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Donor T-cell-derived interleukin-22 promotes thymus regeneration and alleviates chronic graft-versus-host disease in murine allogeneic hematopoietic cell transplant

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ABSTRACT

Defect of thymus results in poor posttransplant immune recovery and dysfunction of immune tolerance after allogeneic hematopoietic cell transplants (allo-HCT). Improving thymus regeneration represents a potential strategy to accelerate recovery of T-cell immunity. IL-22 was reported to mediate thymus regeneration after injury. In this study, we found donor T-cell is a major source of IL-22 in allotransplant recipient. Through applying IL-22 knock out (IL-22KO) mice in allo-HCT, we found donor T-cell derived IL-22 promotes thymus regeneration in association with increased level of intra-thymic IL-22. IL-22KO T-cell-transplanted recipients show deficient thymus recovery which is reversed by injection of exogenous IL-22. T-cell derived IL-22 promotes proliferation of thymic epithelial cells (TECs) in vitro. In addition, donor T-cell derived IL-22 increases expression level of Aire in the thymus and decreases skin chronic graft-versus-host disease (GVHD). Furthermore, short-term use of exogenous IL-22 posttransplant accelerates recovery of thymus without increasing severity of acute GVHD. Our data indicate that cross-talk between T-cell and TECs is an important mechanism to mediate reconstitution of T-cell immunity after allo-HCT.

1. Introduction

Chronic graft-versus-host disease (GVHD) is an autoimmune-like disorder characterized by autoantibody production and activation of pro-fibrotic pathways, and is a major cause of late morbidity and mortality following allogeneic hematopoietic cell transplant (HCT) [1,2]. Significant progresses had improved our understanding on pathogenesis of chronic GVHD [3]. New strategy for treating chronic GVHD is still urgently needed in clinical practices due to lack of efficiently targeted therapies and successful clinical translation of preclinical studies [2].

Chronic GVHD is featured by dysregulation of immune tolerance during immune reconstitution. Thymus is the primary organ in establishing central immune tolerance of the host through expressing autoimmune regulator (Aire) and inducing differentiation of regulatory T cells (Tregs) [4,5]. However, drugs and radiation give the recipient thymus pretransplant damage delaying immune recovery [6]. Defective thymic function is considered as an important mechanism which leads to release of autoreactive T-cell after allogeneic HCT [7,8]. Thus, boosting function of damaged thymus is a promising strategy to reconstitute immune tolerance of the host. Thymic epithelial cells (TECs) provide the basic microenvironment for T-cell development [9]. Strategies to enhance recovery of TECs potentially accelerated recovery of T-cell number [10].

Interleukin-22 (IL-22) was recently reported to promote recovery of damaged TECs during HCT [11,12]. As a member of IL-10 family of cytokines, IL-22 mediates inflammation and homeostasis of epithelial cells. IL-22 is mainly produced by T helper cells and binds IL-22 receptor expressed on epithelial cells [13]. IL-22 increases thymocytes count in association with accelerated recovery of TECs. We found that IL-22 exerts proliferative effect on TECs via a JAK/STAT3/myeloid cell leukemia sequence 1 (Mcl-1) pathway (article in press). In addition, recent studies reported both pro-inflammatory and anti-inflammatory effects of IL-22 in acute GVHD [14,15]. We previously identified IL-22-

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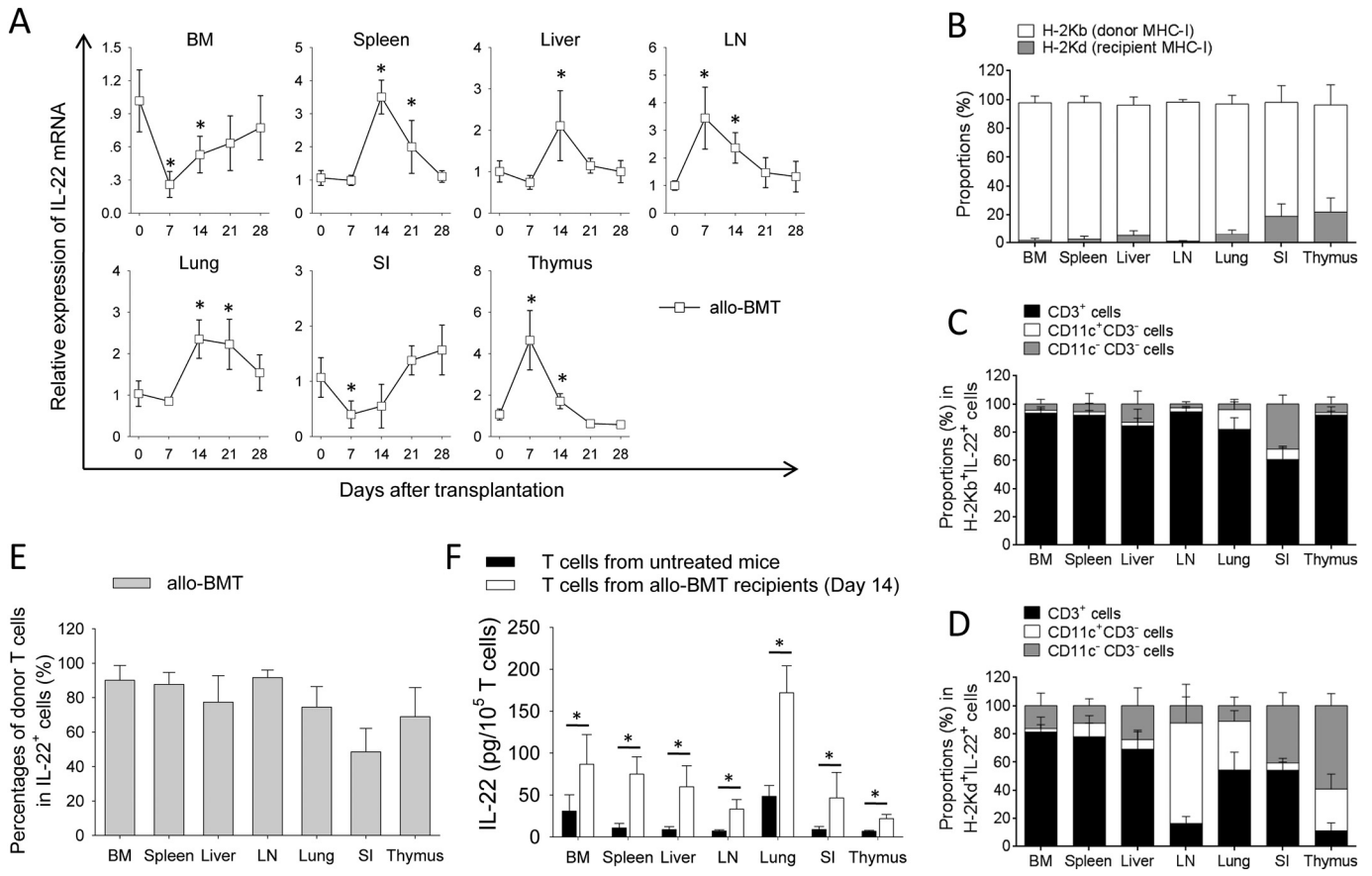

