Interleukin 37 expression protects mice from colitis

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IL-37, a newly described member of the IL-1 family, functions as a fundamental inhibitor of innate inflammation and immunity. In the present study, we examined a role for IL-37 during experimental colitis. A transgenic mouse strain was generated to express human IL-37 (hIL-37tg), and these mice were subjected to dextran sulfate sodium (DSS)-induced colitis. Despite the presence of a CMV promoter to drive expression of IL-37, mRNA transcripts were not present in colons at the resting state. Expression was observed only upon disruption of the epithelial barrier, with a six- to sevenfold increase ($P = 0.02$) on days 3 and 5 after continuous exposure to DSS. During the development of colitis, clinical disease scores were reduced by 50% ($P < 0.001$), and histological indices of colitis were one-third less in hIL-37tg mice compared with WT counterparts ($P < 0.001$). Reduced inflammation was associated with decreased leukocyte recruitment into the colonic lamina propria. In addition, release of IL-1β and TNFα from ex vivo colonic explant tissue was decreased 5- and 13-fold, respectively, compared with WT ($P \leq 0.005$), whereas IL-10 was increased sixfold ($P < 0.001$). However, IL-10 was not required for the anti-inflammatory effects of IL-37 because IL-10-receptor antibody blockade did not reverse IL-37-mediated protection. Mechanistically, IL-37 originating from hematopoietic cells was sufficient to exert anti-inflammatory effects because WT mice reconstituted with hIL-37tg bone marrow were protected from colitis. Thus, IL-37 emerges as key modulator of intestinal inflammation.

cytokine | intestine | inflammatory bowel disease

Inflammatory bowel disease (IBD) results from environmental factors (e.g., bacterial antigens) triggering a dysregulated immune response in genetically predisposed hosts. IBD is often characterized by an imbalance between the effector and regulatory arms of intestinal immunity, with a preponderance of proinflammatory cytokines (1). Although TNF blockade induces clinical remission in 50–70% of patients with IBD (2), sustained remission rates decrease after 1 y (3). Furthermore, the efficacy of manipulating other cytokines (e.g., IL-6, IL-10, and IL-11) has been limited (4, 5). Thus, alternative biological therapies that target additional pathways of the chronic inflammatory cascade should be evaluated. Tilting the balance toward the proregulatory arm is an attractive strategy, yet systemic IL-10 administration resulted in limited efficacy (6). Of note, although anti-inflammatory cytokines such as IL-10, IL-1Ra, and TGFβ are elevated in IBD, chronic inflammation continues uncontrolled (7, 8).

Of the 11 members of the IL-1 family, 7 are agonists (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ) and 2 are naturally occurring receptor antagonists (IL-1Ra and IL-36Ra). IL-37b (formerly IL-1F7b) is the last member of the IL-1 family without a well-defined function (9, 10). Different from most IL-1 family members, IL-37 has emerged as an anti-inflammatory cytokine. At the present time, a mouse homolog has not been identified; however, five splice variants of human IL-37 have been described (IL-37a–f) (10–13). The IL-37a isoform has a unique N terminus encoded by exon 3 (11), whereas the short isoforms IL-37c, IL-37d, and IL-37e lack exon 4, exon 2, or both exons, respectively. None of the N-terminal sequences encoded by exon 1 contain a prodomain, and it remains unclear whether IL-37 is processed by caspase-1 to an active form (10, 14). In fact, multiple N termini have been reported upon expression of full-length IL-37 in mammalian cells (14, 15). Although there are reports that IL-37 binds to the IL-18 receptor α-chain without signaling (14, 15), there is no evidence that IL-37 acts as a receptor antagonist for IL-18 (14, 16). In contrast to an extracellular role for IL-37, ~25% of LPS-induced endogenous IL-37 translocates to the nucleus (17).

The anti-inflammatory properties of IL-37 have been demonstrated in vitro. Mouse RAW macrophages transfected with IL-37 displayed markedly reduced levels of LPS-induced IL-1α, IL-6, TNF, and CXCL2 (18, 19). Similar reductions in LPS- and IL-1–stimulated cytokines were observed in human THP-1 macrophages and A549 epithelial cells. Furthermore, siRNA treatment of these cell lines targeting endogenous IL-37 resulted in significant increases in 13 proinflammatory cytokines, including IL-1α, IL-1β, IL-6, TNFα, and GM-CSF. In addition, expression of IL-37 in THP-1 cells resulted in a marked reduction of several intracellular kinases important for transducing proinflammatory signals, such as focal adhesion kinase (FAK), STAT1, p38 MAPK, and c-jun (19). IL-37 is also inducible in human peripheral blood mononuclear cells (17). Kumar and colleagues have identified IL-37 protein expression in tonsils, skin, esophagus, placenta, and melanoma as well as carcinomas of the breast, prostate, colon, and lung, albeit at low levels (10, 14).

