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A combination regimen of low-dose bortezomib and rapamycin prolonged the graft survival in a murine allogeneic islet transplantation model

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ABSTRACT

As the first FDA-approved proteasome inhibitor drug, bortezomib has been used for the treatment of multiple myeloma and lymphoma. However, its effects alone or in combination with other immunosuppressants on allogeneic islet transplantation have not been reported so far. In this study, we showed that the short-term combination treatment of low-dose bortezomib and rapamycin significantly prolonged the survival of islet allografts. Short-term treatment of low-dose (0.05 mg/kg or 0.1 mg/kg) bortezomib reduced the MHC class II expression in dendritic cells (DCs) of alloantigen-sensitized mice, and prolonged the islet allograft survival for up to 50 days in diabetic mice. Notably, when bortezomib was combined with rapamycin, it induced islet-specific immunological tolerance which allowed the acceptance of a second graft without additional immunosuppression. This regimen dramatically reduced the alloantigen-specific IFN-γ-producing T cells in the spleen, and increased regulatory T cells both at the graft site and in the spleen. Therefore, we propose that short-term treatment of low-dose bortezomib and rapamycin could be a new tolerance-promoting immunosuppressive regimen for allogeneic islet transplantation.

1. Introduction

Islet transplantation is one of the best therapeutic options for end-stage type 1 diabetes patients with severe hypoglycemia unawareness. However, as in other organs and tissues, immunological rejection is still a significant hurdle for successful islet transplantation [1]. In addition to the health risks such as infection and cancer caused by pan-immunosuppression, several studies have reported that widely used immunosuppressants such as glucocorticoids or calcineurin inhibitors have cytotoxic effects against islet β-cells [2,3]. Therefore, there has been a constant need for the development of tolerance-promoting regimen which could preserve the viability and function of islets following transplantation.

Bortezomib, a selective inhibitor of the 26S proteasome, was FDA-approved for the treatment of relapsed multiple myeloma [4,5]. By blocking the proteosomal degradation of Inhibitor of κB (IκB), it suppresses the activation of nuclear factor-κB (NF-κB) [6,7]. Since NF-κB is a critical transcription factor involved in the expression of various genes associated with immune responses, several studies have demonstrated the immunosuppressive effects of bortezomib. It selectively depleted alloreactive T cells *in vitro* and decreased the secretion of T helper 1 (Th1) cytokines [8]. Besides, bortezomib could modulate the function of DCs: bortezomib treatment led to a skewed phenotypic maturation of DCs in response to stimuli from lipopolysaccharide (LPS) and other endogenous sources with reduced cytokine production [9]. Furthermore, other studies have reported that bortezomib can prevent

Abbreviation: DPT, days post-transplantation; MLR, mixed lymphocyte reaction; FBS, fetal bovine serum

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graft-versus-host disease (GVHD) and cardiac allograft rejection in mouse allogeneic stem cell transplantation models [10,11]. We also previously reported that bortezomib can suppress the activation of rapamycin-resistant memory T cells without affecting regulatory T cell (Treg) viability in non-human primates [12]. Collectively, these immunomodulatory effects suggest the possibility of bortezomib as a promising immunosuppressant candidate for islet transplantation.

Rapamycin acts on mammalian cells through the mammalian target of rapamycin (mTOR) kinase and has immunosuppressive effects. It binds to the immunophilin, FK506 binding protein (FKBP12), and exerts immunosuppressive activity by inhibiting mTOR kinase activity [13]. A number of studies have revealed that rapamycin, unlike other calcineurin inhibitors, promotes the expansion of Tregs and increases the *de novo* induction of Tregs both *in vitro* and *in vivo*, thereby leading to immunological tolerance [14,15]. Conversely, some studies have shown that treatment with high-dose rapamycin has deleterious effects in islet transplantation by impairing β-cell regeneration and reducing islet engraftment [16,17]. Therefore, it would be necessary to build a new combination regimen based on low-dose rapamycin that has tolerogenic effects with minimal β- cell cytotoxicity.

In this study, we examined the possibility of a combination treatment of low-dose bortezomib and rapamycin as an alternative, tolerance-promoting immunosuppressant regimen in allogeneic pancreatic islet transplantation settings. Interestingly, the short-term, combined treatment of bortezomib and rapamycin significantly increased the graft survival and induced the tolerance to islet antigens accompanied by the increase in Tregs and reduction of inflammatory cytokines.

2. Material and methods

2.1. Mice

12- to 16-week-old female C57BL/6NCrSlc (B6; H-2^b), BALB/cCrSlc (BALB/c; H-2^d), and C3H/HeNSlc (C3H; H-2^k) mice were purchased from SLC Inc. (Japan) and maintained under specific pathogen-free conditions according to the guidelines of the Institute of Laboratory Animal Resources Seoul National University (ILAR SNU). All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of SNU.

