Journal of Immunology
Research and Therapy
JIRT, 3(1): 151-157
www.scitcentral.com

ISSN: 2472-727X

Original Research Article: Open Access

IL-27 Receptor Signaling on T cells Augments GVHD Severity through Enhancing Th1 Responses

David Bastian[1], Yuejun Liu[1,2], Yongxia Wu[1], Steven Schutt[1], Hung D Nguyen[1], Anusara Daenthanasanmak[1], M Hanief Sofi[1], Mengmeng Zhang[1], Supinya Iamsuwat[1] and Xue-Zhong Yu[1,3*]

[1]*Department of Microbiology and Immunology, Medical University of South Carolina, USA*

[2]*Institute of Blood and Marrow Transplantation, Department of Hematology, The First Affiliated Hospital of Soochow University, China*

[3]*Department of Medicine, Medical University of South Carolina, USA*

Received February 10, 2018; Accepted May 03, 2018; Published June 10, 2018

ABSTRACT

IL-27 is a heterodimeric cytokine comprised of IL-27p28 and EBI3. As a relatively new member of the IL-12 family, the biological mechanisms associated with the role of IL-27 in the immune response are ambiguous, displaying both proinflammatory and suppressive functions that seem to be dependent on the disease model. A recent report demonstrates that pharmacological blockade of IL-27p28 alleviates graft-versus-host disease (GVHD) in mice. However, the specific role of the IL-27Rα/gp130 signaling complex that forms the IL-27 receptor (IL-27R) on T cells has not been well characterized in the context of allogeneic hematopoietic stem cell transplantation (allo-HCT). Here, we demonstrate that IL-27Rα expression on T cells exacerbates GVHD after allo-HCT, which was consistent across 3 different MHC- mismatched murine models of allo-HCT. Expression of IL-27Rα on T cells was required for acquisition of optimal Th1 effector function and subsequent inhibition of Th2 and T regulatory subsets after allo-HCT. Furthermore, administration of IL-27significantly increased mortality after allo-HCT; suggesting that the suppressive functions linked to IL-27 in T cell responses may be relatively modest in this model. Hence, IL-27Rα signaling on T cells promotes the development of GVHD.

INTRODUCTION

Interleukin-27 (IL-27) is a heterodimeric cytokine belonging to the IL-12 family. IL-27 is comprised of an IL-27p28α-chain and an EBI3β-chain and is the only member of the family that is not secreted as a functional dimer [1,2]. As such, the receptor for IL-27is also heterodimeric and is composed of a unique IL-27 receptor (IL-27R) component, or WSX-1, that forms a complex with gp130 to transduce signaling [3]. Activated dendritic cells (DCs) and monocytes serve as the primary source of p28 and EBI3 [1]. IL-27Rα is expressed in low levels on naïve T cells, but is upregulated on effector and memory T cells [4]. The biological mechanisms associated with the role of IL-27 in the immune response are ambiguous, displaying both proinflammatory and suppressive functions that seem to be dependent on the disease model.

Allogeneic hematopoietic stem cell transplantation (allo-HCT) is an effective means by which to treat a wide variety of diseases resulting from dysfunctional hematopoiesis; ranging from certain immune deficiencies to severe blood diseases and cancers [5]. However, the development of graft-versus-host disease (GVHD) remains the major cause of morbidity and mortality after allo-HCT. Acute GVHD (aGVHD) generally occurs in the first 100 days post allo-HCT and is a result of donor T cell recognition of genetically disparate antigens presented by antigen presenting cells (APCs), which subsequently leads to activation of both the innate and adaptive immune responses against host epithelial tissues; namely the skin, lung, liver and gastrointestinal tract (GI tract) [6].