Fig. 1. Donor T-cell is a major source of IL-22 in allotransplant recipient. In C57BL/6 → BALB/c allo-BMT model, irradiated recipients received an allotransplant. (A) Total RNA was extracted from bone marrow (BM), spleen, lymph nodes (LN), thymus, liver, lung and small intestine (SI) on day 0, 7, 14, 21 and 28 after BMT (n = 3 for each time point). qPCR was applied to analyze IL-22 mRNA levels. Data represent fold changes. *, $p < 0.05$ compared with day 0. (B–E) On day 14 after BMT, lymphocytes were isolated from BM, spleen, LN, thymus, liver, lung and SI (n = 3). Cells were stimulated by PMA and ionomycin with Brefeldin A, followed by intracellular staining with IL-22. (B) After being gated on IL-22$^+$ population, cells were divided into H-2Kd$^+$ population (of recipient origin) and H-2Kb$^+$ population (of donor origin). H-2Kb$^+$IL-22$^+$ cells (C) and H-2Kd$^+$IL-22$^+$ cells (D) were further analyzed for expression of CD3 and CD11c. (E) Percentages of donor T cells in total IL-22$^+$ cells were also analyzed. (F) T cells were sorted from lymphocytes, followed by stimulation with anti-mouse CD3ε and anti-mouse CD28 for 72 h. Supernatant of T-cell culture was delivered to ELISA detection for level of IL-22 (n = 6). Data are shown as mean ± SD, and are compared using unpaired *t*-test. *, $p < 0.05$.

producing T cells during pathogenesis of acute GVHD and found that recipient derived IL-22 ameliorated acute GVHD in mice [16,17]. The role of IL-22 in chronic GVHD is still unclear.

Because T-cell is a potent producer of IL-22, we explored the impact of T-cell derived IL-22 on thymus regeneration and chronic GVHD after allotransplant in mice. We found that donor T-cell derived IL-22 promotes thymus regeneration and decreased chronic skin GVHD post allotransplant.

2. Methods

2.1. Mice

Six-week old wild type C57BL/6 and BALB/c mice were purchased from Charles River (Vital River, Beijing, China). IL-22 knock out (IL-22KO) mice with C57BL/6 background were obtained from Cyagen Biosciences (Suzhou, China). IL-22KO mice were generated using Transcription Activator-Like Effector Nuclease (TALEN). All these mice were bred in a special pathogen free room. All procedures regarding animal care and experiments were approved by the Experimental Animal Care and Use Committee of Xuzhou Medical University.

2.2. Cell culture

Murine thymic epithelial cell line mTEC1 (derived from BALB/c mice), kindly provided by Prof. Yu Zhang (Peking University, Beijing) [18], was cultured in RPMI-1640 medium (Sigma-Aldrich, Shanghai, China) supplied with 10% FBS (Biowest, Nuaillé, France). Freshly isolated T cells from tissues were cultured in RPMI-1640 medium supplied with 10% FBS, anti-mouse CD3ε (145-2C11) (BioLegend, San Diego, CA) and anti-mouse CD28 (37.51) (Biolegend). Co-culture of mTEC1 and T-cell was performed in a Transwell with Polycarbonate Membrane Insert (Corning, NY, USA) for 72 h in the presence of anti-IL-22 (Poly5164) or isotype control antibody (Biolegend). T cells were cultured in the upper chamber and mTEC1 cells were plated in the lower chamber.

2.3. Reagent

Recombinant murine IL-22 (rmIL-22) was purchased from PeproTech (210-22, Rocky Hill, NJ, USA).

2.4. Allogeneic bone marrow transplantation

Allogeneic bone marrow transplantation (BMT) was performed as we previously described [19]. In C57BL/6 → BALB/c allogeneic BMT