2.2. Immunization of naïve C57BL/6 mice with BALB/c splenocytes

For pre-sensitization, irradiated (20 Gy) 1×10^6 BALB/c splenocytes were injected into naïve C57BL/6 mice on day 0. Afterwards, 0.1 mg/kg of bortezomib was injected i.p. from day 0 to day 3. On day 7, splenocytes from B6 mice were analyzed by flow cytometry and enzyme-linked immunospot (ELISPOT) assay. Irradiated BALB/c or C3H mice splenocytes were used as stimulators for ELISPOT and proliferation assay.

2.3. Diabetes induction

The recipient mice were rendered diabetic by i.p. injections of 125 mg/kg streptozotocin (STZ: Sigma, St Louis, MO) freshly dissolved in citrate buffer for two consecutive days. Islet transplantation was performed when two consecutive non-fasting blood glucose levels in the tail vein were over 350 mg/dl.

2.4. Isolation of islets and transplantation under the kidney capsule

BALB/c islets were isolated using collagenase P digestion followed by Ficoll gradient purification and hand pick-up as previously described [18]. The islets were then stabilized by culturing in RPMI 1640 medium supplemented with 11 mM glucose, 2 mM L-glutamine, 10% FBS and 50 μg/ml gentamycin for 12 h before transplantation. Diabetic recipient mice (B6) were anesthetized with isoflurane. The left kidney was exposed, and 450 islet equivalents (IEQs) of islets were transplanted under the left kidney capsule. 0.05 mg/kg or 0.1 mg/kg bortezomib was injected i.p. every day from DPT 0 to DPT 3. 1 mg/kg rapamycin was orally administered every day from DPT 0 to DPT 13. The blood glucose levels were determined with blood samples obtained from snipped tails between 09:00 AM and 11:00 AM under non-fasting conditions using a portable glucose meter (LifeScan, Inc., Milpitas, CA).

The islet graft function was considered as satisfactory when the non-fasting blood glucose level was below 200 mg/dl. To confirm that the reversal of diabetes was not due to the restoration of recipient pancreas function, survival nephrectomies around DPT 100 were performed.

2.5. Skin transplantation

Full-thickness tail skins obtained from BALB/c and C3H donors were transplanted simultaneously to graft beds on the left flank of the anesthetized recipient B6 mice and covered with Vaseline gauze and Band-Aid (Johnson & Johnson, New Brunswick, NJ). Bandages were removed after 7 days, and grafts were examined every 2 days for 3 weeks and weekly thereafter. The graft was scored as rejected when less than 30% of viable tissue remained and visible inflammation ended.

2.6. Flow cytometry

Cells (1×10^6) were stained in PBS buffer containing 1% of BSA and 0.5% FBS for flow cytometry. 1 μg of FcγR blocker (2.4G2) was added 30 min before the fluorochrome- conjugated antibody staining. All conjugated antibodies were obtained from BD Pharmingen (San Diego, CA) including anti-mouse CD4, CD8, CD25, CD11c, CD80, CD86, CD40 and CD44. The appropriate antibody was added and incubated for 30 min at 4 °C before washing. The appropriate isotype control antibodies were also included as negative standards. Intracellular FoxP3 staining was performed using FoxP3 anti-mouse/rat FoxP3 staining set (eBioscience, San Diego, CA) according to the manufacturer's instructions. After washing, the cells were analyzed using either FACSCanto II (BD Biosciences, San Jose, CA) or FACScan (BD Biosciences) and analyzed with the CELLQuest software (BD Biosciences).

2.7. ELISPOT

The number of cytokine-producing cells was measured using ELISPOT assay. Briefly, Millipore multiscreen HA plates (Merck, Burlington, MA) were coated with 3 μg/ ml of anti-mouse IL-2, IL-4, IFN-γ, and IL-10 monoclonal antibodies (BD pharmingen) by incubating overnight at 4 °C. 1×10^6 murine splenocytes were added per well and incubated at 37 °C in a 5% CO_2 incubator for 24 h to detect IFN-γ-, IL-2-, and IL-10-producing cells or for 48 h to detect IL-4-producing cells. After incubation, the wells were washed and incubated overnight at 4 °C with biotinylated anti-IFN-γ, IL-2, IL-4, and IL-10 antibody (BD Pharmingen, San Diego CA, USA). After washing, the wells were incubated with 100 μl PBS containing avidin-alkaline phosphatase (Thermo Fisher Scientific, Waltham, MA) for 1 h at 37 °C. The bound cytokine was visualized by incubation with 5-bromo 4-chloro-3-indolyl phosphate/nitrobule tetrazolium (BCIP/NBT) substrate (Thermo Fisher Scientific). The number of spots was counted using AID ELISPOT reader (AID, Strassberg, Germany).