A recent report demonstrates that pharmacological blockade of IL-27p28 alleviates GVHD in mice [7]. However, the specific role of the IL-27R/gp130 signaling complex that forms the IL-27 receptor on T cells during GVHD

Corresponding author: Xue-Zhong Yu, Department of Microbiology and Immunology, MSC 955, Medical University of South Carolina, 86 Jonathan Lucas Street, Charleston, SC 29425-5090, USA, Tel: (843) 792-4756; E-mail: yux@musc.edu

Citation: Bastian D, Liu Y, Wu Y, Schutt S, Nguyen HD, et al. (2018) IL-27 Receptor Signaling on T cells Augments GVHD Severity through Enhancing Th1 Responses. J Immunol Res Ther, 3(1): 151-157.

development is still unclear. Hence, we evaluated the role of IL-27R signaling in T cell responses to alloantigen across multiple MHC- mismatched models of allo-HCT and found that IL-27Rα expression promotes T cell pathogenicity attributable to augmented Th1 effector function.

MATERIALS AND METHODS

Mice

C57BL/6 (B6; H-2^b), BALB/c (H-2^d), B6-Ly5.2 (H-2^b), B6D2F1 (B6 x DBA2) F1 (H-$2^{b/d}$) were purchased from NCI. IL-27R KO and BALB. B mice were purchased from Jackson Labs. All animals were housed in specific pathogen-free conditions in the America Association for Laboratory Animal Care-accredited Animal Resource Center at the Medical University of South Carolina (MUSC). The Institutional Animal Care and Use Committee of MUSC approved all work.

GVHD models

Using an X-RAD 320 irradiator, lethally irradiated recipient BALB/c (650cGy), BALB. B (900cGy) or B6D2F1 (1200cGy) recipients were transplanted with 5 × 10^6 T cell depleted bone marrow (TCD-BM) alone or TCD-BM plus 1-3 × 10^6 WT or IL-27R KO T cells and monitored for survival and body weight loss as previously described [8-11]. T cells were purified from pooled spleen and lymph node cells by negative selection to remove non-T cells including B cells, natural killer (NK) cells, DCs, macrophages, granulocytes and erythroid cells. Briefly, non-T cells were magnetically labeled with biotin-conjugated Abs against CD45R (B220), CD49b (DX5), CD11b (Mac-1) and Ter-119, followed by anti-biotin MicroBeads (Miltenyi Biotech, Auburn, CA). Isolation of T cells was achieved by negative selection. Bone marrow (BM) was harvested from tibia and femurs and T cells were depleted through complement lysis of Thy1.2+ cells.

For experiments involving adenoviral production of IL-27, mice were injected intramuscularly with 2 × 10^{11} DRP of vectors 7 days prior to BMT with either vector control or IL-27AAV [12].

Flow cytometry and intracellular cytokine staining

Mononuclear cells were isolated from recipient spleen or liver as previously described and stained for surface markers and intracellular cytokines using standard flow cytometric protocols [10,11]. Stained cells were analyzed using FACSDiva software, LSR II (BD Biosciences, San Jose, CA) and FlowJo (Tree Star, Ashland, OR).The following Abs were used for cell-surface staining: anti-CD4-V450, -APC and -PEcy7 (BD Biosciences), anti-CD8-PEcy5, -APCcy7 and -AF700 (BD Biosciences,); anti-CD45.1-FITC and -APC (BD Biosciences). Intracellular staining was carried out using anti-IFN-γ-PE or Per-cp 5.5 (XMG1.2; BD Biosciences), anti-IL-4-PE (11B11; BD Pharmingen), anti-IL-5-PE (TRFK5; BD Pharmingen), anti-Foxp3-PE (FJK-16s; eBioscience).

Statistics

For comparison of recipient survival among groups in GVHD experiments, the log-rank test was used to determine statistical significance. To compare body weight changes and cytokine levels, a Student t test was performed.

