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A Role for IL-27 in Limiting T Regulatory Cell Populations

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IL-27 is a cytokine that regulates Th function during autoimmune and pathogen-induced immune responses. Although previous studies have shown that regulatory T cells (Tregs) express the IL-27R, and that IL-27 inhibits forkhead box P3 upregulation in vitro, little is known about how IL-27 influences Tregs in vivo. The studies presented in this article show that mice that overexpress IL-27 had decreased Treg frequencies and developed spontaneous inflammation. Although IL-27 did not cause mature Tregs to down-regulate forkhead box P3, transgenic overexpression in vivo limited the size of a differentiating Treg population in a bone marrow chimera model, which correlated with reduced production of IL-2, a vital cytokine for Treg maintenance. These data identify an indirect role for IL-27 in limiting Treg pool.

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Interleukin-27 is a member of the IL-6/IL-12 family of cytokines, a group of factors remarkable for their pleiotropic effects on Th function, differentiation, and development (1–3). IL-27 is a heterodimer composed of the IL-27p28 and EBV-induced 3 (EBI3) subunits that signals through a receptor consisting of WSX-1 and gp130 (4, 5). Ligation of the receptor complex activates STAT proteins that promote immune-regulatory gene expression programs (4–8). Initially, IL-27 was described as a proinflammatory molecule produced by APCs that supports Th1 responses (4). Naïve T cells exposed to IL-27 activated STAT1 and upregulated T-box expressed in T cells and IL-12Rβ2 expression, rendering these cells sensitive to IL-12 signals (6–8). Subsequent reports revealed that IL-27 can also limit inflammation. Thus, when mice deficient in WSX-1 were infected with Toxoplasma gondii or Trypanosoma cruzi, they mounted a protective Th1 response and controlled parasite replication, yet succumbed acutely to a lethal T cell-mediated inflammatory disease, suggesting that IL-27 has a suppressive role during these infections (9, 10). Other studies in various models of inflammation have expanded our understanding of the anti-inflammatory properties of IL-27 in Th1 (11–13), Th2 (14–16), and Th17 responses (17–19).

Although we appreciate that IL-27 can have both proinflammatory and anti-inflammatory effects in vivo, the biologic function of IL-27 during immune homeostasis remains unclear. WSX-1−/− and IL-27p28−/− animals do not display any overt immunologic defects, suggesting that IL-27 signaling is not required for the development of a normal immune system. However, there is evidence that IL-27 may have a role in modulating the regulatory T cell (Treg) population. Tregs are vital in preventing autoimmunity and uncontrolled inflammation in the steady state, and they suppress inappropriate immune responses through a variety of mechanisms (20–22). Accordingly, there is significant interest in identifying the factors that can influence Treg homeostasis and function (23). Naïve CD4+ T cells cultured with TGF-β (24) and IL-2 (25) in vitro upregulate the transcription factor Foxp3, indicating a differentiation to the Treg fate (26, 27). When these inducible Tregs are exposed to IL-27 during differentiation, Foxp3 upregulation is inhibited (28–30). Furthermore, natural Tregs (nTregs) express high levels of WSX-1 (31). In addition, IL-27 can inhibit the production of IL-2 (32, 33), a vital factor that supports the generation and maintenance of the Treg pool (34–39). In order to investigate the role that IL-27 plays in Treg homeostasis, we used a transgenic mouse model in which the IL-27p28 and EBI3 subunits are overexpressed [IL-27 transgenic (Tg) mice]. IL-27 Tg mice succumbed at 8–11 wk of age to a systemic inflammatory condition, characterized by immune pathology in multiple tissues, increased percentages of activated T cells, and elevated cytokine levels. In accordance with this uncontrolled inflammation, IL-27 Tg mice lacked Treg in lymphoid organs. In vitro and in vivo studies showed that IL-27 did not cause Foxp3 downregulation in mature nTregs; however, in a bone marrow (BM) chimera model, when IL-27 was present during the differentiation or generation of the Treg pool, re-constitution of the Treg population was inhibited. Consistent with these findings, IL-27 Tg mice had a marked defect in their capacity to produce IL-2, an important cytokine for Treg homeostasis. These data suggest that IL-27 can limit the generation or maintenance of the Treg population by inhibiting IL-2 production and thus promote inappropriate inflammation.