2.8. Mixed lymphocyte reaction (MLR)

Splenocytes from B6 recipient mice were used as the responder cells. Prior to the experiments, stimulator cells (splenocytes from BALB/c or C3H mice) were irradiated with 20 Gy of γ-ray. Co-culture was then performed by adding 1×10^5 stimulator and 2×10^5 responder cells per well (1:2) in a 96-well round-bottom plates. Proliferation was assessed by 1 μCi of [^{3}H]-thymidine (Amersham, Buckinghamshire, UK) incorporation. [^{3}H]-thymidine was added after 24-h, 48-h or 72-h

culture. The wells were cultured for additional 18 h, and then cells were harvested to measure [^{3}H]-thymidine incorporation by a microbeta TriLux luminescence counter (Perkin Elmer, Wellesley, MA). The results were presented as counts per minute (cpm).

2.9. Histological analysis

The left kidneys harboring the islet grafts were removed for histological observation. Tissues were cryofixed using liquid nitrogen as embedded in OCT compound (Leica Biosystems, Wetzlar, Germany). Cryotome-cut sections (5 μm) were acetone-fixed and stained with hematoxylin-eosin (H&E) or immunohistochemical staining using appropriate antibodies (anti-insulin, FITC-anti-CD4, and Alexa Fluor 546-anti-FoxP3). Stained insulin was detected with DAB Peroxidase (HRP) Substrate Kit (Vector Laboratories, Burlingame, CA) and viewed under a light microscope (AxioCam; Carl Zeiss, Germany). Immunofluorescence was viewed with a confocal microscope (LSM 510 META; Carl Zeiss, Germany), and cell counting was performed using ImageJ software (National Institutes of Health, Bethesda, MD).

2.10. Statistical analyses

The statistical significance of the differences between experimental groups was determined using Student's *t*-test or Mantel-Cox test and the findings were regarded as significant when the two-tailed P-values were < 0.05. ∗, $P < 0.05$; ∗∗, $P < 0.01$.

3. Results

3.1. Low-dose bortezomib treatment modulated the DC phenotype

First, to test the immunosuppressive effects of low-dose of bortezomib *in vivo*, B6 mice pre-sensitized with irradiated BALB/c splenocytes were injected with 0.1 mg/kg of bortezomib for 4 consecutive days. Since NF-κB is the main transcription factor for DC maturation, we checked the maturation state by measuring expression levels of MHC Class II and other co-stimulatory molecules in CD11c$^+$ DCs. Interestingly, low-dose bortezomib decreased only the MHC class II expression, not the other co-stimulatory molecules expressed on DCs (Fig. 1A). Unlike other studies showing the suppressive effects of bortezomib on alloreactive T cells by high-dose treatments, the short-term treatment of low-dose bortezomib had an insignificant effect on the percentages of splenic effector-memory cells (CD4$^+$CD44^{high} and CD8$^+$CD44^{high}), and the number of alloantigen-specific IFN-γ-producing T cells (Fig. 1B and C). Based on these results, we presumed that low-dose bortezomib could suppress DCs by altering its MHC II expression *in vivo*.

3.2. The combined treatment of bortezomib and rapamycin significantly prolonged the islet graft survival

Next, we tested the efficacy of low-dose bortezomib alone or in combination with rapamycin in the islet transplantation model. Despite the low-dose (0.1 mg/kg), the bortezomib-treated group showed prolonged graft survival compared to the control group (Fig. 2A; 0.05 mg/kg group: $P = 0.1$, 0.1 mg/kg group: $P = 0.0036$). To test whether the transplanted islets were responsible for maintaining normal blood glucose levels, the islet graft from one mouse (0.05 mg/kg bortezomib-treated) was removed by nephrectomy. As expected, the blood glucose level dramatically increased up to 400 mg/dl on the day after graft removal. To further test if this mouse has generated tolerance to BALB/c islets, the mouse was re-transplanted with a second graft (islets from BALB/c donors). However, as shown in Fig. 2B, this second graft was not accepted.