RESULTS

IL-27R is required for T cells to induce GVHD

Given the recent findings that IL-27p28 exacerbates graft-versus-host disease (GVHD), we hypothesized that targeting the alpha receptor subunit of the IL-27 receptor (IL-27Rα) specifically on T cells would result in a reduction in GVHD severity after allogeneic bone marrow transplantation (allo-BMT). In order to decipher the role of IL-27Rα on T cells, we initially tested the ability of IL-27R deficient T cells to cause GVHD in a MHC-matched but minor histocompatibility antigen (miHA) mismatched murine BMT model, C57BL/6 to BALB.B. Recipients that received T cells deficient for IL-27Rα developed less severe GVHD, as shown by a significantly higher survival percentage across experiments compared to WT controls **(Figure 1A)**; which correlated with significantly improved body weight maintenance among groups that received IL-27Rα deficient T cells **(Figure 1B)**. Hence, T cells deficient for IL-27Rαhave a compromised ability to induce GVHD in a MHC-matched model of allo-BMT.

IL-27R signaling augments Th1 responses

IL-27 was initially reported to be involved in Th1 differentiation [1]. Therefore, we hypothesized that a decrease in IFNγ production by T cells might be responsible for the alleviated GVHD burden seen in the MHC-matched BMT model **(Figures 1A and 1B)**. Consistent with this hypothesis, we found a significant decrease in the percentage of IFNγ+ T cells in the spleen **(Figures 2A and 2C)** and liver **(Figures 2B and 2D)** of cohorts that received IL-27RαKO T cells 21 days' post BMT. This data demonstrates that IL-27Rα plays a role in T cell pathogenesis during GVHD development and that this pathogenicity is, at least in part, attributable to an IL-27Rα-dependent Th1 effector response.

IL-27R is required for optimal T cell pathogenicity across multiple BMT models

Since we observed that CD4+ T helper cell differentiation was significantly altered in IL-27R deficient T cells after allo-BMT in the B6 to BALB.B model, we hypothesized that a similar reduction in GVHD may also hold true in additional models of allo-BMT. To address this hypothesis, we used both the B6 to B6D2F1 **(Figures 3A and 3B)** haplo identical BMT model as well the B6 to BALB/c **(Figures 3C and 3D)** full MHC-mismatched model. In both models,

we found an increase in survival percentage **(Figures 3A and 3C)** among recipients of IL-27R KO T cells compared to WT controls after allo-BMT, albeit not statistically significant in BALB/c recipients. This data confirms that IL-27R expression on T cells plays a pathogenic role in GVHD development across multiple murine BMT models.

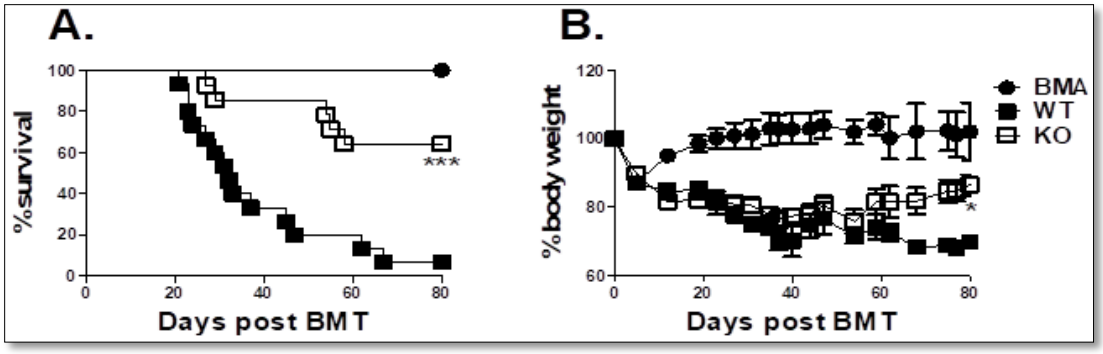

Figure 1. Role of IL-27R on T cells in miHA-mismatched GVHD. Lethally irradiated BALB.B mice were transplanted with 5×10^6 TCD-BM alone (BMA) or plus 3×10^6 purified T cells from WT B6 or IL-27R KO mice. Percentages of recipient survival (A) and body weight loss (B) are shown. Data shown is pooled from 2 replicate experiments with a total of 4 BALB.B mice that received TCD-BM alone and 10 BALB.B mice per group that received T cells.
** p<0.05; *** p<0.001*