Materials and Methods

Mice

C57BL/6 mice were obtained from the Jackson Laboratory, and CD45.1 congenic mice were obtained from Taconic Farms. Foxp3GFP mice have been described previously (40). Mice that overexpress either IL-27p28 or EBI3 were generated by Zymogenetics. The murine IL-27p28 or EBI3 open reading frames were amplified by PCR, and the resulting cDNA was cloned into an Eμ lck transgene expression vector (41), driving expression in T and B cells. Expression cassettes were microinjected into B6C3F1 oocytes fertilized by C57BL/6 males, using procedures described pre-
viously (42). Stable lines were generated by breeding founders to C57BL/6 mice. Hemizygous female EB13 Tg mice were bred with hemizygous male IL-27p28 Tg mice to produce IL-27 Tg mice that carry overexpressed alleles of IL-27p28 and EB13. Mice were maintained in specific pathogen-free conditions at the University of Pennsylvania animal facility, according to institutional and federal regulations.

Pathology

Tissues were fixed in buffered paraformaldehyde and embedded in paraffin. Sections were cut and stained with H&E, trichrome, or periodic acid Schiff-Alien Blue by the University of Pennsylvania School of Veterinary Medicine Pathology Laboratory. Images were captured using a Nikon Eclipse E600 microscope, a Photometrics Cool Snap EZ CCD camera, and Nikon NIS Elements software.

Flow cytometry, intracellular staining, and cell sorting

Single-cell suspensions were prepared from spleens, lymph nodes (LN), periportal exudate cells (PECs), and thymus, and cells were surface stained with the following Ab: FITC anti-CD3e (145-2C11) and anti-CD69 (H1.2F3), PerCP-Cy5.5 anti-CD45.1 (A20), Pacific Blue anti-CD3e (17A2), allophycocyanin anti-CD25 (PC61.5), anti-CD45.2 (104), and anti-CD62L (MEL-14), allophycocyanin–eFluor 780 anti-CD25 (PC61.5), anti-CD45.2 (104), and anti-CD62L (MEL-14), PE anti-CD25 (PC61.5) and anti-CD69 (H1.2F3), and PE-Cy7 anti-CD44 (IM7) supplied by eBioscience; Pacific Orange anti-CD8α (53-6-7) and PE-Texas Red anti-CD62L (MEL-14) supplied by Invitrogen; PerCP-Cy5.5 anti-CD4 (RM4-5) and anti-CD8α (53-6-7), PE anti-CD45.1 (A20), PE-Cy5 anti-CD44 (IM7), and PE-Cy7 CD69 (H1.2F3) supplied by BD Biosciences; and Alexa Fluor 700 anti-CD3 (17A2) supplied by BioLegend. To stain for Foxp3 or GFP, the eBioscience Foxp3 staining set was used according to manufacturer instructions, and cells were stained with the following Ab: eFluor 450 anti-Foxp3 (FJK-16s) and purified rabbit polyclonal anti-GFP (eBioscience) and Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen). To stain for intracellular cytokines, cells were stimulated for 4 h with 50 ng/ml PMA (Sigma), 500 ng/ml ionomycin (Sigma), 10 μg/ml brefeldin A (Sigma), and 1:1500 GolgiStop (BD Biosciences). Cells were surface stained, fixed in 2% paraformaldehyde, permeabilized in permeabilization buffer according to the manufacturer’s instructions (BD Biosciences), and stained intracellularly with the following Abs: FITC anti–TNF-α (MP6-XT22) and PE-Cy7 anti–IFN-γ (XM1.2) supplied by eBioscience. For cell sorting, cells were surface stained with anti-CD4 and anti-CD8α, and CD4+CD8–GFPlow cells were sorted by the University of Pennsylvania Flow Cytometry Core, using a three-laser, six-color FACSSaria (BD Biosciences). For flow cytometry, a four-laser, 18-color LSR II (BD Biosciences) or a three-laser, eight-color FACSCanto (BD Biosciences) were used to acquire data. FlowJo 8.7.1 (Tree Star) was used to analyze data.

