 26). In addition, our surprising finding of species-specificity may relate to a previous report of the induction of species-specific memory against xenogeneic islets (27). In this model, repeated transplant with islets from unrelated donors from the same species induced accelerated rejection. Although increased IgG antibody was formed against the graft, this did not mediate the accelerated rejection, which was T cell dependent. Similarly in our case, B cells were not required for the species-specific tolerance and actually served to prevent its induction.

These findings relate to those reported for B cell depletion in a murine model of arthritis in which anti-CD20 treatment led to CD4⁺CD25⁺Foxp3⁺ cell expansion (28). At present, the mechanism by which B lymphocytes impede CD4⁺ Treg accumulation is not known. Nonetheless, B cell depletion may open new opportunities for immune modulation that can be captured with a second agent although it is also likely that not all B cells are detrimental and that some may enhance regulation. In anti-CD20 treated mice, there are likely to be residual B cells, some of which may contribute to immune tolerance. Whether such combination or serial therapies could enhance CD4⁺ Treg production in human islet recipients or human autoimmune disease awaits further study.

Overall, we have demonstrated the potential for species-specific xenogeneic tolerance. Practically, the induction of human islet tolerance in mice can promote more rapid and

incisive studies of human islets in a model system with diminished technical and financial barriers as compared to immunodeficient recipients, which may make these studies accessible to a wider range of investigators. We have further identified a CD4⁺ Treg dependent tolerance mechanism and a role for B cells in opposing this process. This finding has additional, immediate clinical relevance in on-going studies to evaluate the capacity of B cell directed therapies to induce immune tolerance.

Acknowledgments

This work was supported by R01AI057851(J.F.M.), the National Natural Science Foundation of China(No. 81172832), Sichuan Youth Science and Technology Foundation (2013JQ0020) and Special Program for Sichuan Youth Science and Technology Innovation Research Team to G.Z., and by NIH K08DK090146 and R03DK097410 (D.J.M.).

Abbreviations

GFP	green fluorescent protein
HLA	human leukocyte antigen
H&E	hemotoxin & eosin
IEQ	islet equivalent
MST	mean survival time
nPOD	Network for Pancreatic Organ Donors
STZ	streptozotocin
T1D	Type 1 diabetes
Tregs	T regulatory cells

References

1. Simonis-Bik AM, Nijpels G, van Haften TW, et al. Gene variants in the novel type 2 diabetes loci CDC123/CAMK1D, THADA, ADAMTS9, BCL11A, and MTNR1B affect different aspects of pancreatic beta-cell function. *Diabetes*. 2010; 59:293–301. [PubMed: 19833888]
2. Guo S, Dai C, Guo M, et al. Inactivation of specific beta cell transcription factors in type 2 diabetes. *J Clin Invest*. 2013; 123:3305–3316. [PubMed: 23863625]
3. Pugliese A. The multiple origins of Type 1 diabetes. *Diabet Med*. 2013; 30:135–146. [PubMed: 23181718]
4. Bellin MD, Laguna T, Leschyshyn J, et al. Insulin secretion improves in cystic fibrosis following ivacaftor correction of CFTR: a small pilot study. *Pediatr Diabetes*. 2013; 14:417–421. [PubMed: 23952705]
5. Grarup N, Rose CS, Andersson EA, et al. Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. *Diabetes*. 2007; 56:3105–3111. [PubMed: 17827400]
6. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. *N Engl J Med*. 2009; 360:1646–1654. [PubMed: 19369670]
7. Campbell-Thompson M, Wasserfall C, Kaddis J, et al. Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. *Diabetes Metab Res Rev*. 2012; 28:608–617. [PubMed: 22585677]