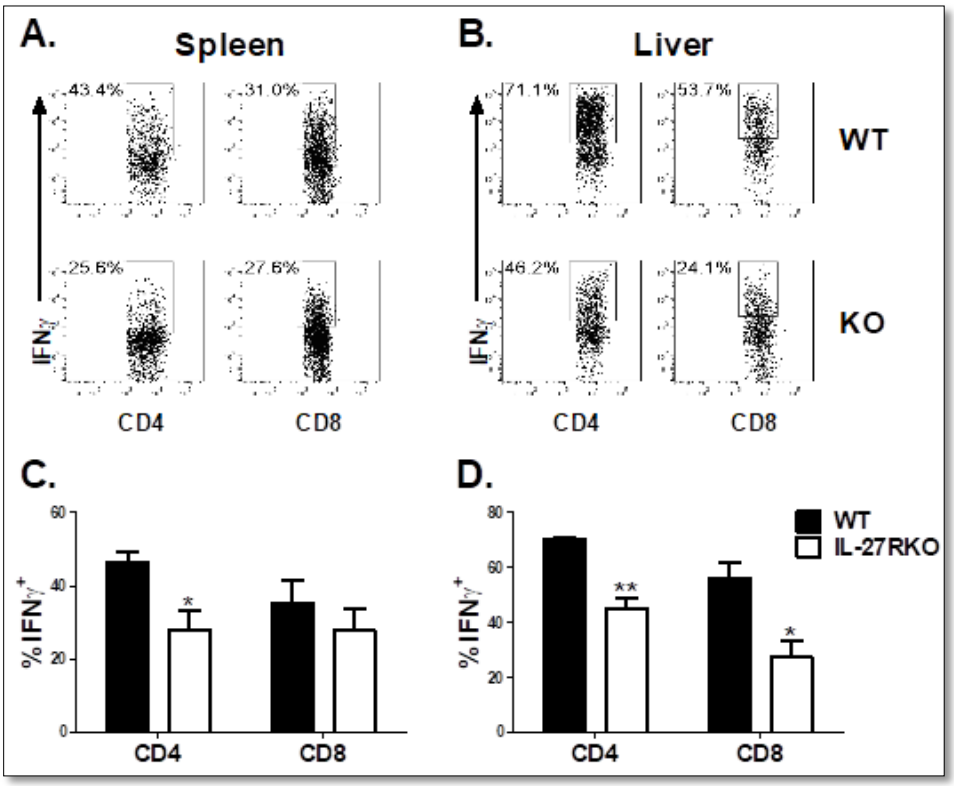

Figure 2. Effect of IL-27R on donor T cells in response to miHA. Lethally irradiated BALB.B mice were transplanted with 5×10^6 $Ly5.1^+$ TCD-BM alone or plus 3×10^6 purified T cells ($Ly5.2^+$) from WT B6 or IL-27R KO mice. Twenty-one days post-BMT, recipient spleens and livers were collected and mononuclear cells were isolated and stained for surface and intracellular markers for analysis by flow cytometry. Flow cytometry data is depicted for 1 representative mouse per group on $IFN\gamma^+$ expression among gated $Ly5.1^-$ $CD4^+$ or $CD8^+$ cells in the spleen (A) and liver (B). Average percentages of $CD4^+$ or $CD8^+$ T cells positive for IFNγ among gated $Ly5.1^-$ $CD4^+$ or $CD8^+$ cells in the spleen (C) and liver (D) are shown. Data from 1 representative experiment with 4 mice per group is shown. The total number of mice analyzed in these experiments in the WT group was 3 and the total number group in the IL-27R KO group was 4.
** p<0.05; ** p<0.01*