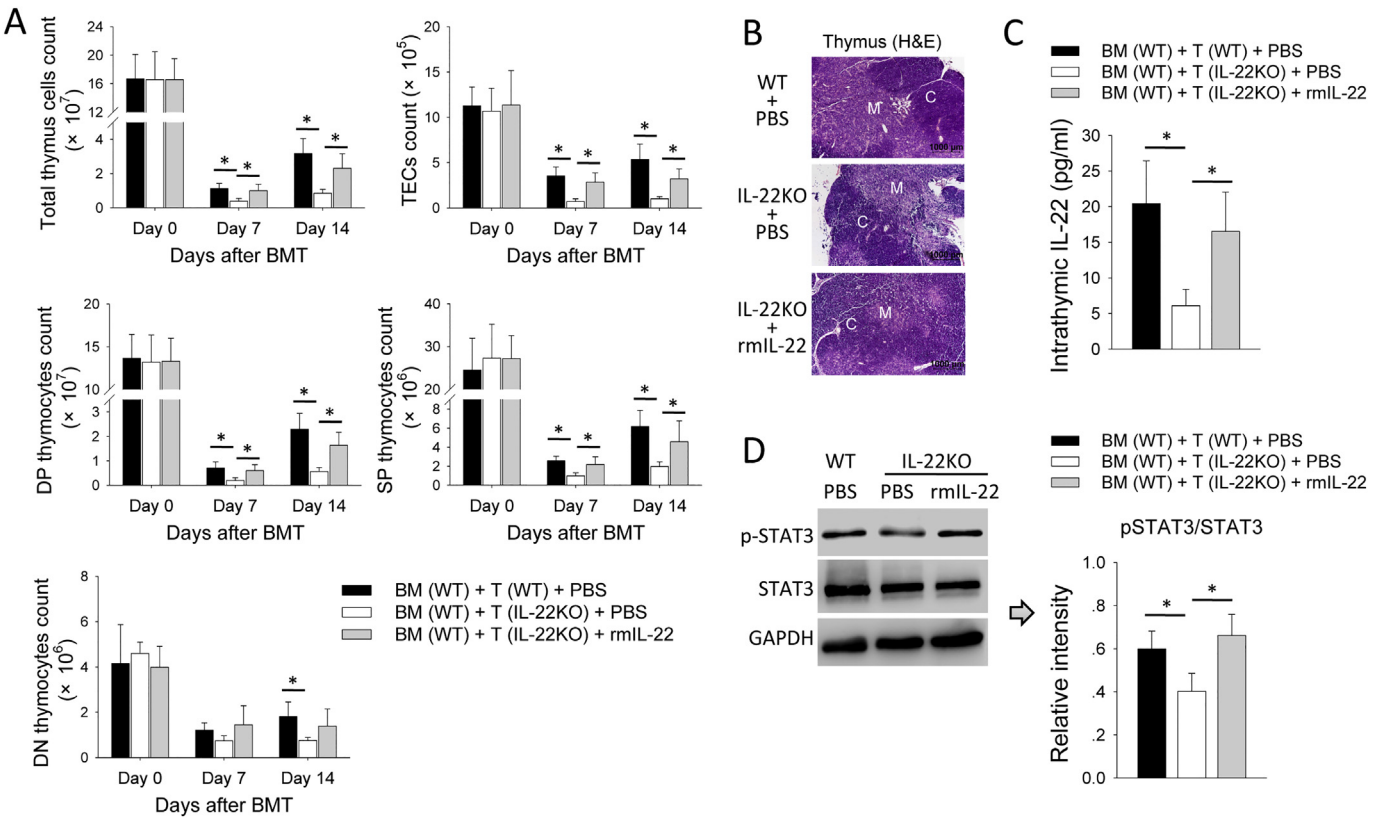

Fig. 2. Donor T cell-derived IL-22 accelerates thymus recovery of recipients. In C57BL/6 → BALB/c allo-BMT model, recipients were transfused with T-cell-depleted bone marrow cells from wild type donors. Recipients were co-transfused with splenic T cells isolated from wild type or IL-22KO donors. Recipients were treated with PBS or rmIL-22 (0.1 mg/kg) on days 0, 2, 4, 6 and 8 after BMT. (A) Flow cytometry was applied to analyze proportions of thymic epithelial cells (TECs), double positive (DP), single positive (SP) and double-negative (DN) thymocytes in total thymus cells (n = 4). Phenotypes of each cell types in flow cytometry: TECs, $CD45^-EpCAM^+$; DP thymocytes, $CD45^+CD4^+CD8^+$; SP thymocytes, $CD45^+CD4^+CD8^-$ and $CD45^+CD4^-CD8^+$; DN thymocytes, $CD45^+CD4^-CD8^-Lin^-$. (B–D) Thymus samples were obtained on day 14 after BMT. (B) H&E staining was performed on tissue slides of thymus (n = 3). One representative figure was shown. Scale bar: 1000 μm. (C) Thymus was mildly ground in 1 ml of PBS, and supernatant was subjected to ELISA analysis for concentration of IL-22 (n = 3). (D) Protein samples, isolated from thymus, were subjected to western blot analysis with indicated antibodies (n = 3). Relative intensity of bands was analyzed by Image J software. Data are shown as mean ± SD, compared using one-way ANOVA test. *, $p < 0.05$.

model, 8-week BALB/c mice were used as recipients, while 8-week C57BL/6 mice were used as donors. BALB/c recipients received a 7.5 Gy TBI from a ^{137}Cs source followed by injection with bone marrow cells (5×10 [6]/mouse) from donors. For analyzing role of donor T-cell-derived IL-22 in BMT, recipients were injected with wild type T-cell-depleted bone marrow cells (TCD-BM) (5×10^6/mouse) together with splenic T cells (5×10^5/mouse) from wild type or IL-22KO donors. T cells were sorted using a Pan T Cell Isolation Kit II (Miltenyi Biotec, San Diego, CA). Recipients were injected intraperitoneally with PBS or rmIL-22 (0.1 mg/kg) after BMT.

2.5. Masson staining and H&E staining

Masson staining was performed on paraffin slides of skin tissues with a Trichrome Stain Kit (HT15, Sigma-Aldrich). H&E staining was performed as previously described [19].

2.6. Assessment of GVHD

Severity of acute GVHD was assessed base on the clinical scores, which comprises five parameters as previously described [20]. Skin chronic GVHD was analyzed by applying a scoring system based on changes of skin pathology including cellular infiltration, fat loss, dermal fibrosis and epidermal interface changes, which were indicated by H&E staining and Masson staining [1].

2.7. Lymphocyte isolation

Bone marrow cells were obtained by flushing femurs with PBS. Spleen, lymph nodes and thymus tissues were mildly ground to release of lymphocytes which were isolated using Lymphocyte Separation Medium (DAKEWE, Shanghai, China). Liver tissue was mildly ground to obtain a single cell suspension followed by centrifugation in Percoll buffer (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). Lung tissue was cut into small pieces and digested in 1 mg/ml collagenase type-I (Sigma-Aldrich) and 1 mg/ml DNase I for 60 min at 37 °C. The single cell suspension was centrifuged in Percoll buffer. Peyer patches were removed from the small intestine sample and remaining tissues cut into small pieces and sequentially digested with 5 mM EDTA, 1 mg/ml collagenase type-I with 0.5 mg/ml collagenase type-V with 1 mg/ml DNase I and 0.5 mg/ml Dispase with 1 mg/ml collagenase type-I. The single-cell suspension was then centrifuged in Percoll buffer.