nTreg cultures in vitro

Sorted nTreg (CD4+CD8–GFPlow) from Foxp3 GFP mice were cultured with plate-bound 1 μg/ml anti-CD3, soluble 1 μg/ml anti-CD28, 10 μg/ml anti–IFN-γ (XM1.2), 1 μg/ml anti–IL-4 (5AB6) (all produced in house), and 50, 100, or 200 U/ml IL-2 for 72 h, with or without 10 ng/ml rIL-6 (eBioscience) or 50 ng/ml rIL-27 (Amgen).

Isolation of CD4+CD25+ cells and cell transfer

CD4+CD25+ cells were isolated from single-cell suspensions from spleen and peripheral LN of CD45.1+ female mice using the CD4+CD25+ cell isolation kit (Miltenyi Biotech) according to the manufacturer’s instructions. Recipient mice were anesthesia using isofluorine gas, and 1.5 × 106 cells were transferred i.v.

BM chimeras

For donor cell isolation, single-cell suspensions from BM of 8- to 9-wk-old IL-27 Tg mice or wild-type (WT) littermates were prepared. BM was isolated by flushing the femur and tibia, lysing RBCs, and depleting T cells using the BM90.2 negative selection kit (Miltenyi Biotech) according to the manufacturer’s instructions. CD45.1 recipient mice were lethally irradiated with 950 cGy using a Nordion GammaCell irradiator, anesthetized with ketamine administered i.p., and given 5 × 106 donor cells i.v. Chimeric mice were treated with sulfamethoxazole/trimethoprim for 2 wk after reconstitution and were analyzed at 5 wk after reconstitution.

Rules-based medicine and ELISA

Serum was collected from 7- to 8-wk-old IL-27 Tg mice and WT littermates and sent to Rules Based Medicine for 60-parameter Ag analysis. Serum samples were mixed with capture microsphere multiplexes and incubated for 1 h at 25°C. Biotinylated reporter Ab for each multiplex were added and incubated for 1 h at 25°C, and multiplexes were developed for 1 h at 25°C with an excess of streptavidin-PE. Prepared samples were analyzed using a Luminex 100 (Luminex Corporation) and proprietary analysis software (Rules Based Medicine). For each multiplex, calibrators and controls were tested, and values for each Ag were determined with four and five parameters and with weighted and unweighted, curve-fitting algorithms. For supernatant samples, 1 × 106 cells were stimulated with soluble 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 for 24 or 48 h, and supernatants were collected. Supernatants from in vitro cultures were analyzed by ELISA, using the following Ab pair: anti–IL-2 (JES6-1A12) and biotinylated anti–IL-2 (JES6-5H4; eBioscience). ELISA plates were developed using ABTS substrate solution (KPL Protein Research Products) and visualized using a microplate reader (Molecular Devices).

Statistical analyses

Nonparametric Mann–Whitney, unpaired, two-tailed Student t test, and one-way, nonparametric Kruskal-Wallis ANOVA with Dunn’s comparison after testing were used to determine statistical significance; p < 0.05 was considered significant.