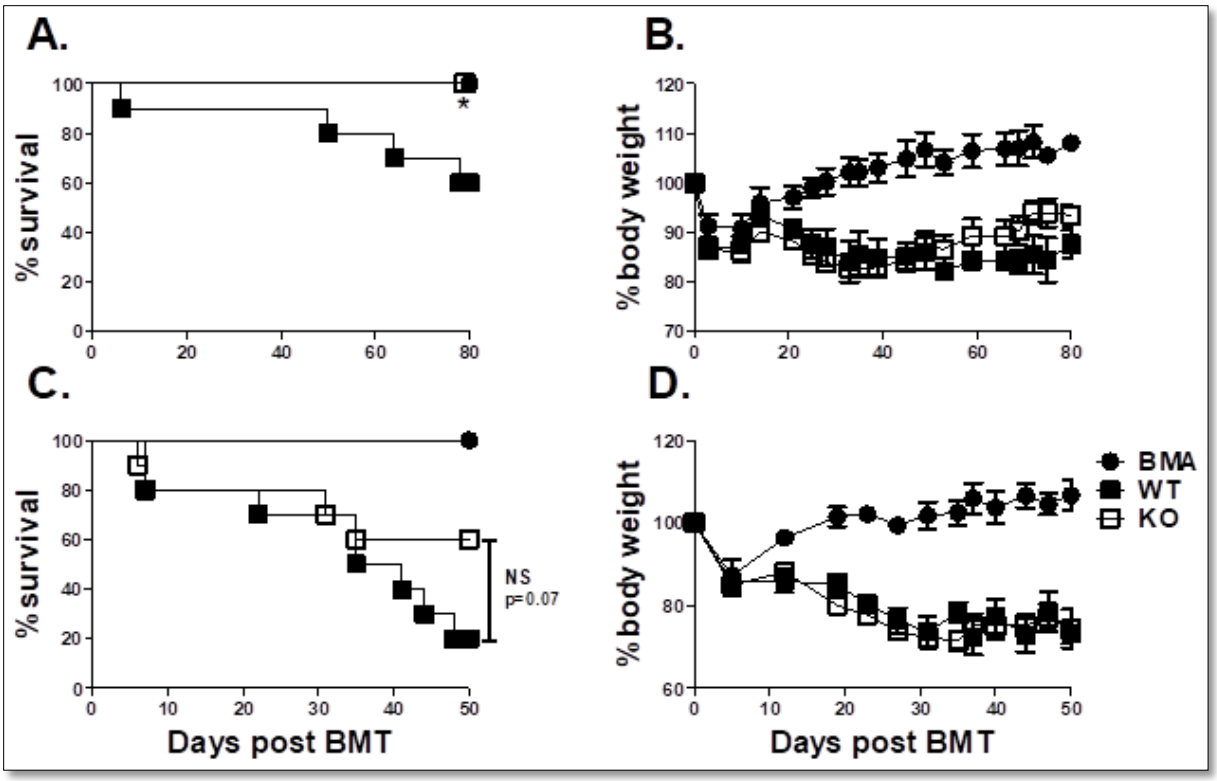

Figure 3. Role of IL-27R on T cells in haplo-identical or fully mismatched models of GVHD. Lethally irradiated B6D2F1 (A, B) or BALB/c (C, D) mice were transplanted with 5×10^6 TCD-BM alone or plus 3×10^6 (B6D2F1) or 1×10^6 (BALB/c) purified T cells from WT B6 or IL-27R KO mice. Percentages of recipient survival (A, C) and body weight loss (B, D) are shown.

*Data shown is pooled from 2 replicate experiments with a total of 4 BALB/c or B6D2F1 mice that received TCD-BM alone and 10 BALB/c or B6D2F1 mice per group that received T cells. * $p<0.05$*

IL-27R expression on T cells inhibits differentiation toward Th2 and T regulatory subsets after allo-BMT