In the current study, we examined the role of IL-37 with a transgenic mouse strain generated to express human IL-37 (hIL-37tg) during acute dextran sulfate sodium (DSS)-induced colitis (20). We determined expression of IL-37 with the onset of colonic injury and the effect of IL-37 expression on clinical parameters and histological indices of colitis. Furthermore, changes in leukocyte recruitment and local cytokine production were assessed. Last, we generated bone marrow (BM) chimeric mice to ascertain whether myeloid-derived IL-37 was sufficient to exert anti-inflammatory effects in vivo.

Results

Transgenic IL-37 Expression Protects Mice from Clinical Signs of Colitis. To evaluate whether IL-37 might protect mice from intestinal inflammation, DSS or water vehicle was administered to WT and hIL-37tg mice for 7 d. Body weights of mice that received water vehicle remained stable during the 7-d period. By contrast, WT mice given DSS lost over 12% more weight than hIL-37tg mice did (83 ± 2% vs. 97 ± 4%, $P < 0.01$) (Fig. 1*A*). In addition, the disease activity index (DAI), a composite score comprising clinical signs of colitis (weight loss, stool consistency, and bleeding), was significantly higher in WT mice compared

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The authors declare no conflict of interest.

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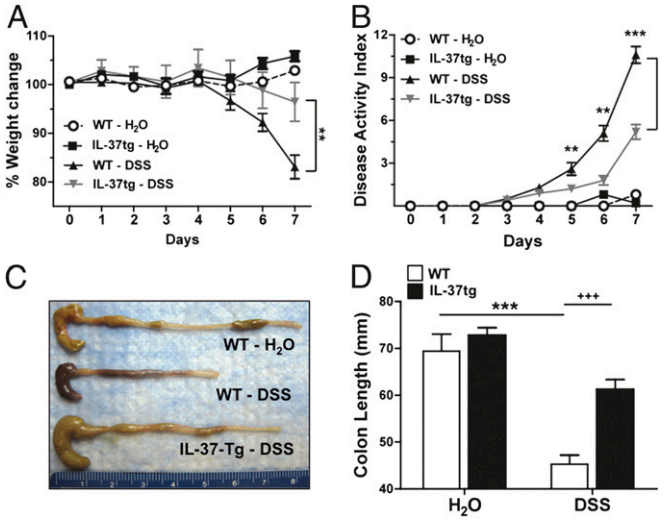

Fig. 1. Reduced severity of DSS colitis in hIL-37tg mice. DSS or water vehicle was administered to WT and IL-37tg mice ad libitum in drinking water for 7 d. (*A*) Weight change during treatment was expressed as percentage change from day 0. (*B*) Clinical DAI (*SI Methods*). (*C*) Macroscopic images of colons from indicated treatment cohorts. (*D*) Colon lengths were assessed at necropsy on day 7. Colon lengths were assessed at necropsy on day 7. Data are expressed as mean ± SEM. (*A*) $^{**}P < 0.01$, $^{***}P < 0.001$ vs. IL-37tg. (*B*) $^{***}P < 0.001$ vs. WT-H_2O, $^{+++}P < 0.001$ vs. WT-DSS (n = 19–27 mice from four independent experiments).