2.8. Thymic stromal cell isolation

Thymic stromal cells isolation was performed as previously described [21]. Freshly dissected thymus was cut into small pieces, which were mechanically dissociated in DMEM containing 2% FBS. After being centrifuged, the total pellets were digested with 0.5 mg/ml collagenase type IV (Thermo Fisher Scientific, Waltham, MA, USA) and 1 mg/ml DNase I (Sigma-Aldrich, Shanghai, China) for 30 min at 37 °C. After the first digestion, the remaining fragments were digested using

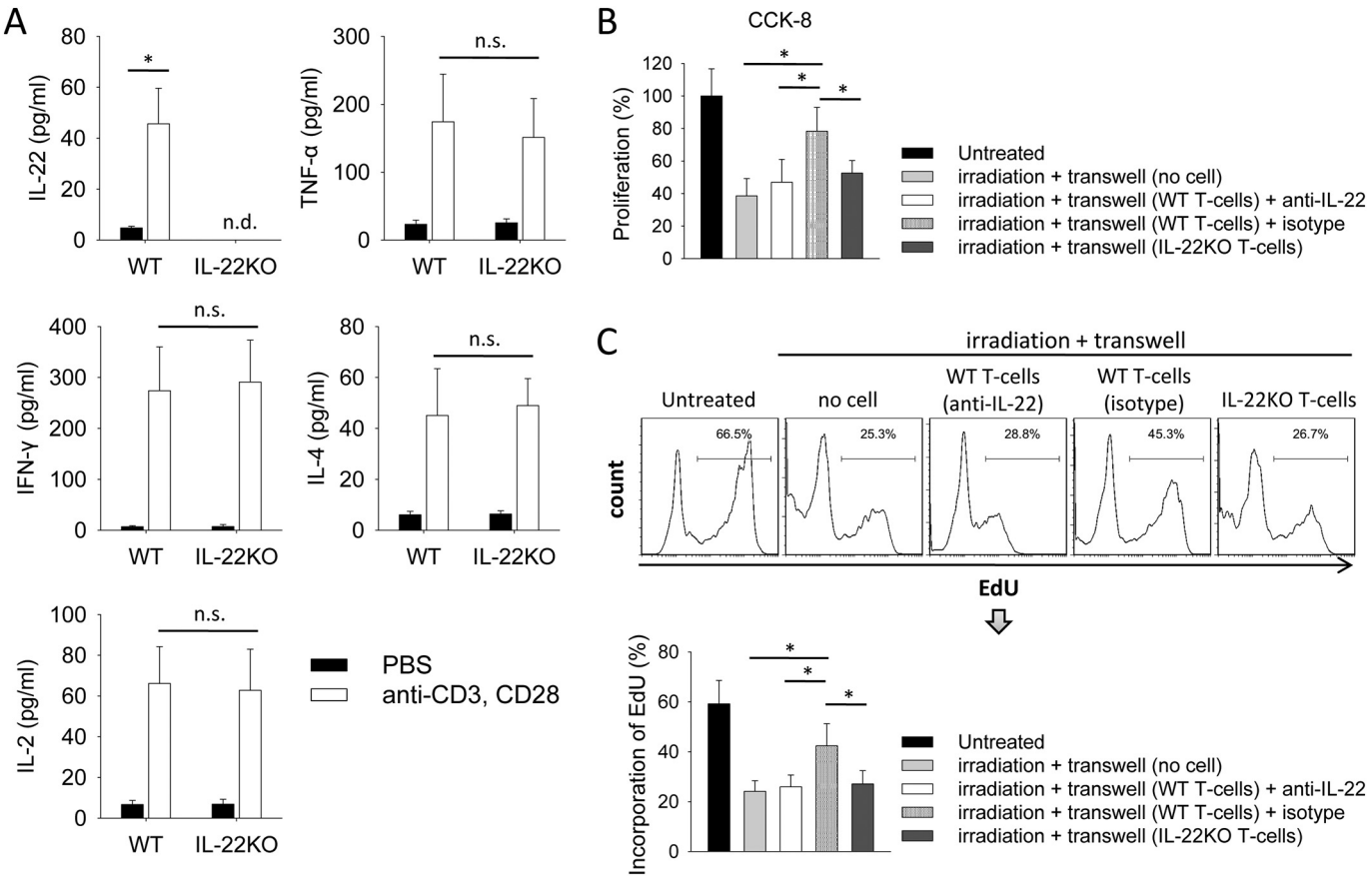

Fig. 3. Donor T cell-derived IL-22 stimulates proliferation of TECs in vitro. (A) T cells were isolated from spleen of wild type or IL-22KO mice, followed by stimulation with anti-mouse CD3ε and anti-mouse CD28 for 72 h. Supernatant of T-cell culture was delivered to cytometry bead array and ELISA analyses for levels of IL-22, IL-2, IL4, IFN-γ and TNF-α (n = 6). (B–C) T cells and mTEC1 cells were co-cultured in a Transwell for 72 h in the presence of anti-IL-22 (200 ng/ml) or isotype control antibody. mTEC1 cells, treated by 7.5 Gy irradiation, were cultured in lower chamber. T cells, isolated from spleen of wild type or IL-22KO mice, were stimulated with anti-mouse CD3ε and anti-mouse CD28 in the upper chamber. (B) Viability of cells was measured by CCK-8 assay (n = 6). (C) Proliferation of cells were assessed using EdU incorporation assay (n = 3). Data are shown as mean ± SD, and are compared using unpaired *t*-test or one-way ANOVA test. *, $p < 0.05$; n.s., no significance.

the same protocol once again. After the second digestion, all the cells were collected, followed by a transient digestion with 1 mg/ml dispase (Sigma-Aldrich). All released cells were harvested and suspended in PBS supplied with 2% FBS and 5 mM EDTA.