Our previous results indicate that IL-27R expression on T cells can promote GVHD after allo-BMT and that this is attributable to an increased Th1 response. In order to corroborate this mechanism by which IL-27R on T cells influences the development of GVHD, we analyzed T cell proliferation and differentiation in the spleen and liver of BALB/c recipients 21 days' post BMT **(Figure 4)**. In the spleen, we saw a significant decrease in the percentage of IFNγ produced by CD4+ T cells in recipients that received IL-27R KO T cells compared to WT controls. Additionally, IL-27R KO CD4+ T cells isolated from the spleen produced a significantly higher percentage of IL-4/5 and had a significantly increased percentage of Foxp3 expression **(Figures 4A and 4B)**. Consistently, recipients of IL-27R KO T cells had a significantly higher percentage of CD4+IL-4/5+ T cells in the liver **(Figures 4C and 4D)**. These data indicate that T cells deficient for IL-27R are skewed away from Th1 differentiation and instead differentiate intoTh2 and T regulatory subsets, a pattern which would be manifested by reduced GVHD. However, we also noted significant increases, albeit among few cells, in IL-17 production by IL-27R KO CD4+ T cells in both the spleen **(Figures 4A and 4B)** and the liver **(Figures 4C and 4D)**.

Administration of IL-27 exacerbates GVHD

Our results specifically demonstrate that IL-27R expression on T cells augments GVHD. Taken together with previous literature implicating IL-27p28 as a proinflammatory mediator of GVHD development, we sought to delineate whether the cytokine IL-27 could augment T cell mediated GVHD. To address this question, we injected either an adenoviral vector expressing the IL-27 heterodimer (IL-27AAV) or empty vector into BALB/c recipients 7 days prior to BMT. Lethally irradiated recipient mice were then transplanted with B6 T cells and TCD-BM as described in **Figure 1** and monitored for survival **(Figure 5A)** and body weight loss **(Figure 5B)**. Recipient mice that were treated with IL-27AAV had significantly higher mortality than those that received empty vector **(Figure 5A);** providing further support for the notion that IL-27 signaling on T cells promotes the development of GVHD.