To further complement the effects of low-dose bortezomib, we added low-dose (1 mg/kg) rapamycin treatment in the same transplantation setting. Islets graft survivals were significantly prolonged both in 0.05 mg/kg bortezomib + rapamycin ($P = 0.0011$) and 0.1 mg/kg bortezomib + rapamycin groups compared to the control group ($P = 0.001$). Although it was not statistically significant, the combination of rapamycin and bortezomib increased the graft survival compared to bortezomib-alone-treated groups (Fig. 2A and C). In 0.1 mg/kg bortezomib + rapamycin-treated group, 4 of 6 mice maintained normoglycemia over 100 days whereas 0.1 mg/kg bortezomib-alone-treated group, 2 of 6 mice maintained normoglycemia over 100 days (Supplementary Fig. 1) Moreover, mean graft survival date was increased from 24 to 58 by adding rapamycin in 0.05 mg/kg bortezomib treated group (Fig. 2A–C). To determine whether the immunological tolerance was induced by low-dose of bortezomib + rapamycin treatment, the graft was removed from the recipient mouse which had maintained the normal blood glucose levels for more than 100 days, and a second graft (islets from BALB/c donors) was re-transplanted into the contralateral kidney (Fig. 2C). Interestingly, the mouse re-transplanted with the second graft maintained normoglycemia for 50 days without any immunosuppression (Fig. 2C). To determine if this tolerance was systemic, the BALB/c and C3H (third party) along with B6 (control) skin grafts were transplanted to the flank of the second graft-recipient. The C3H skin graft was rejected on DPT 14 (Fig. 2D). The rejection of BALB/c skin graft was a little delayed, but the tissue was eventually rejected on DPT 18. Unexpectedly, as shown in Fig. 2C, the rejection of BALB/c skin seemed to result in the rejection of second islet graft since the blood glucose level returned to hyperglycemia (20 days

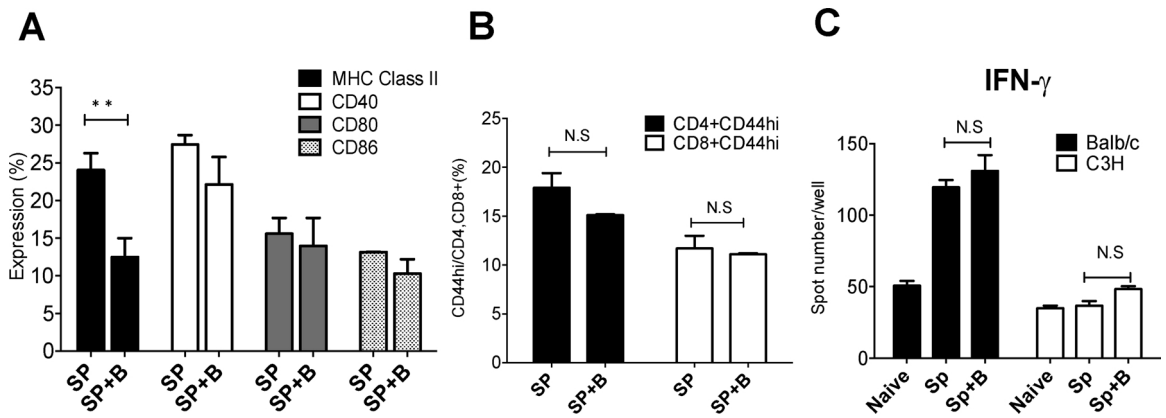

Fig. 1. Effects of low-dose bortezomib on DCs. (A) MHC Class II, CD40, CD80, and CD86 expressions on CD11c$^+$ DCs were analyzed 4 days after the last bortezomib injection. (B) CD44 expression on T cells were measured after botezomib treatment. (C) IFN-γ-producing cell numbers were estimated by ELISPOT assay. Irradiated BALB/c and C3H splenocytes (stimulators) were added and co-cultured with B6 splenocytes (responders). SP, splenocytes-injected; B, bortezomib. n = 3.