Results

Generation of IL-27 Tg mice

To investigate how IL-27 influences the immune system and shapes the Treg pool during the steady state, mice that overexpress the IL-27p28 and EB13 subunits of IL-27 were developed. First, individual transgenic mice that express either IL-27p28 or EB13 alone were generated using a construct that targets overexpression to T and B cells (Supplemental Fig. 1A). Splenocytes from IL-27p28 Tg mice expressed high levels of IL-27p28 mRNA and normal levels of EB13 mRNA (compared with WT splenocytes), and splenocytes from EB13 Tg mice expressed low levels of IL-27p28 mRNA and high levels of EB13 mRNA (Supplemental Fig. 1B). Overexpression of IL-27p28 was restricted to T and B cells (data not shown). IL-27p28 and EB13 Tg mice appeared grossly normal and lived a normal life span. Compared with WT littermates, IL-27p28 Tg mice had slightly elevated numbers of T cells in the spleen, but normal Treg numbers, and EB13 Tg mice had T cell counts comparable to WT mice (Supplemental Fig. 1C). IL-27p28 Tg mice were bred to EB13 Tg mice to create mice that overexpress both subunits (IL-27 Tg mice). Splenocytes from IL-27 Tg mice expressed high levels of both IL-27p28 mRNA and EB13 mRNA (Supplemental Fig. 1B).

IL-27 Tg mice develop and succumb to an inflammatory disease

IL-27 Tg neonates appeared normal; however, starting at 5 wk of age, IL-27 Tg mice began to show signs of disease, characterized by weight loss, dry skin, and alopecia. By 8–11 wk of age, these mice succumbed to this condition (Fig. 1A). Histologic analysis of 10-wk-old IL-27 Tg mice revealed marked pathologic changes and inflammation in multiple tissues. Immune infiltrates were observed in the liver, lungs, pancreas, kidney, and seminal vesicles; hyperkeratosis, acanthosis, and follicular atrophy were observed in the skin; fibrosis was evident in the lung; and the colon showed evidence of goblet cell mucin production and edema in the submucosa and lamina propria (Fig. 1B, Supplemental Fig. 2A). Analysis of IL-27 Tg mice at 7–8 wk of age showed lymphopenia in the spleen and LN, but these mice had normal PEC counts (Fig. 2A). IL-27 Tg mice had high percentages of total CD8+ T cells in the spleen and PEC (Supplemental Fig. 2B). In addition, compared with WT littermates, IL-27 Tg mice had a higher percentage of CD4+ and CD8+ T cells in the spleen, LN, and PEC that expressed the early activation marker CD69 (Fig. 2B), and a higher percentage of CD8+ and CD4+ T cells in the spleen that produced IFN-γ after stimulation with PMA and ionomycin (Fig. 2C).
CD4+ T cells from IL-27 Tg mice did not demonstrate increased expression of IL-17 or RORγt (Supplemental Fig. 2D). Finally, multiple parameter analysis for a panel of serum Ag showed that IL-27 Tg mice had elevated levels of numerous cytokines, including IFN-γ, IL-10, IL-5, IL-6, and TNF-α (Fig. 2D, Supplemental Fig. 3).

**IL-27 Tg mice are deficient in Tregs**

Previous studies have indicated that IL-27 has proinflammatory activities (4, 6–8), and this property could potentially account for the inflammatory disease that affects IL-27 Tg mice. However, this uncontrolled inflammation is also consistent with a defect in the Treg compartment, because murine models of Treg deficiency—
including scurfy mice (43–45), Foxp3−/− mice (46), and IL-2−/− and IL-2R−/− mice (34–37, 39)—have a similar inflammatory phenotype. When expression of the Treg transcription factor Foxp3 was analyzed, 7-wk-old IL-27 Tg mice almost completely lacked CD4+Foxp3+ Tregs in the spleen, LN, PEC, and thymus, by frequency (Fig. 3A) and total number (Fig. 3B). Treg deficiency was also present at 1.5 and 10 wk of age (data not shown). Because Tregs have a critical role in limiting inflammation, the lack of Tregs in IL-27 Tg mice is likely a major factor in the development of inflammatory disease in these animals.