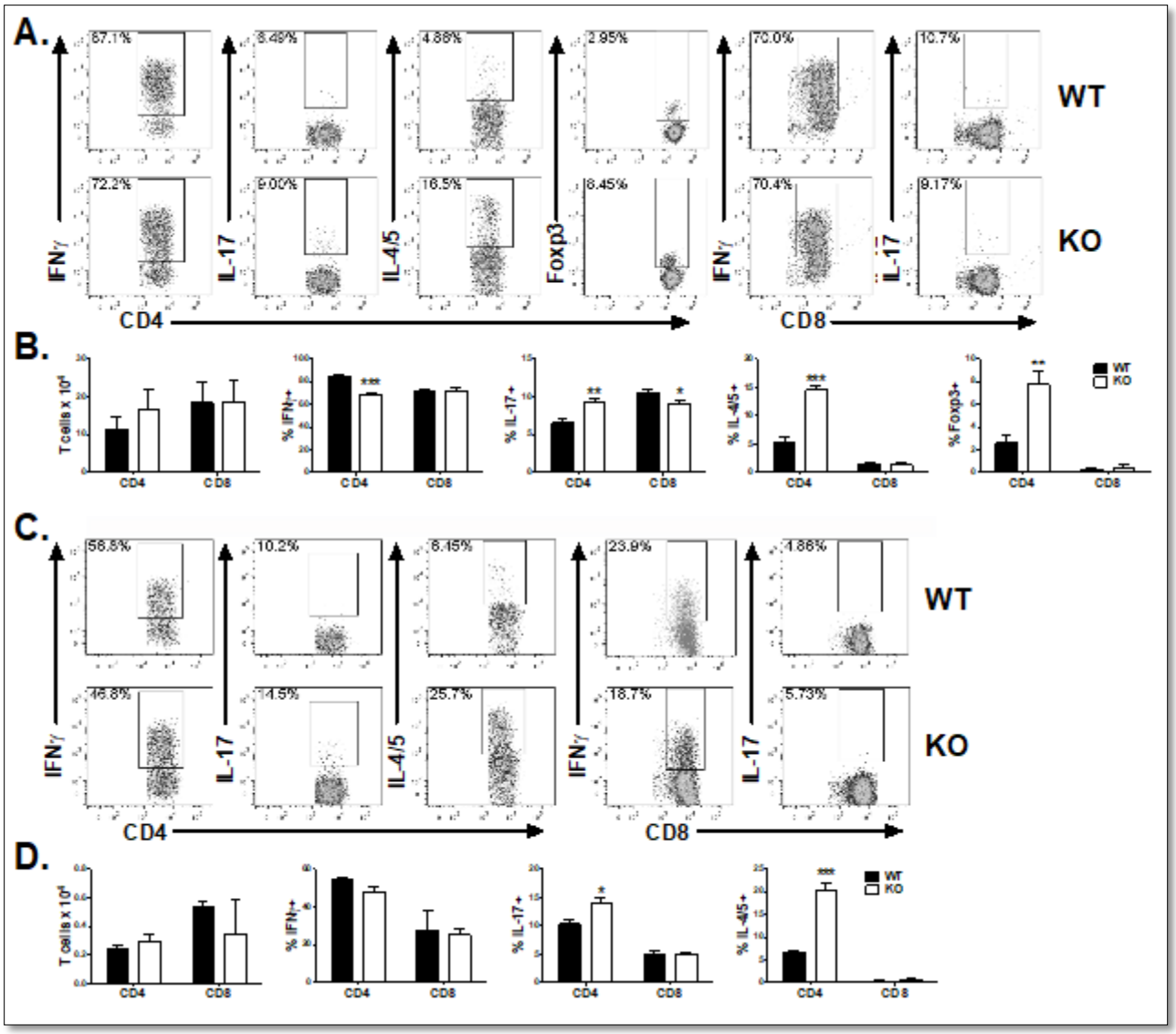

Figure 4. Effect of IL-27R on donor T cells during GVHD development. Lethally irradiated BALB/c mice were transplanted with 5 × 10^6 Ly5.1$^+$ TCD-BM alone or plus 1x10^6 purified T cells (Ly5.2$^+$) from WT B6 or IL-27R KO mice. 21 days post-BMT, recipient spleens and livers were collected and mononuclear cells were isolated and stained for surface and intracellular markers for analysis by flow cytometry. Flow cytometry data is depicted for 1 representative mouse per group for percentages of IFNγ$^+$, IL-4/5$^+$, IL-17$^+$ or Foxp3$^+$ (Tregs) among gated H2Kb$^+$ Ly5.1$^-$ CD4$^+$ or CD8$^+$ cells in the spleen (A) and liver (C). Average percentages of CD4+ or CD8+ T cells positive for IFNγ, IL-4/5, IL-17 or Foxp3 among gated H2Kb$^+$ Ly5.1$^-$ CD4$^+$ or CD8$^+$ cells in the spleen (B) and liver (D) are shown.

*Data from 1 out of 2 replicate experiments with 4 mice per group are shown. The total number of mice analyzed in these experiments in the WT group was 8 and the total number group in the IL-27R KO group was 6. * $p<0.05$; ** $p<0.01$, *** $p<0.001$*

DISCUSSION

In the context of autoimmunity, IL-27 has been implicated in both pro- and anti-inflammatory responses. Taken together with the recent report advocating for pharmacological blockade of p28 as a potential therapy for GVHD, our results unambiguously demonstrate that IL-27 signaling on T cells exacerbates GVHD after allo-HCT.

In this study, we observed a consistent increase in GVHD severity; not only in a MHC-matched model of allo-BMT, but also in MHC complete-mismatched as well MHC haploidentical BMT model in cohorts that received T cells expressing IL-27R. Mechanistically, we observed that Th1 responses were augmented in cohorts that received IL-27R competent T cells, while Th2 and Treg differentiation were significantly decreased, consistent with previous reports. Of

note, the observed increase in IL-4/5 production by donor T cells was quite dramatic. This is supported by previous studies which implicate IL-27 signaling as a critical regulator of T-bet and IL-12Rβ2 expression in T cells and further demonstrate that these Th1-promoting factors consequently suppress the master Th2 transcription factor, GATA3 [13].