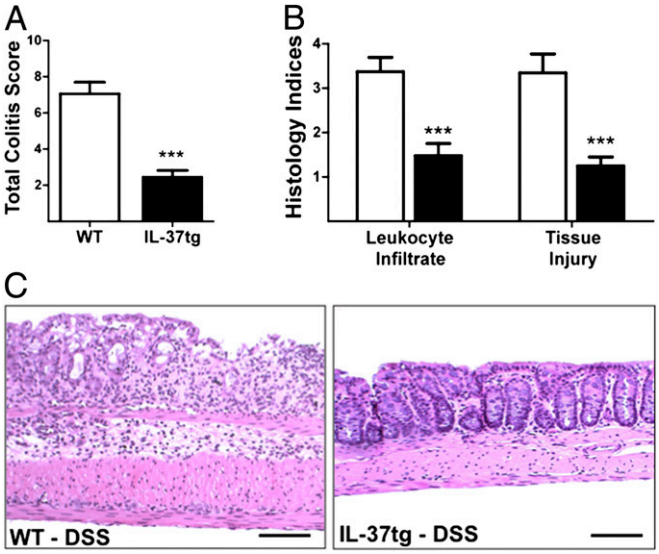

Fig. 2. Amelioration of histological indices in hIL-37tg mice subjected to DSS colitis. (*A* and *B*) Histological assessment of colitis severity demonstrated amelioration of all assessed parameters in IL-37tg mice compared with WT mice. Data are expressed as mean ± SEM. $^{***}P < 0.001$ vs. WT (n = 27 mice per cohort from four independent experiments). (*C*) Representative micrograph images of colons from indicated treatment cohorts. (Scale bars: 200 μm.)

with hIL-37tg mice that received DSS (10.6 ± 0.6% vs. 5 ± 0.5%, $P < 0.0001$) (Fig. 1*B*).

Reduced colon length serves as a surrogate macroscopic marker of colonic injury, and IL-37tg mice exposed to DSS display marked protection compared with WT counterparts (61 ± 2 mm vs. 45 ± 2 mm, $P < 0.001$) (Fig. 1 *C* and *D*). Thus, hIL-37tg mice were protected from all clinical indices of DSS colitis.

Transgenic hIL-37 Expression Ameliorates Histological Indices of Colitis. Colonic tissues from DSS-treated mice were examined to determine whether clinical signs of colitis correlated with histological severity. hIL-37tg mice exhibited decreased total histological scores (2.4 ± 0.4% vs. 7 ± 0.6%, $P < 0.001$) and its components, namely leukocyte infiltrates (1.4 ± 0.2% vs. 3.2 ± 0.3%, $P < 0.001$) and tissue injury (1.3 ± 0.2 vs. 3.3 ± 0.4, $P < 0.001$), compared with WT mice (Fig. 2 *A* and *B*). In addition, there was a significant reduction in leukocyte infiltrates, preservation of epithelial cell integrity, decreased edema, and reduced hyperplasia of the colonic muscularis propria in hIL-37tg mice (Fig. 2*C*). Thus, hIL-37 exerts a potent protective effect from DSS-induced intestinal injury.

IL-37 Expression Is Inducible and Correlates with Intestinal Barrier Breakdown. In mouse and human cell lines transfected with IL-37, there is no detectable expression unless cells are stimulated with LPS (17). To determine whether the breakdown of the intestinal barrier induces the expression of the IL-37 transgene, colonic tissue was assessed for IL-37 mRNA transcripts after exposure to DSS. Although baseline expression was minimal, IL-37 expression progressively increased after DSS exposure, reaching a fivefold increase by day 3, with peak levels on day 5 (sevenfold, $P = 0.02$) (Fig. 3*A*), coinciding with the onset of histologically evident colitis (Fig. 3 *B* and *C*). Thereafter, levels decreased. Thus, an insult such as chemical injury, which results in epithelial breakdown and influx of bacterial antigens, was required for the induction of IL-37.

IL-37 Suppresses Colonic TNF and IL-1β Production and Induces IL-10. Endogenous IL-37 reduces the production of several proinflammatory cytokines in vitro and in vivo (19). To assess whether IL-37 had a similar capacity to modulate cytokine production within colonic tissues after DSS injury, we measured the cytokines released from colonic explant cultures harvested at day 7 in the indicated experimental cohorts. IL-37 suppressed release of TNFα by 13-fold ($P < 0.001$) and IL-1β by 5-fold ($P < 0.001$), whereas the expression of IL-17, IL-6, and CXCL1 (KC) were unaffected (Fig. 4). The inhibition of TNFα and IL-1β was contrasted by a sixfold increase of IL-10 ($P < 0.001$) (Fig. 4), compared with production from DSS-treated WT colonic tissues. Thus, expression of IL-37 not only suppresses TNFα and IL-1β but also induces IL-10, an effect that is unique to this in vivo model.