**IL-27 does not cause nTregs to downregulate Foxp3 in vitro or in vivo**

One possible explanation for the loss of Tregs observed in IL-27 Tg mice was that IL-27, like its cousin IL-6 (47), could cause nTregs to downregulate Foxp3 expression. To test this hypothesis in vitro, nTregs were isolated by cell sorting from mature nTregs to downregulate Foxp3 expression. To test this Tg mice was that IL-27, like its cousin IL-6 (47), could cause Tregs have a critical role in limiting inflammation, the lack of Tregs in IL-27 Tg mice is likely a major factor in the development of inflammatory disease in these animals.

**FIGURE 4.** nTregs exposed to IL-27 did not downregulate Foxp3. A, Foxp3GFP+. nTregs did not downregulate Foxp3 after 72 h of culture with IL-27, but they did downregulate Foxp3 after culture with rIL-6. Plots were gated on CD3+CD4+ cells. Data represent SEM for IL-2 and IL-2 plus rIL-27 from four independent experiments, and a representative plot of IL-2 plus rIL-6 (IL-2 versus IL-2 plus rIL-6, p = 0.6857). Eleven days after WT CD4+CD25+ cells were transferred to 7-wk-old WT or IL-27 Tg recipients, there was a similar percentage (B) of congenic cells that were Foxp3+ cells and total number (C) of CD45.1+CD4+Foxp3+ T cells in the spleen and LN, regardless of recipient genotype. Plots were gated on CD3+ CD45.1+CD4+ cells. Data represent SEM for seven WT and eight IL-27 Tg mice from three independent experiments. All p values (*) were calculated with an unpaired, two-tailed Mann–Whitney U test. B, Spleen p = 0.2857, LN p = 0.7789. C, Spleen p = 0.1893, LN p = 0.0939.

**FIGURE 3.** IL-27 Tg mice were deficient in Tregs. IL-27 Tg mice had low percentages (A) and total numbers (B) of Foxp3+ Tregs in the spleen, LN, PEC, and thymus at 7–8 wk of age. Plots were gated on CD3+CD4+ cells. Data represent SEM for 10 WT and nine IL-27 Tg mice from five independent experiments (thymus data represent SEM for eight WT and seven IL-27 Tg mice, from four independent experiments). All p values (*) were calculated with an unpaired, two-tailed Mann–Whitney U test. A, Spleen p < 0.0001, LN p < 0.0001, PEC p < 0.0001, thymus p = 0.0003. B, Spleen p < 0.0001, LN p < 0.0001, PEC p < 0.0001, thymus p = 0.0003.

Foopx3 expression on the congenic cells was examined. In WT recipients, ~40% of CD45.1+CD4+ cells in the spleen and LN expressed Foxp3 (Fig. 4B), indicating that a proportion of the transferred Foxp3+ cells either downregulated Foxp3 or died, or that a population of Foxp3+ cells expanded. However, WT and IL-27 Tg recipients had a similar percentage (Fig. 4B) and number (Fig. 4C) of CD45.1+CD4+Foxp3+ cells in the spleen and LN, suggesting that while the transferred congenic cells displayed decreased Foxp3 in a WT recipient compared with the input, this decrease was similar in an IL-27 Tg recipient. These findings, in agreement with in vitro data, indicate that the loss of Tregs in IL-27 Tg mice is not due to Foxp3 downregulation. Of note, transfer of a small number of CD4+CD25+ cells to IL-27 Tg mice did not ameliorate liver and pancreas inflammation (Supplemental Fig. 4A), or decrease T cell activation (Supplemental Fig. 4B), and the transferred mice succumbed to inflammatory disease, similar to nontransferred controls.

**IL-27 limits Treg expansion in a BM chimera model**

Previous studies have reported that IL-27 blocks the generation of inducible Tregs in vitro by inhibiting Foxp3 upregulation (28–30). To determine whether this type of inhibition correlates with the loss of Tregs in IL-27 Tg mice, a BM chimera model was used in which lethally irradiated WT congenic mice were reconstituted with WT or IL-27 Tg BM. At 5 wk after irradiation and reconstitution, the T cell compartment is composed of ~50% donor-derived and 50% host-derived cells, and the host-derived population contains a high proportion of radio-resistant Foxp3+ cells...
that can reconstitute the Treg compartment in irradiated mice given Treg-deficient BM (48, 49). Thus, this model provides a system to assess whether IL-27 modulates Foxp3 expression in a cell-extrinsic manner during reconstitution of the Treg compartment.