2.9. Flow cytometry

For analyzing intra-thymic cells, isolated thymus cells were stained with anti-CD45 (30-F11), anti-EpCAM (G8.8), anti-CD4 (RM4-5), anti-CD8a (53-6.7) and anti-lineage (Lin) antigen. Lin antigen cocktail contains anti-CD3ε (145-2C11), anti-Gr-1 (RB6-8C5), anti-B220 (RA3-6B2), anti-TER119 (TER-119), anti-NK1.1 (PK136) and anti-CD11b (M1/70).

For analyzing IL-22-producing cells, lymphocytes were stimulated with PMA (Sigma-Aldrich) and ionomycin (Sigma-Aldrich) in the presence of Brefeldin A (BD Biosciences, San Jose, CA, USA) for 4 h. Cells were harvested and processed with a Fixation/Permeabilization Solution Kit (BD Biosciences). Cells were stained with the following antibodies: anti-CD45 (30-F11), anti-CD3ε (145-2C11), anti-CD11c (N418), anti-H-2Kd (SF1-1.1), anti- H-2Kb (AF6-88.5) and anti-IL-22 (Poly5164).

For analyzing incorporation of EdU, mTEC1 cells were processed with an EdU Andy Fluor™ 647 Flow Cytometry Assay Kit (A008, GeneCopoeia, Guangzhou, China) per manufacturer's instructions. Cells were acquired and analyzed on an LSRFortessa flow cytometer (BD Biosciences).

2.10. Enzyme linked immunosorbent assay (ELISA)

For detecting intra-thymic levels of IL-22 protein, thymus was mildly ground in 1 ml of PBS, and supernatant was collected for further analysis. IL-22 concentrations in thymus supernatants and supernatants from cell cultures were measured using a mouse IL-22 ELISA kit (eBioscience, Thermo Fisher Scientific).

2.11. Western blot analysis

Whole cell proteins were extracted from thymus using Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA, USA). Western blot was performed with the following antibodies: Phospho-STAT3 (Tyr705), STAT3 and GAPDH (D16H11). All the antibodies were purchased from Cell Signaling Technology.

2.12. Cytometry bead array (CBA)

Supernatant concentrations of IL-2, IL4, IFN-γ and TNF-α were measured using CBA (BD Biosciences).

2.13. Cell viability

Viability of cells was measured with a Cell Counting Kit-8 (CCK-8, CK04) from DOJINDO (Tokyo, Japan).