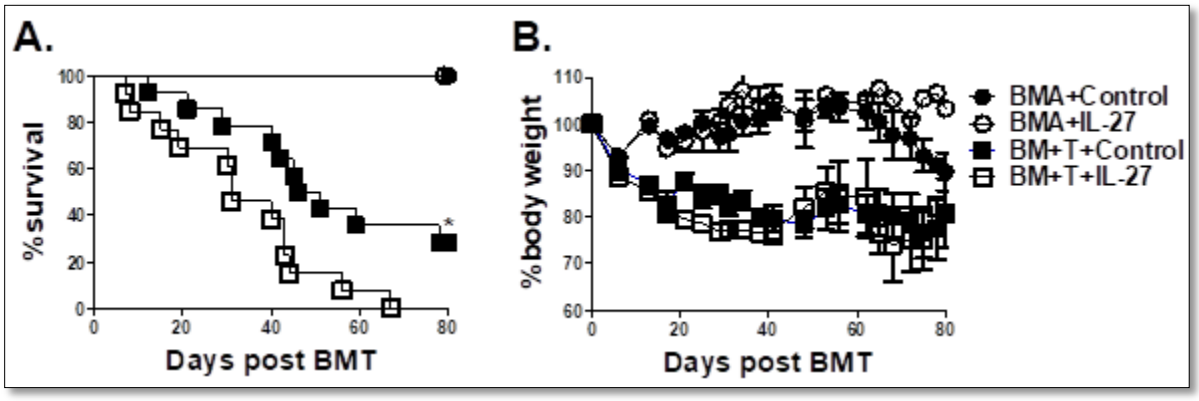

Figure 5. Effect of IL-27 administration in GVHD. Lethally irradiated BALB/c mice were transplanted with 5 × 10^6 TCD-BM alone or plus 1 × 10^6 purified T cells from WT B6 or IL-27R KO mice. Percentages of recipient survival (A) and body weight loss (B) are shown. Mice were injected intramuscularly with 2 × 10^{11} DRP of vectors 7 days prior to BMT with either vector control or IL-27AAV.

*Data shown is pooled from 3 replicate experiments with a total of 6 BALB/c mice that received TCD-BM alone with vector control or IL-27AAV and 14 BALB/c mice per group that received T cells plus vector control and 13 BALB/c mice per group that received T cells plus IL-27AAV. * $p<0.05$*

Our results indicate that IL-17 production was significantly decreased in mice that receivedIL-27R expressing T cells, which could be a potential explanation for why we did not observe a significant difference in body weight maintenance throughout our experiments. The increase in IL-17 production by IL-27R deficient T cells could be potentially explained by the significant reduction in IFNγ production, which has been reported to negatively regulate Th17 differentiation and, hence, IL-17 production [14]. Rather, the decreased function of Th1 cells, which hypothetically would alleviate GVHD, was offset by an increased Th17 response, resulting in Th17-mediated pathology and subsequently no difference in weight maintenance could be observed. This is supported by reports demonstrating IL-27a signaling negatively regulates Th17 differentiation using other models of autoimmunity [15,16].

In addition to demonstrating that IL-27 signaling on T cells promotes the development of GVHD, we investigated the effect of IL-27 administration after allo-BMT. In these experiments, we observed that excess IL-27 significantly increased GVHD severity. Hence, our results address the role of IL-27 in GVHD in 2 different ways, and further substantiate the claim that IL-27 signaling exacerbates GVHD. In conclusion, we provide additional evidence that IL-27 signaling is detrimental in GVHD and advocate that this pathway could be a potentially efficacious therapeutic target in clinical settings.

ACKNOWLEDGEMENT

We thank Dr. Xue-feng Bai for providing recombinant AAV vector to express IL-27. We thank the members of DLAR and the Flow CytometryCore Facility at the Medical University of South Carolina. The project described was supported in part by the NIH National Center for Advancing Translational Sciences (NCATS) through Grant Numbers TL1 TR001451 and UL1 TR001450.This work is partially supported by R01 CA169116, CA118116, CA169116 and R21 CA192202 (to X.-Z.Yu).

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