In irradiated WT mice reconstituted with WT BM, ∼8% of donor-derived CD3^+CD4^+ cells in the spleen were Foxp3^+, whereas ∼25% of host-derived CD3^+CD4^+ cells were Foxp3^+ (Fig. 5A). The LN and thymus specimens from these mice showed similar patterns of Foxp3^+ expression (Fig. 5A). In contrast, irradiated WT mice reconstituted with IL-27 Tg BM had a low percentage of donor-derived CD3^+CD4^+ T cells that were Foxp3^+ cells in all organs examined, and a marked reduction in the percentage of host-derived, CD3^+CD4^+ cells that were Foxp3^+ (Fig. 5A). The defect in the percentage of Foxp3^+ cells in both the donor- and host-derived T cell compartments in irradiated WT mice given IL-27 Tg BM was also apparent when total numbers of Foxp3^+ cells were calculated (Fig. 5B). Thus, irradiated WT mice reconstituted with IL-27 Tg BM could not generate a normal Treg pool from donor-derived T cells. In addition, and in a cell-

extrinsic manner, IL-27 prevented host-derived cells from reconstituting the Treg compartment, leading to Treg deficiency similar to that observed in whole IL-27 Tg mice. Accordingly, irradiated WT mice reconstituted with IL-27 Tg BM developed systemic inflammation that mirrored the disease in IL-27 Tg mice in the spleen and pancreas (Fig. 5C), and these mice succumbed to this condition (Fig. 5D).

**IL-27 Tg mice are IL-2 deficient**

IL-2 is a vital factor for the proper development of the Treg compartment. In the absence of IL-2, or subunits of its receptor, mice are Treg deficient and die of systemic inflammation (34–39). Thus, the ability of IL-27 to inhibit IL-2 production (32, 33) may be one mechanism by which IL-27 limits the size of the Treg population. To determine whether IL-27 overexpression affects IL-2 production, cells from the spleen, LN, and thymus from WT or IL-27 Tg mice were isolated, stimulated for 4 h with PMA and ionomycin, and intracellular cytokine staining was used to assess IL-2 production. Whereas WT CD4^+ T cells from all tissues examined produced IL-2, a significantly lower percentage of IL-27