8. Scalea J, Hanecamp I, Robson SC, et al. T-cell-mediated immunological barriers to xenotransplantation. *Xenotransplantation*. 2012; 19:23–30. [PubMed: 22360750]
9. Schachner RD, Ricordi C, Inverardi L. Xenogeneic discordant islet transplantation: natural antibodies and complement in a human-to-rat model. *Transplant Proc*. 1995; 27:3318. [PubMed: 8539969]
10. Huang X, Moore DJ, Mohiuddin M, et al. Inhibition of ICAM-1/LFA-1 interactions prevents B-cell-dependent anti-CD45RB-induced transplantation tolerance. *Transplantation*. 2008; 85:675–680. [PubMed: 18337659]
11. Deng S, Moore DJ, Huang X, et al. Cutting edge: transplant tolerance induced by anti-CD45RB requires B lymphocytes. *J Immunol*. 2007; 178:6028–6032. [PubMed: 17475825]
12. Lazarovits AI, Poppema S, Zhang Z, et al. Prevention and reversal of renal allograft rejection by antibody against CD45RB. *Nature*. 1996; 380:717–720. [PubMed: 8614467]
13. Auersvald LA, Rothstein DM, Oliveira SC, et al. Anti-CD45RB treatment prolongs islet allograft survival in mice. *Transplantation proceedings*. 1997; 29:771. [PubMed: 9123520]
14. Moore DJ, Huang X, Lee MK, et al. Resistance to anti-CD45RB-induced tolerance in NOD mice: mechanisms involved. *Transplant international : official journal of the European Society for Organ Transplantation*. 2004; 17:261–269. [PubMed: 15138669]
15. Arefanian H, Tredget EB, Rajotte RV, et al. Combination of anti-CD4 with anti-LFA-1 and anti-CD154 monoclonal antibodies promotes long-term survival and function of neonatal porcine islet xenografts in spontaneously diabetic NOD mice. *Cell Transplant*. 2007; 16:787–798. [PubMed: 18087999]
16. Ashkenazi E, Baranovski BM, Shahaf G, et al. Pancreatic islet xenograft survival in mice is extended by a combination of alpha-1-antitrypsin and single-dose anti-CD4/CD8 therapy. *PLoS One*. 2013; 8:e63625. [PubMed: 23717456]
17. Sutherland RM, McKenzie BS, Zhan Y, et al. Anti-CD45RB antibody deters xenograft rejection by modulating T cell priming and homing. *Int Immunol*. 2002; 14:953–962. [PubMed: 12147632]
18. Rayat GR, Gill RG. Indefinite survival of neonatal porcine islet xenografts by simultaneous targeting of LFA-1 and CD154 or CD45RB. *Diabetes*. 2005; 54:443–451. [PubMed: 15677502]
19. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*. 2006; 441:235–238. [PubMed: 16648838]
20. Deng S, Markmann JF, Rickels M, et al. Islet alone versus islet after kidney transplantation: metabolic outcomes and islet graft survival. *Transplantation*. 2009; 88:820–825. [PubMed: 19920782]
21. Deng S, Moore DJ, Huang X, et al. Antibody-induced transplantation tolerance that is dependent on thymus-derived regulatory T cells. *J Immunol*. 2006; 176:2799–2807. [PubMed: 16493036]
22. Zhao G, Moore DJ, Lee KM, et al. An unexpected counter-regulatory role of IL-10 in B-lymphocyte-mediated transplantation tolerance. *Am J Transplant*. 2010; 10:796–801. [PubMed: 20199511]
23. Wang R, Kozhaya L, Mercer F, et al. Expression of GARP selectively identifies activated human FOXP3+ regulatory T cells. *Proc Natl Acad Sci U S A*. 2009; 106:13439–13444. [PubMed: 19666573]
24. Zhou X, Bailey-Bucktrout SL, Jeker LT, et al. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nat Immunol*. 2009; 10:1000–1007. [PubMed: 19633673]
25. Wise MP, Bemelman F, Cobbold SP, et al. Linked suppression of skin graft rejection can operate through indirect recognition. *J Immunol*. 1998; 161:5813–5816. [PubMed: 9834057]
26. Kleijwegt FS, Roep BO. Infectious tolerance as candidate therapy for type 1 diabetes: transfer of immunoregulatory properties from human regulatory T cells to other T cells and proinflammatory dendritic cells. *Crit Rev Immunol*. 2013; 33:415–434. [PubMed: 24099301]
27. Triponez F, Oberholzer J, Morel P, et al. Xenogeneic islet re-transplantation in mice triggers an accelerated, species-specific rejection. *Immunology*. 2000; 101:548–554. [PubMed: 11122459]
28. Hamel KM, Cao Y, Ashaye S, et al. B cell depletion enhances T regulatory cell activity essential in the suppression of arthritis. *J Immunol*. 2011; 187:4900–4906. [PubMed: 21948985]