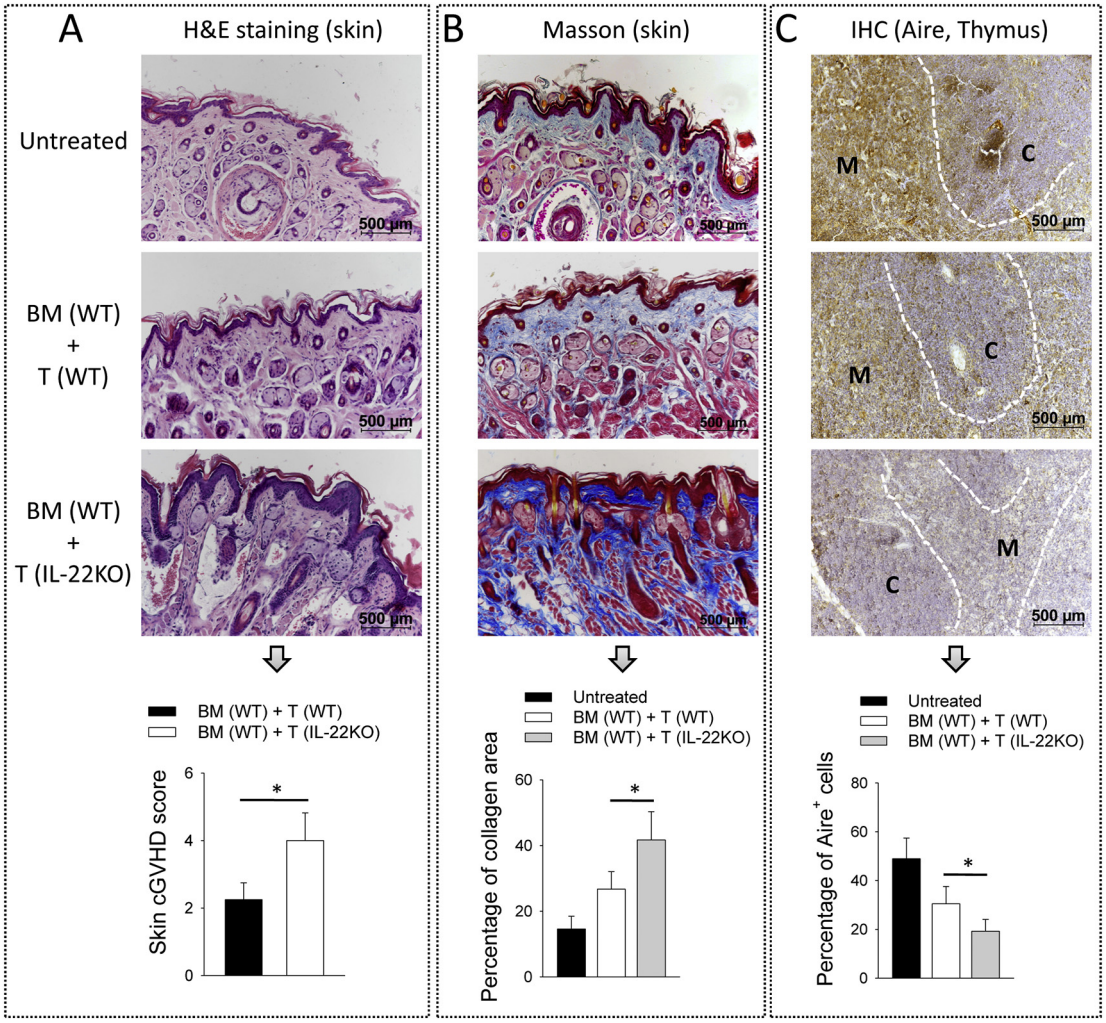

Fig. 4. Donor T cell-derived IL-22 alleviates chronic skin GVHD post allotransplant. For analyzing role of donor T-cell-derived IL-22 in allo-BMT, recipients were injected with wild type T-cell-depleted bone marrow cells together with splenic T cells from wild type or IL-22KO donors. (A–B) Skin tissues were obtained on day 60 posttransplant. H&E staining (A) and Masson staining (B) were performed on paraffin slides of skin tissues (n = 3). Skin chronic graft-versus-host disease (GVHD) was scored according to a scoring system. Collagen deposit, visualized by Masson staining, was indicated by percentage of collagen area, which was measured by Image J software. Scale bar: 500 μm. (C) Thymus samples were obtained on day 60 posttransplant (n = 3). Intra-thymic expression of Aire was detected by immunohistochemistry. Percentage of Aire$^+$ cells was analyzed by Image J software. Scale bar: 500 μm. Data are shown as mean ± SD, compared using unpaired *t*-test or one-way ANOVA test. *, $p < 0.05$.

2.14. Immunohistochemistry

To detect expression of Aire, immunohistochemistry was performed on paraffin slides of thymus. Anti-AIRE (orb135062) was from Biorbyt (Cambridge, UK). Peroxidase-conjugated goat anti-rabbit IgG (ZSGB-BIO, Beijing, China) was used as second antibody. DAB substrate was used for visualization (ZSGB-BIO).

2.15. Quantitative polymerase chain reaction

Total RNA were isolated from tissues using TRIzol (Thermo Fisher Scientific). cDNA was synthesized and subjected to quantitative polymerase chain reaction (qPCR) analysis. qPCR was performed with a LightCycler 480 SYBR Green I Master qRT-PCR kit (Roche, Mannheim, Germany). GAPDH was used as a normalization gene. The results were analyzed by using the method of comparison on $-\Delta\Delta C_T$ values. The following primers were synthesized from Invitrogen (Thermo Fisher Scientific, Shanghai, China): *Il22* (TCGCCTTGATCTCTCCACTC and GCTCAGCTCCTGTCACATCA), and *Gapdh* (TTGATGGCAACAATCTC CAC and CGTCCCGTAGACAAAATGGT). qPCR was performed on an LC480 cycler (Roche).

2.16. Statistics

Survival was compared using log-rank test. Data were presented as mean ± standard deviation (SD). Comparisons of means were performed with unpaired Student *t*-test or one-way ANOVA test followed by Bonferroni correction. P-values < 0.05 were considered significant.

3. Results

3.1. Donor T-cell is a major source of IL-22 in allotransplant recipient

To analyze kinetics of IL-22 level in recipients of murine allogeneic HCT, we measured IL-22 mRNA in bone marrow, spleen, lymph nodes, liver, lung, small intestine and thymus weekly after transplantation. Levels of IL-22 mRNA increased during day 7 and day 14 in spleen, liver, lymph nodes, lung and thymus. In contrast, levels of IL-22 mRNA decreased during day 7 and day 14 in small intestine and bone marrow (Fig. 1A).

Since T cells, myeloid cells and some innate immune cells are capable of producing IL-22 [13], we analyzed IL-22-producing cells of recipients in murine allogeneic HCT. More than 90% of IL-22-producing