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**FIGURE 5.** Irradiated WT mice reconstituted with IL-27 Tg BM were Treg deficient. Irradiated WT mice reconstituted with IL-27 Tg BM had low percentages (A) and total numbers (B) of both donor (CD45.2) and host (CD45.1) Foxp3^+ Tregs in the spleen, LN, and thymus at 5 wk after irradiation and reconstitution. Plots were gated on CD3^+CD4^+CD45.2^+ or CD3^+CD4^+CD45.1^+ cells. Data represent SEM for 11 WT and IL-27 Tg mice from three independent experiments. All p values (*) were calculated with an unpaired, two-tailed Mann–Whitney U test. A, Spleen donor p = 0.005, spleen host p < 0.0001, LN donor p < 0.0001, LN host p < 0.0001, thymus donor p = 0.0013, thymus host p = 0.0001. B, Spleen donor p < 0.0001, spleen host p = 0.0001, spleen total p = 0.0001, LN donor p < 0.0001, LN host p = 0.0002, LN total p = 0.0005, thymus donor p < 0.0001, thymus host p = 0.0878, thymus total p = 0. C, Irradiated WT mice reconstituted with IL-27 Tg BM had inflammatory infiltrates in the liver and pancreas (→) at 5 wk after irradiation and reconstitution, as assessed by H&E staining. Images displayed are representative of eight irradiated WT mice reconstituted with WT or IL-27 Tg BM (scale bars, 100 μm in liver images and 50 μm in pancreas images). D, Irradiated WT mice reconstituted with IL-27 Tg BM died. Data represent 10 WT and IL-27 Tg mice; p = 0.0012 by Kaplan and Meier log rank test.
Tg CD4+ T cells stained positively for IL-2 (Fig. 6A). The total number of CD4+ T cells in the spleen and LN that produced IL-2 was also greater in WT compared with IL-27 Tg mice (Fig. 6B). There was an equivalent number of CD4+IL-2+ cells in the thymus of WT and IL-27 Tg mice (Fig. 6B), reflecting an increased percentage of single positive CD4+ T cells in the IL-27 Tg thymus. When splenocytes from WT irradiated mice reconstituted with WT or IL-27 Tg BM were examined for IL-2 production by intracellular cytokine staining, a greater percentage of host-derived and total CD4+ T cells from WT mice reconstituted with WT BM produced IL-2 compared with those from mice reconstituted with IL-27 Tg BM (Fig. 6C). When numbers of donor-derived, host-derived, and total CD4+IL-2+ cells in the spleen were calculated, WT mice reconstituted with WT BM had higher numbers of IL-2 producers in all three categories compared with WT mice reconstituted with IL-27 Tg BM (Fig. 6D). Finally, when WT and IL-27 Tg splenocytes, or splenocytes from irradiated WT mice reconstituted with WT or IL-27 Tg BM, were stimulated with soluble anti-CD3 and anti-CD28, there was a major decrease in the amount of IL-2 in culture supernatants from whole IL-27 Tg mice and in WT mice that received IL-27 Tg BM compared with that in supernatants from whole WT or WT mice that received WT BM (Fig. 6E). These data suggest that the loss of Tregs and the inflammatory disease observed in IL-27 Tg mice is associated with an IL-2 deficiency, which is consistent with the major role of IL-2 in Treg generation and maintenance.

**Discussion**

The data presented in this study provide new evidence of a role for IL-27 in modulating the size of the Treg pool in vivo. The mechanisms that control Treg homeostasis have been studied intensively, but many questions still remain regarding how this critical cell population is regulated. Various murine models that lack Tregs—including the scurfy Foxp3 mutants (43–45), Foxp3−/− mice (46), mice in which Foxp3 can be ablated (50), and IL-2−/− and IL-2Rβ−/− mice (34–37, 39)—have underscored the strict requirement for Foxp3+ Tregs in controlling inflammation and autoimmunity. Comparing the course of disease in different Treg-deficient models serves to define how each of these factors influences the Treg pool. For example, scurfy mutants that lack...
functional Foxp3 and Foxp3^{−/−} mice have the most severe disease and succumb to multiorgan inflammation prior to weaning, emphasizing that Foxp3 is indispensable for Treg establishment (43–46). In contrast, IL-2^{−/−} and IL-2Rb^{−/−} possess a Treg population, exhibit unique patterns of organ-specific inflammation, and succumb to disease well after weaning (34–37, 39), suggesting that although IL-2 signaling may not be required for Foxp3 upregulation, it is vital for normal Treg homeostasis. The data in this study show that compromised IL-2 production in response to IL-27 signaling is associated with Treg deficiency, yet the phenotype of IL-27 Tg mice is different from that of IL-2^{−/−} and IL-2Rb^{−/−} mice. IL-27 Tg mice live slightly longer (Fig. 1A) than IL-2^{−/−} and IL-2Rb^{−/−} mice, and IL-27 Tg mice are lymphopenic (Supplemental Fig. 2B), whereas IL-2^{−/−} and IL-2Rb^{−/−} mice exhibit lymphoproliferation (35). These differences highlight the complex factors that underlie regulation of the Treg pool. Further examination and comparisons of murine models of Treg deficiency will provide insight into how these cells are regulated during homeostasis.