Figure 1. Anti-CD45RB promotes long-term survival of xenogenic islet graft, which is enhanced by B cell depletion and is species-specific

(A) Human islets were transplanted under the kidney capsule of B6 mice or B6 μ MT $^{-/-}$ mice and treated with anti-CD45RB or left untreated. In immune-sufficient B6 recipients, some mice were treated also with anti-CD20 mAb (250 μ g, ip, on day 0) alone or in combination with anti-CD45RB. In control mice or anti-CD20 therapy alone, islet xenografts were rejected rapidly (MST = 10.0 ± 2.5 days, MST = 11.8 ± 1.6 days, respectively). 26.6% of grafts survived over 125 days after anti-CD45RB therapy (MST > 77.0 ± 28.8 days) in B6 recipients. In B6 μ MT $^{-/-}$ recipients, anti-CD45RB treatment resulted in significant prolongation of islet xenograft survival (MST > 109.9 ± 30.3 days) in comparison with control group, $p < 0.0001$. 100% of grafts showed long-term survival with anti-CD45RB plus anti-CD20 antibody treatment ($p < 0.0001$ anti-CD45RB or combination vs. control, $p < 0.01$ anti-CD45RB vs. combination). (B) 120 days after the first human islet transplant into B6 μ MT $^{-/-}$ recipient tolerized with anti-CD45RB, nephrectomy was performed and hyperglycemia was recovered. Nephrectomized mice were then divided into two groups. One group ($n = 5$) received islets from a second human donor, which were permanently accepted. In a second group ($n = 3$) receiving baboon islets, all islets were rejected.

A-I

J-L

Figure 2. Histochemistry of grafts from transplanted recipients

Human pancreatic islets were transplanted under the kidney capsule of Foxp3-GFP B6 mice (A–I, magnification 100×; J–L, magnification 200×) in the presence or absence of treatment with anti-CD45RB (A–I) or anti-CD45RB/anti-CD20(J–L). Graft staining of un-

treated recipient at day 7 post transplant (A, D, G), graft staining of antibody-treated recipients at day 14 post transplant (B, E, H) and staining of grafts from tolerant mice more than 100 days post transplant (C, F, I) are shown. In untreated mice, histological analysis of grafts at day 7 post-transplant showed moderate lymphocyte infiltration (A) which was predominately composed of T lymphocytes as indicated by CD3 staining (D). Further immunohistological analysis revealed very few Foxp3⁺ cells around the grafts (G). In anti-CD45RB treated mice at day 14 post-transplant, hemotoxilin & eosins staining showed minimal cellular infiltration within the grafts (B). Although CD3 staining presented moderate T lymphocyte infiltration, the majority of these T cells were revealed to be Tregs as indicated by the significant Foxp3⁺ positive staining pattern (E, H) and some were collected into larger “nests” in long-term tolerant mice (dotted line in F). CD3 staining is further highlighted in higher magnification insets represented by the boxed area in D and E. In long-term surviving grafts (more than 100 days post-transplant), lymphocytic infiltration continued to accumulate. A feature of these long surviving grafts was the presence of a “nest” of lymphocytes that was not seen in untreated grafts or treated animals at early time points (F). Immunohistochemical analysis showed that these accumulations consisted mainly of Foxp3⁺ cells (I). Additional analysis of Tregs by immunofluorescence revealed that control mice and anti-CD20 alone treated mice showed negligible Foxp3⁺ GFP cells (J and K), which was remarkably enhanced by combined anti-CD45RB/anti-CD20 treatment (L). A–C, Hematoxylin & eosin; D–F, CD3 immunohistochemistry (brown dot); G–I, Foxp3 (blue dot) and insulin (brown dot) immunohistochemistry staining; J–L, Foxp3-GFP (green dots indicated by arrow head). In all cases, representative images of all grafted mice analyzed are shown.

Figure 3. CD4 Tregs promote induction and maintenance of islet xenografts
 μ MT^{-/-} mice received islet xenografts with anti-CD45RB treatment as in Figure 1 to induce long-term graft survival. Administration of anti-CD25 at day 6 and day 1 before transplantation led to rejection of all xenografts (shown in A). Administration of anti-CD25 at day 63 and 68 post-transplantation resulted in xenograft rejection in 2 of 5 (40%) recipients (shown in B).