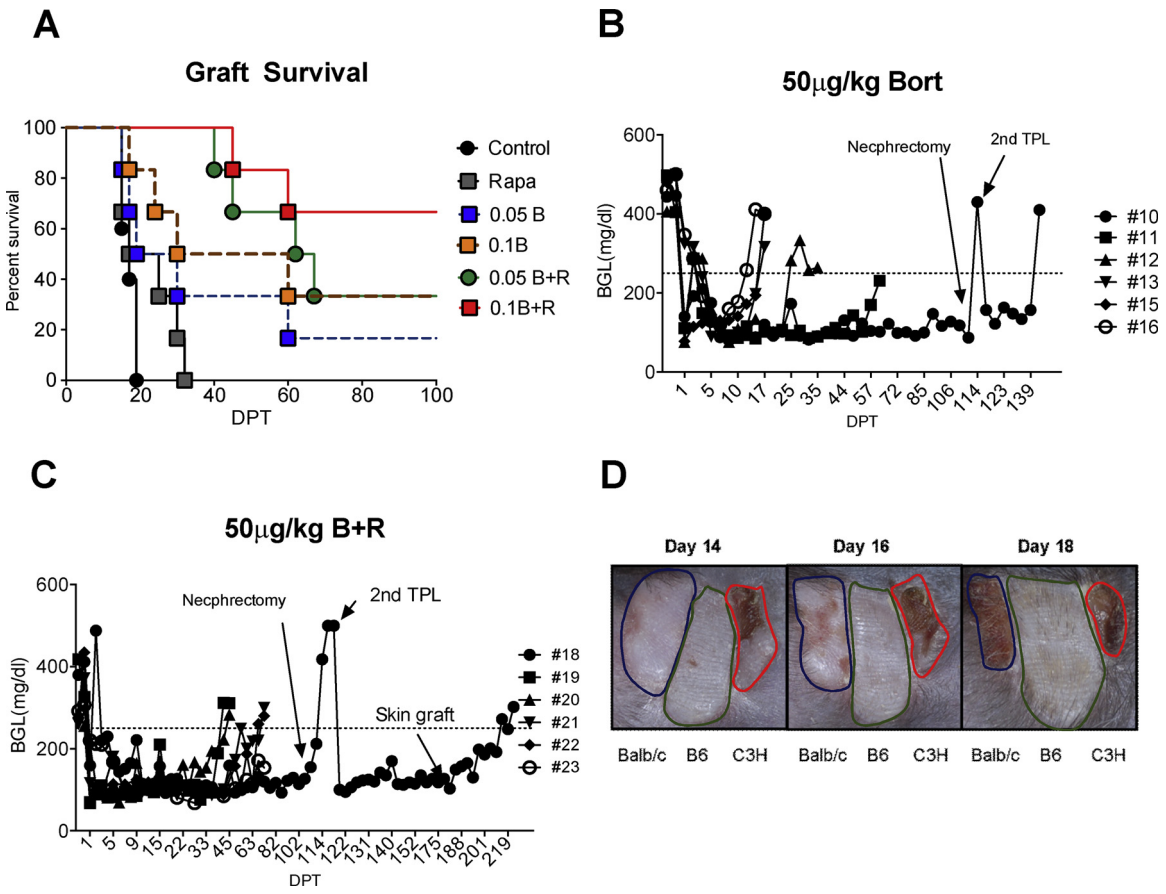

Fig. 2. Bortezomib-alone and combination regimen prolonged the graft survival in allogeneic islet transplantation. A) Graft survival in the different immunosuppressive regimen-treated mice (control group; n = 5, immunosuppressant-treated groups = 6). Non-fasting blood glucose levels of (B) bortezomib-treated mice and (C) bortezomib and rapamycin-treated mice. (B–D) Removal of the transplanted islets was performed between DPT 90–110 in the primary graft-carrying mice. After the recurrence of hyperglycemia, re-transplantation was performed with a second islet graft under the capsule of the contralateral kidney. (D) BALB/c and C3H skin were transplanted to the second graft-recipients. Blue, green, and red region denote the skin grafts of BALB/c, B6, and C3H mouse, respectively. B, bortezomib; R, rapamycin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

after skin rejection). Therefore, we concluded that this combination therapy induced tolerance to islet-specific antigens, and its suppressive effect was insufficient to block robust skin graft rejection.

3.3. Immunological features of T cells from bortezomib and rapamycin-treated mice

Numerous studies have indicated that Th1 cells are the leading players of graft rejection in various transplantation models and IFN-γ plays a crucial role through the activation of cytotoxic $CD8^+$ T cells [19,20]. Therefore, we next checked whether bortezomib alone or in combination with rapamycin could reduce Th1 and IFN-γ-producing cells. Splenocytes from the alloislet-recipient mice which maintained normoglycemia for over 60 days were stimulated *in vitro* with irradiated BALB/c splenocytes, and ELISPOT analysis was performed. As indicated in Fig. 3A, IFN-γ-producing cells were hardly detectable in the combination regimen group. Although the reduction of IFN-γ-producing cells was also observed in the mice treated with bortezomib alone, it was less pronounced than that of the combination regimen group. Interestingly, other cytokine-producing cells were not dramatically changed in the combination regimen group. We also examined the BALB/c-specific T cell response of bortezomib + rapamycin-treated recipient mice by MLR. As shown in Fig. 3B, the T cells from these mice had a reduced proliferative response only towards the BALB/c antigen, and not to the third party C3H antigen. In accordance with these results, the islets of bortezomib-alone-treated group were found to be less intact than that of the combination group (Fig. 3C and D). Thus, these results indicated that the combined therapy induced a profound BALB/c-specific T cell hyporesponsiveness.

3.4. Regulatory T cells were increased both in the spleen and the graft site following the combination therapy

The importance of FoxP3-expressing $CD4^+CD25^+$ Tregs for preventing allograft rejection has been emphasized in many preclinical and clinical models of transplantation [21,22]. Thus, we analyzed the proportion of Tregs in the spleen and the graft site of bortezomi**b**-treated mice which had long-term graft survival. As shown in Fig. 4A, the mice treated with bortezomib alone had an increased proportion of $CD25^-FoxP3^+$ T cells compared to the control mice, but there was insignificant change in the $CD25^+FoxP3^+$ T cells. Meanwhile, the mice treated with the combined regimen showed significant increase in $CD25^+FoxP3^+$ T cells. As indicated in Fig. 4B and C, at the graft site of mice treated with the combination regimen a considerable number of $FoxP3^+$ T cells (67 ± 6.5%) were observed around the graft. Although the mice treated with bortezomib alone also showed the presence of $FoxP3^+$ T cells (50 ± 2%) near the graft site, the numbers were fewer than those seen in the combination regimen group (Fig. 4B and C). Therefore, these results indicated that the combination treatment induced increases of the Treg cells both in the secondary lymphoid organ and at the graft sites, which were positively related with graft survival.