Previous work provides evidence that IL-27 influences Tregs in vivo (51, 52). IL-27 Tg mice have been generated that expressed a fused version of IL-27p28 and EBI3 in the liver, and the phenotype in these mice displayed some similarity to that described in our studies, with pathologic changes in the liver and shortened survival. That report concluded that IL-27 overexpression caused potentially lethal BM dysfunction; however, the authors of this study did not examine Foxp3 expression, and the inflammatory phenotype observed in these mice suggests that autoimmune or inappropriate inflammation may be present (51). In addition, recent work from Cox et al. (52) established a role for IL-27 in limiting Treg populations in vivo in a murine transfer model of colitis. The authors reported that when transferred T cells lacked WSX-1, a larger percentage of transferred cells upregulated Foxp3, thus ameliorating colonic inflammation. The authors hypothesized that increased IL-2 production in the absence of WSX-1 was not responsible for increased Treg frequencies. However, this finding does not preclude an IL-2-dependent mechanism in our study, because the authors did not assess Treg conversion in the absence of IL-2 (52).

The study of various models of Treg deficiency will be useful in defining mechanisms that contribute to the inflammation that develops in response to a loss of Tregs. In particular, study of the IL-2^{−/−} and IL-2Rb^{−/−} mouse models has led to a better understanding of how IL-2 signaling regulates T cells. Formerly, IL-2 was considered to be an essential T cell growth factor required for the expansion of effector Th populations in vivo (53, 54). However, the fact that IL-2^{−/−} and IL-2Rb^{−/−} mice exhibit a lymphoproliferative disease showed that IL-2 signaling is not absolutely required to support Th effector expansion or autoimmunity and dysregulated inflammation (34–39). Likewise, in IL-27 Tg mice the ability of IL-27 to suppress IL-2 is associated with a Treg deficit, which is consistent with the development of systemic inflammation, but the proinflammatory activities of IL-27 may also influence the inflammation that develops. In agreement with initial studies on IL-27, which described the cytokine as a proinflammatory factor that enhanced Th1 responses (4, 6–8), IL-27 Tg mice have increased IFN-γ levels (Fig. 2C, Supplemental Fig. 2C), and IL-27 Tg T cells were more likely to express the transcription factor T box expressed in T cells (data not shown). In addition, although IL-2 is not required for T cell expansion, competition for IL-2 in IL-27 Tg mice might influence the generation of the T cell response in the inflamed environment. Thus, in this model, IL-27 could limit Treg populations and contribute to the Th1-type inflammation and the effector T cell response that is associated with the Treg deficiency.

Although our studies of IL-27 Tg mice identify a role for overexpression of this cytokine in Treg homeostasis, there is no evidence to date that IL-27 signaling occurs in the thymus and shapes the Treg pool of normal WT mice. Mice deficient in IL-27 signaling do not exhibit obvious changes in Treg numbers in the steady state or during inflammation, raising the question of why these cells express the IL-27R (31). Regardless, our data do not elucidate Treg differentiation pathways in the intact thymus; rather, they highlight a mechanism by which the Treg compartment could be manipulated, particularly when the Treg pool is expanding, as in the BM chimera model described in this study. There are numerous situations in which Tregs are deleterious to the initiation or establishment of a desirable immune response, particularly during vaccination or cancer immunotherapy (55–60). In these scenarios, IL-27 therapy delivered systemically or to the local site of the immune response could help to limit Tregs and support the generation of the desired immune response. Indeed, IL-27 therapy in a murine model of neuroblastoma limited IL-2-induced Treg expansion in the tumor microenvironment and correlated with enhanced tumor killing (61). Whereas the balance between regulated inflammation and autoimmunity is always a consideration, the studies presented in this article describe a new mechanism by which the Treg population can be modulated, thereby indicating a pathway that could be manipulated therapeutically.

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**Disclosures**

The authors have no financial conflicts of interest.

**References**


IL-27 INFLUENCES Treg HOMEOSTASIS