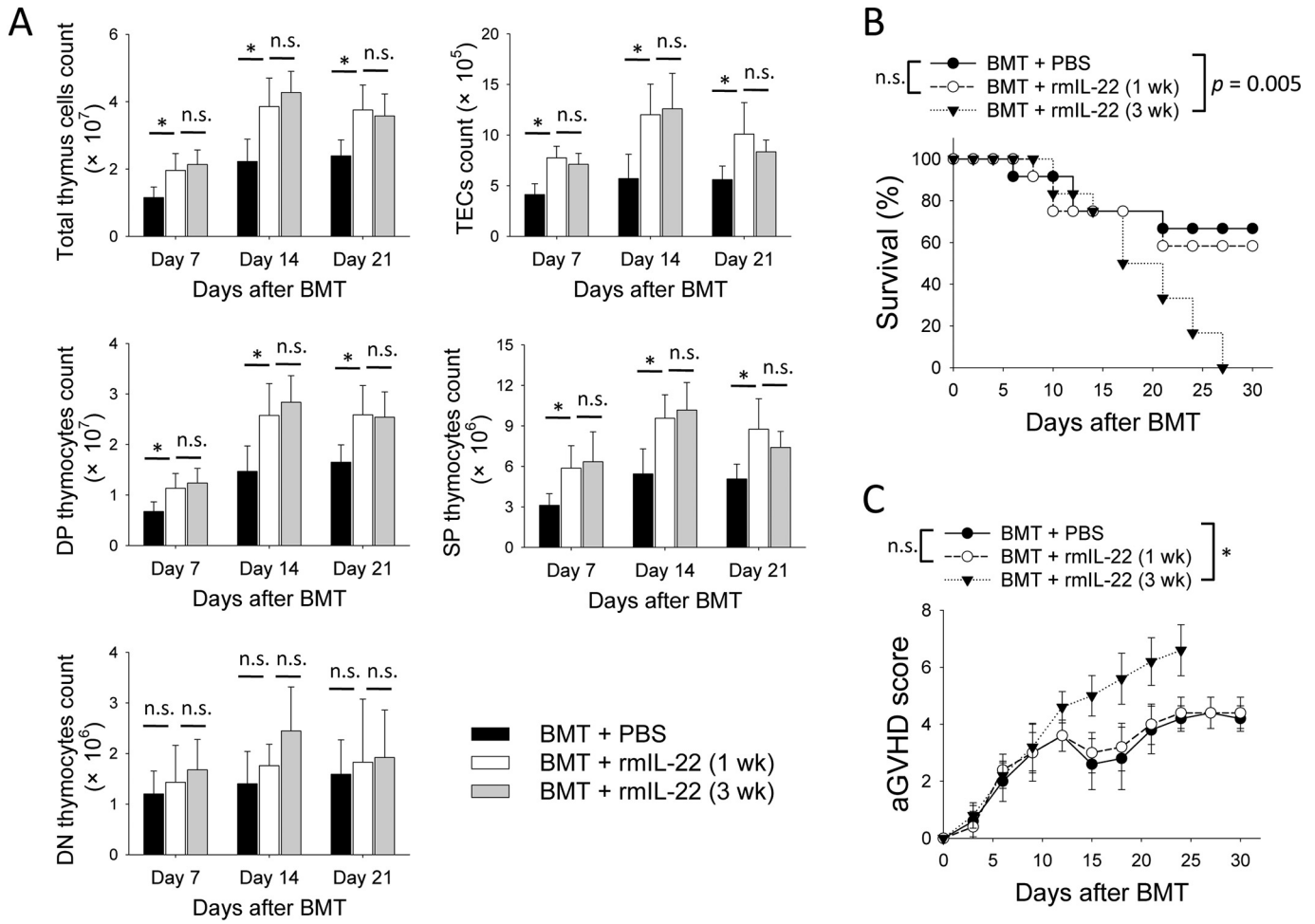

Fig. 5. Short-term use of exogenous IL-22 accelerates recovery of thymus. In C57BL/6 → BALB/c allo-BMT model, irradiated recipients received an allotransplant. Recipients were injected with PBS or rmIL-22 (0.1 mg/kg) on every other day after transplantation for up to 1 week or 3 weeks. (A) Flow cytometry was applied to analyze proportions of TECs, DP, SP and DN thymocytes in total thymus cells (n = 4). Survival (n = 24) (B) and acute GVHD sores (n = 10) (C) of recipients were continuously monitored. Survival was compared using log-rank test. Data are shown as mean ± SD, and are compared using one-way ANOVA test. *, $p < 0.05$; n.s., no significance.

cells were of donor origin in bone marrow, spleen, lymph nodes, liver and lung tissues, and around 80% of IL-22-producing cells were of donor origin in small intestine and thymus (Supplementary Fig. S1 and Fig. 1B). Among the donor derived cells in these tissues, most IL-22-positive cells were T cells ($CD3^+$), whereas a small part of IL-22-positive cells were myeloid cells ($CD11c^+CD3^-$) and non-T and non-myeloid cells ($CD11c^-CD3^-$). In addition, donor $CD11c^-CD3^-$ cells were also major producers of IL-22 in small intestine (Fig. 1C). Although T cells, myeloid cells and $CD11c^-CD3^-$ cells from the recipient were capable of producing IL-22 in these tissues (Fig. 1D), they took very small part in the total IL-22-positive cells. Thus, donor T-cell is a major source of IL-22 in immune organs and target tissues of GVHD (Fig. 1E). We further analyzed IL-22-producing potency of T-cell in bone marrow, spleen, lymph nodes, liver, lung, small intestine and thymus (Fig. 1F). T cells from allotransplant recipients produced more IL-22 than untreated mice derived-T-cells did. These results indicate that donor T-cell is a potent producer and a major source of IL-22 in allotransplant recipient.

3.2. Donor T cell-derived IL-22 mediates regeneration of thymus of recipients

To determine the impact of donor T-cell-derived IL-22 on thymus regeneration, we injected recipients with wild type T-cell-depleted bone marrow cells together with wild type T cells or IL-22KO T cells. Mice received IL-22KO T-cells had fewer thymus cells and TECs on days 7 and 14 posttransplant compared with those receiving wild type T cells. Injection of rmIL-22 improved recovery of thymus cells and TECs of IL-22KO T-cell-treated recipients on day 7 and day 14 posttransplant. Increased numbers of thymus cells were attributed to DP thymocytes and SP thymocytes (Fig. 2A and B). IL-22KO T-cell-treated recipients had lower level of intra-thymic IL-22 than wild type T-cell-treated ones, and injection of rmIL-22 increased level of intra-thymic IL-22 of IL-22KO T-cell-treated recipients (Fig. 2C). IL-22 was shown to activate JAK/STAT pathway [11]. We found that IL-22KO T-cell-treated recipients had lower levels of intra-thymic phosphorylated STAT3 on day 14 compared with mice receiving wild type T cells, which were reversed by injection of rmIL-22 to IL-22KO T-cell-treated recipients (Fig. 2D). These results indicate that donor T-cell-derived IL-22 improves regeneration of thymus of recipients.

3.3. Donor T cell-derived IL-22 stimulates proliferation of TECs in vitro

To confirm IL-22-mediated proliferative effect on TECs, we performed in vitro study by co-culturing activated T cells with irradiation-treated mTEC1, a murine thymic epithelial cell line. After being stimulated with anti-CD3 and anti-CD28 antibodies, wild type T cells produced more IL-22, which were not detectable in IL-22KO T cells. Wild type T cells produced comparable levels of TNF-α, IFN-γ, IL-4 and

IL-2 as IL-22KO T cells did (Fig. 3A). Next, we analyzed proliferation of irradiation-treated mTEC1 using cell counting assay and EdU incorporation assay. Irradiation reduced proliferation of mTEC1, which was reversed by co-culture with activated wild type T cells. This proliferative effect of wild type T cells was blocked by an IL-22 antibody. Similarly, IL-22KO T cells failed to induce a proliferative effect on irradiation-treated mTEC1 (Fig. 3B and C).

3.4. Donor T cell-derived IL-22 alleviates chronic skin GVHD post allotransplant

Given that IL-22 improves recovery of thymus [11], increasing T cells might be involved in pathogenesis of GVHD. We assessed severity of GVHD in mice receiving an allotransplant. Transplantation with IL-22KO T cells did not affect survival and severity of acute GVHD as compared with wild type T-cell-transplanted recipients (Supplementary Fig. S2). However, IL-22KO T-cell-transplanted recipients showed more severe chronic skin GVHD than wild type T-cell-transplanted ones, which was indicated by chronic GVHD scores (Fig. 4A) and collagen deposition (Fig. 4B). Because chronic GVHD is an autoimmune-like disease, we analyzed expression of Aire in thymus, an important regulator of autoimmune diseases [22]. Levels of intra-thymic Aire were decreased in IL-22KO T-cell-transplanted recipients (Fig. 4C). These results indicate that donor T cell-derived IL-22 alleviates chronic GVHD in association with improved thymus function.