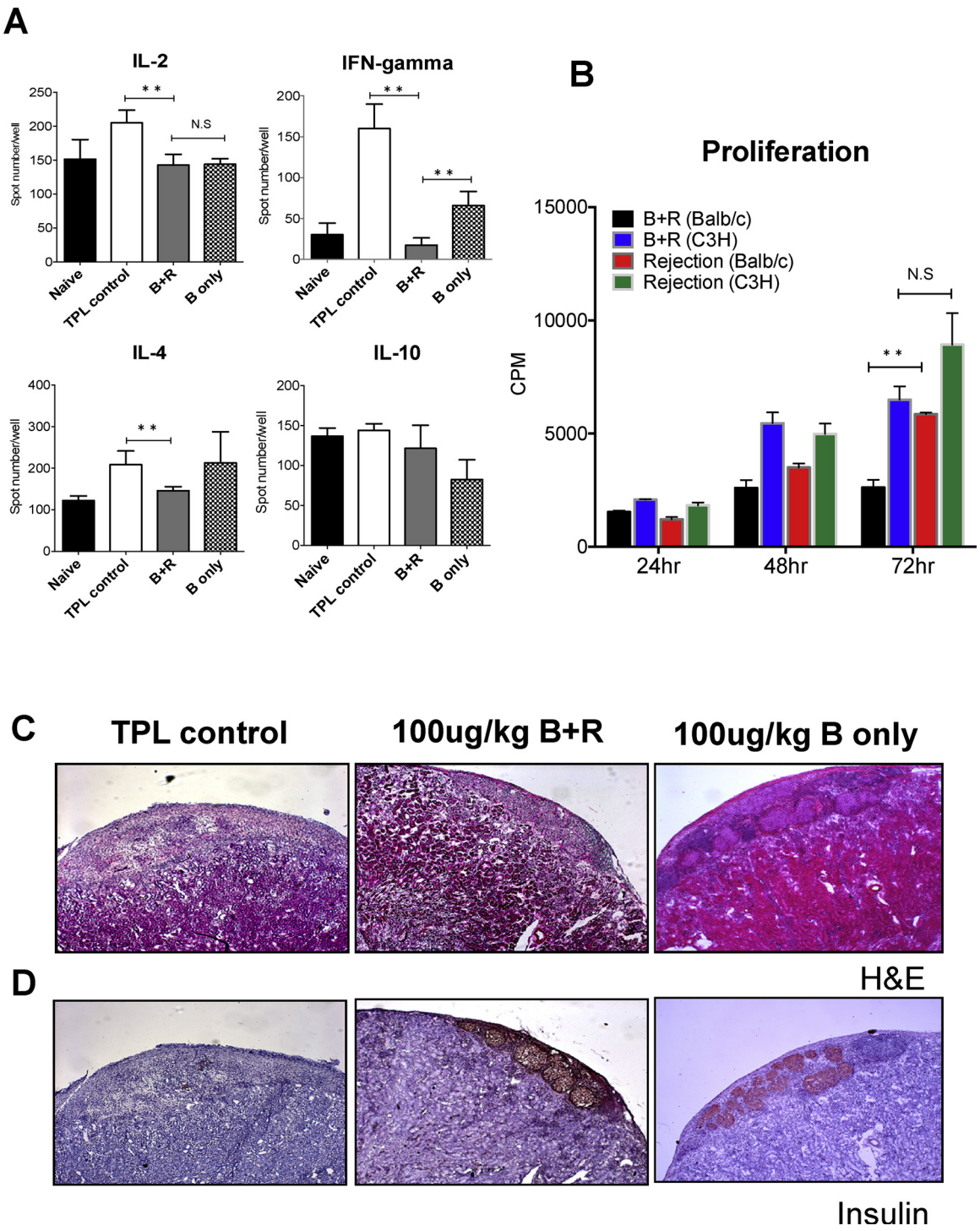

Fig. 3. Bortezomib and rapamycin-treated mice have more intact grafts and show hyporesponsiveness to alloantigen. (A) Splenocytes were isolated from naïve (n = 3), control (rejected, n = 3), bortezomib (0.1 mg/kg, n = 3), and combination regimen-treated mice (n = 3) and ELISPOT assay was performed. All experiments were performed in duplicates. Bars represent mean ± SD. (B) MLR results with co-culture of B6 splenocytes (2×10^5) from either the combination regimen-treated (n = 3) or the rejected control (n = 3) and 1×10^5 irradiated BALB/c or C3H splenocytes. MLR proliferative results are expressed as cpm. (C) H&E staining of the islet-implanted kidney. The grafts were removed on DPT 17 (TPL control) or DPT 60 (bortezomib-treated and combination regimen-treated). (D) Expression of Insulin (brown) in the graft. B, bortezomib; R, rapamycin. Data are representative results of three independent experiments.