Decreased Leukocyte Recruitment to the Colonic Lamina Propria of DSS-Treated IL-37tg Mice. We profiled the subsets of leukocytes within the colonic lamina propria of WT and hIL-37tg mice via flow cytometry. We observed an all-encompassing effect, with a significant reduction of the absolute counts for all subsets evaluated (Fig. 5*A*). An increase in the percentage of $CD11c^{high}$ $MHCII^{+}$ dendritic cells (Fig. 5*B*; $P < 0.001$), $CD11c^{low}MHCII^{+}$ macrophages ($P = 0.05$), $Siglec\text{-}F^{high}GR1^{neg}$ eosinophils ($P < 0.001$), and $Siglec\text{-}F^{neg}GR1^{high}$ neutrophils ($P < 0.001$) was observed in DSS-treated WT mice compared with vehicle-treated mice (WT-H_2O). However, transgenic expression of IL-37 uniformly reduced recruitment of all assessed leukocyte populations ($P \leq 0.05$) to the colonic lamina propria.

Hematopoietic-Derived IL-37 Is Sufficient to Protect from DSS Colitis. Steady-state levels of IL-37 mRNA in the peripheral blood and lung tissues of hIL-37tg mice is low or absent, despite a constitutively active CMV promoter (19), and this absence is also observed in colonic tissues (Fig. 2). Because the transgene showed no tissue-specific expression, we generated BM chimeric mice to ascertain the contribution of hematopoietic- and stromal-derived IL-37 on its protective effect during experimental colitis.

We first assessed crypt architecture and proliferation at discrete intervals from 1 to 56 d postirradiation, before the induction of DSS colitis. Colonic sections from irradiated and nonirradiated mice were immunostained for Ki67. Compared with controls, colonic sections from mice both 1 and 3 d post-treatment displayed significant irradiation damage, as measured

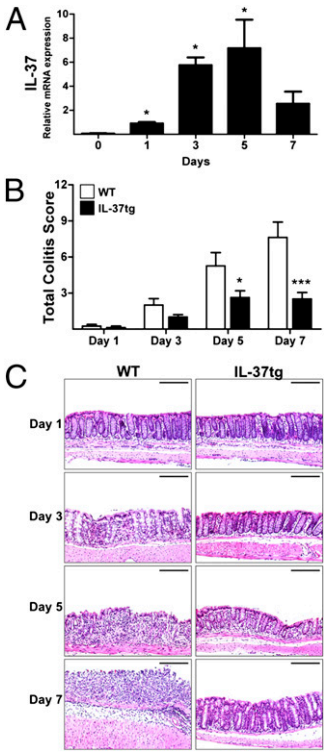

Fig. 3. Induction of IL-37 mRNA correlates with intestinal barrier breakdown. (*A*) Time course of IL-37 mRNA transcripts within colonic tissues during DSS colitis. (*B*) Colonic tissues from DSS-treated WT and hIL-37tg mice were harvested on indicated days, and the severity of colitis was assessed as described in *Methods*. (*C*) Representative micrographs of the progression of colitis at the indicated time points in WT and IL-37tg mice. Data are expressed as mean ± SEM (n = 4 mice per time point from two independent experiments). $^{*}P < 0.05$, $^{***}P < 0.001$ vs. day 0 (*A*) or WT (*B*). (Scale bars: 200 μm.)

by a loss of Ki67^{+} crypt cells (day 1: 37.3 ± 2% vs. 22.6 ± 2.5%; day 3: 37.3 ± 2% vs. 21.6 ± 2.7%, $P = 0.002$) (Fig. S1 *A–D*). However, after 56 d of reconstitution, no gross morphologic differences in colons were detected between irradiated and nonirradiated mice (day 56: 37.3 ± 2% vs. 35.7 ± 2.8%, $P = 0.4$) (Fig. S1 *A* and *D*).

To confirm BM reconstitution, CD45.1 C57BL6/J (WT) mice were used as donors or recipients of hIL-37tg BM. WT mice receiving hIL-37tg BM (IL-37tg–WT) were designated "hematopoietic," whereas hIL-37tg mice receiving WT BM (WT–IL-37tg) were termed "stromal." To control for the effects of irradiation/reconstitution, two additional control groups were generated by transferring WT BM into WT recipients (WT-WT) and hIL-37tg BM into hIL-37tg recipients (IL-37–Tg–IL-37tg). Flow cytometry confirmed >95% reconstitution for all experimental cohorts (Fig. 6*A*). At 8 wk after reconstitution, DSS colitis was induced. WT mice that received WT BM (WT-WT) or IL-37tg mice receiving WT BM (WT–IL-37tg) lost weight (83 ± 0.2% and 80 ± 3%, respectively) and exhibited significantly higher DAI scores (9 ± 1 and 10.8 ± 0.6) (Fig. 6 *B* and *C*), whereas those that received hIL-37tg BM (IL-37tg–WT, hematopoietic) had significant protection from weight loss (93 ± 1% vs. 83 ± 0.2% in WT→WT-DSS, $P < 0.005$) and each of the clinical indices of colitis (5 ± 0.6% vs. 9 ± 0.9% in WT→WT-DSS, $P < 0.001$). Thus, hematopoietic-derived IL-37 mediates the protective properties of this cytokine in colitis. Whether this is the mechanism of protection by IL-37 in native conditions where the gene is not transgenically expressed remains to be elucidated.