3.5. Short-term use of exogenous IL-22 accelerates recovery of thymus

Since donor T cell-derived IL-22 mediated thymic regeneration after injury, we tested if use of exogenous IL-22 would accelerate recovery of thymus after allogeneic HCT in mice. One-week injection of rmIL-22 posttransplant increased counts of total thymus cells, TECs, DP thymocytes and SP thymocytes. However, prolonged use (three weeks) of rmIL-22 had no additive effect on recovery of thymus cells (Fig. 5A). One-week injection of rmIL-22 had no impact on survival and acute GVHD score of recipients. Of note, three-week injection of rmIL-22 aggravated acute GVHD of recipients (Fig. 5B and C). These results indicate that short-term use of exogenous IL-22 accelerates recovery of thymus without increasing severity of acute GVHD.

4. Discussion

In this study, we found donor T-cell-derived IL-22 stimulates proliferation of TECs and improves regeneration of thymus of allotransplant recipients. Donor T cell-derived IL-22 alleviates chronic skin GVHD. Short-term use of exogenous IL-22 posttransplant accelerates recovery of thymus without increasing severity of acute GVHD.

TECs directly contact with and provide growth signals for developing thymocytes. Therefore, promoting recovery of TECs represents a promising strategy in boosting repair of damaged thymus [10,23]. IL-22 has drawn increasing interest in recent years due to the regulatory functions in inflammation and epithelial cells [13]. IL-22 has been reported to mediate recovery of thymus [11,12]. We previously found that up-regulation of intra-thymic IL-22 correlated with regeneration of thymus in mice treated by total body irradiation, and deletion of DP thymocytes triggered up-regulation of intra-thymic IL-22 [21]. IL-22 is mainly produced by $CD4^+$ T cells and Group 3 innate lymphoid cells (ILC3) [13]. Myeloid cells such as macrophages and neutrophils were also reported to produce IL-22 [24,25]. In the setting of allogeneic HCT, our results indicate that donor T-cell is a major source of IL-22. Others reported that ILC3 is a potent IL-22 producer in murine allogeneic HCT [11,12]. We also observed a non-T and non-myeloid population of cells which were capable of producing IL-22 in the small intestine and thymus. Nonetheless, donor T cells isolated from immune organs and target tissues of GVHD produced substantial amount of IL-22. T-cell-derived IL-22 mediated recovery of TECs, which in turn promoted differentiation of thymocytes. We showed that cross-talk between T-cell and TECs is an important mechanism to mediate reconstitution of T-cell immunity. In addition, thymus is considered as a target organ in GVHD [6,26]. Our results showed that donor T-cell-derived IL-22 did not cause damage to thymus but rather played a protective role here.

Preclinical studies had reported the protective effect of IL-22 on epithelium [27]. We and others previously found that level of intra-thymic IL-22 increased shortly and transiently after thymic injury [11,21]. It remains unclear how to translate this protective effect of IL-22 into clinical practices. IL-22 exerts both pro-inflammatory and anti-inflammatory effects in acute GVHD [14,28]. We found that donor T-cell derived IL-22 promoted thymus recovery and alleviated skin chronic GVHD, which suggested that use of exogenous IL-22 might alleviate chronic GVHD. One concern is that use of IL-22 is at risk of increasing acute GVHD. Here, we showed that short-term use of exogenous IL-22 posttransplant accelerates recovery of thymus without increasing severity of acute GVHD. However, prolonged use (three weeks) of IL-22 did not further promote thymus recovery but rather increased severity of acute GVHD. Thus, use of IL-22 should be carefully conducted in allogeneic HCT regarding dosage and timing. Nevertheless, recombinant human IL-22 is being tested in clinical trials for treating hepatitis (NCT02655510) and gastrointestinal GVHD (NCT02406651), which would provide us more valuable information about use of exogenous IL-22 in allogeneic HCT.

GVHD remains a devastating challenge to allotransplant recipients. $CD4^+$ T cells and their related cytokines play central roles in pathogenesis of GVHD [29,30]. Some groups reported both protective and pathogenic roles of IL-22 in acute GVHD [14,15]. The function of IL-22 in chronic GVHD is still unclear. IL-22 exerts both pro-inflammatory and anti-inflammatory effects in different immune scenarios [13], which depends on dynamic of IL-22 production, donor or recipient origin, and involved target tissues [28]. We found that donor T-cell-derived IL-22 did not affect severity of acute GVHD but decreased skin chronic GVHD. Aire is mainly expressed on medullar TECs, which mediate negative selection function of thymus [4,22]. IL-22 increased level of intra-thymic Aire, which might improve negative selection function of thymus and prevent release of autoreactive T cells. Repairing function of thymus with IL-22 might represent a novel strategy to treat autoimmune diseases. IL-22 was also reported to alleviate chronic inflammation in other autoimmune disease and organ transplant [31,32].

5. Conclusions

In conclusion, we show donor T-cell derived IL-22 promotes thymic regeneration and decreased chronic skin GVHD post allotransplant. Short-term use of exogenous IL-22 posttransplant accelerates recovery of thymus without increasing severity of acute GVHD. Our findings may have implications for treatment of chronic GVHD in human.

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Authorship statement

K.X. contributed to the concept and design, analyzed data and revised the manuscript. B.P. performed experiments, analyzed data, wrote the manuscript and helped to design experiments. F.Z. and Z.L. performed experiments and analyzed data (BMT model and cell culture).

L.L., L.S., F.X., R.F. and M.X. performed part of experiments. L.Z. provided experimental expertise. B.P., F.Z. and Z.L. contributed equally to this study.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2018.12.023.

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