4. Discussion

In the present study, we examined the applicability of a low-dose bortezomib and rapamycin combination as a new immunosuppressant regimen for the allogeneic islet transplantation. So far, most of the clinical islet transplantations were done through the liver, but this is not the best site in murine models since hemostasis failures frequently occur due to the limited breadth of murine portal venule. Thus, in the current study, we chose the kidney subcapsular space as the graft site since islet transplantation under the mouse kidney capsule was widely accepted in murine models. In this model, low-dose bortezomib and rapamycin treatment significantly prolonged the islet graft survival and induced islet-specific immunological tolerance.

In the genetically engineered mouse model for multiple myeloma,

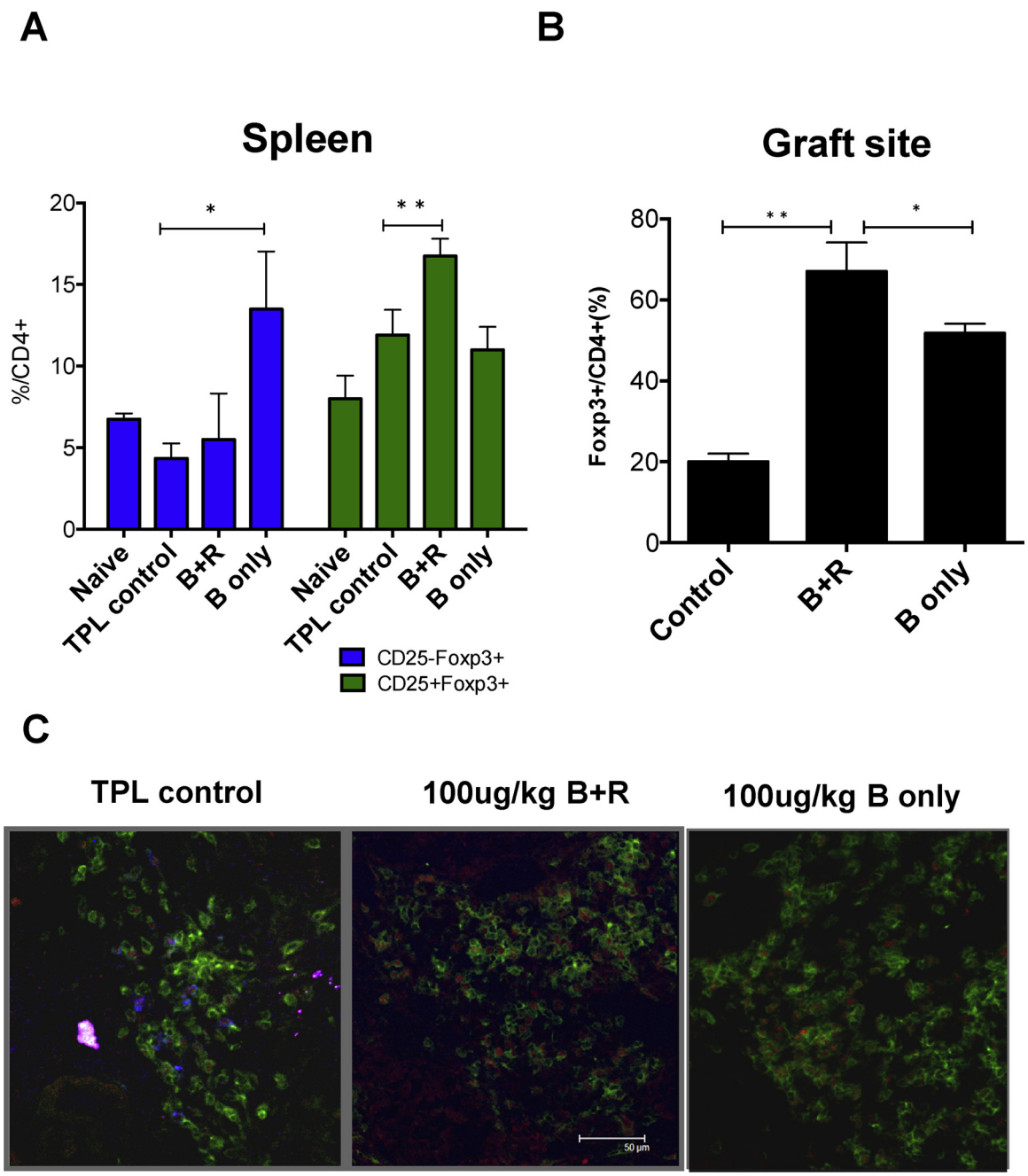

Fig. 4. FoxP3$^+$ cells were significantly increased in the spleen and at the graft site of the combination regimen-treated mice. (A) Regulatory T cell proportion among the splenic CD4$^+$ T cells was analyzed by flow cytometry. n = 3 for each group (B) The islet-infiltrating CD4$^+$ and FoxP3$^+$ cells were quantified by microscopic counting at 400×. Bars represent mean ± SD. n = 3 for each group (C) Confocal images of immunofluorescence-stained tissue with CD4 (green) FoxP3 (red) Abs. The grafts from the rejected mice or mice which maintained a normal glucose level over 60 days were sectioned. The scale bar in the middle applies universally. B, bortezomib; R, rapamycin. Data are representative results of three independent experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

an initial dose (0.3 or 1.0 mg/kg) of bortezomib was administrated twice per weekly for 4 weeks [23]. Clinically, bortezomib is usually administered intravenously on the days 1, 4, 8, and 11 for the treatment of multiple myeloma with a 3-week interval to give patients intermittent time for recovery [24]. Since the goal of our study was to minimize the cytotoxic effects of bortezomib, we treated mice with lower-doses of bortezomib (0.1 or 0.05 mg/kg) than generally used for a shorter period. Indeed, this short-term treatment with low-dose bortezomib did not affect the insulin secretion and islet survival *in vivo* (data not shown).

Interestingly, although we used low dose, bortezomib definitely increased the graft survival when combined with rapamycin (Fig. 2A). Moreover, one mouse in the combination regimen-treated mice accepted the second islet graft, maintaining normoglycemia over 50 days without any other immunosuppressant (Fig. 2C). However, this mouse was tolerant only to alloislet antigens since this mouse did not accept BALB/c skin graft. The absence of the Tregs initially present at the removed islet graft was not the leading cause of skin rejection, because the transplanted skin graft was also rejected when islet graft was intact (data not shown). Unlike the combination regimen-treated mice, the bortezomib-alone-treated mouse which maintained normoglycemia for over 100 days did not accept the 2nd graft. Thus, this indicates that

bortezomib alone might induce simple accommodation or immunosuppression rather than immunological tolerance.

Another interesting point is that $CD4^+CD25^+FoxP3^+$ T cells were increased in the mice treated with the combination regimen (Fig. 4A). These results were in agreement with previous studies which showed that rapamycin induces the expansion of $CD4^+CD25^+FoxP3^+$ T cells. However, it is not clear whether direct inhibition of NF-κB and mTOR signaling is associated with the induction of FoxP3 expression in T cells. Also, we could not rule out the possibility that bortezomib induced the Tregs by modulating antigen-presentation or stimulation ability of APCs. This is conceivable since DCs from bortezomib-treated mice showed reduced MHC II expression (Fig. 1A).

Furthermore, $FoxP3^+$ T cells were significantly increased at the graft site of mice that received the combination regimen (Fig. 4B and C). Since compelling evidences in the transplantation models has demonstrated the critical role of Tregs at the graft site [25], we believe that these infiltrated Tregs protected the islets from effector T cells. We also expect that persistent IL-2 production in the combination-regimen treated mice contributed to maintaining the Treg population since IL-2 is a critical cytokine for Treg survival [26]. In fact, only IFN-γ-producing cells dramatically reduced in the combination-regimen group while other cytokines such as IL-2, IL-4 or IL-10 were relatively less reduced or not changed (Fig. 3A).

Although we first documented the effects of low-dose bortezomib and rapamycin on prolongation of allogeneic pancreatic islet graft in murine model, further mechanistic studies would be needed to apply this regimen to the clinical setting. In this study, we did not scrutinize the effects of bortezomib on other types of cells since the goal of the study was to test if this regimen prevents the activation of alloreactive T cells while preserving Tregs. NF-κB is a ubiquitously-expressed, pro-inflammatory transcription factor which regulates the expression of over 500 genes. Therefore, T cells could be one of the bortezomib's target. Because bortezomib is currently used to treat multiple myeloma and known to inhibit activated B cells and antibody-secreting cells, B cells could be another target. Also, as already proven in other studies, DCs could be affected by bortezomib because NF-κB also controls the maturation of DCs.

Our data provided the proof-of-principle of the new regimen using low dose bortezomib and rapamycin to target T cells in murine allogeneic islet transplantation model. For the successful clinical translation, testing the efficacy of the regimen in nonhuman primate model would be required to harness the full potential of this regimen.

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Declaration of Competing Interest

The authors confirm that this article content has no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imlet.2019.10.005.

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