Hematopoietic-Derived hIL-37–Mediated Protection in DSS Colitis Is Associated with Decreased IL-1β and TNFα Release but Increased IL-10. Given the improvement of all clinical parameters observed in WT mice receiving hIL-37tg BM, we harvested their colons for macroscopic evaluation at 7 d after continuous administration of DSS. WT and IL-37tg recipients receiving WT BM exhibited significantly decreased colon lengths compared with mice receiving water vehicle (WT-WT: 72.4 ± 2 mm vs. 58 ± 1.4 mm, $P < 0.001$; WT–IL-37tg: 72.4 ± 2 mm vs. 54.7 ± 1.3 mm, $P < 0.001$). In contrast, WT mice receiving IL-37tg BM (IL-37tg–WT–DSS) were markedly protected from DSS-induced colonic shortening (66.3 ± 1 mm) (Fig. 7*A*).

Histological scores for the severity of colitis paralleled the above findings (WT→WT-DSS vs. IL-37tg–WT–DSS: 7.8 ± 1 vs. 2.5 ± 0.5, $P < 0.001$). No protection was observed in hIL-37tg recipients receiving WT BM (WT–IL-37tg–DSS) with a score of 8.5 ± 0.8 (Fig. 7 *B* and *C*).

Cytokine release from colonic explant cultures were assessed in BM chimeric mice. Just as in hIL-37tg mice, WT mice reconstituted with hIL-37tg BM revealed significantly decreased release of IL-1β and TNFα ($P < 0.001$), whereas IL-10 was elevated fivefold ($P < 0.05$) (Fig. 7*D*). Thus, the key cell type that protects from DSS injury in IL-37tg mice is within the hematopoietic, not the stromal, compartment.

An Anti–IL-10 Receptor-Blocking Antibody Does not Affect the Protective Effect of hIL-37 in DSS Colitis. Because IL-10 up-regulation is a hallmark of IL-37 protection in DSS colitis, we blocked

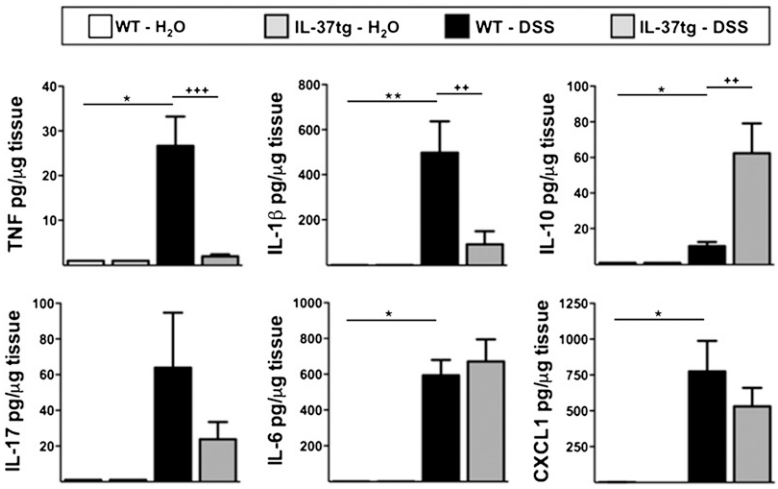

Fig. 4. Levels of colonic IL-10, TNFα, and IL-1β. Colonic tissue explants were harvested at 7 d after continuous DSS exposure and cultured ex vivo at 37 °C for 24 h. Secreted cytokines were assayed from supernatants as described in *Methods* and expressed as picogram of cytokine per milligram of tissue (pg/μg). Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. WT-H_2O vehicle, $^{++}P < 0.001$, $^{+++}P < 0.001$ vs. WT-DSS (n = 19–27 mice from four independent experiments).

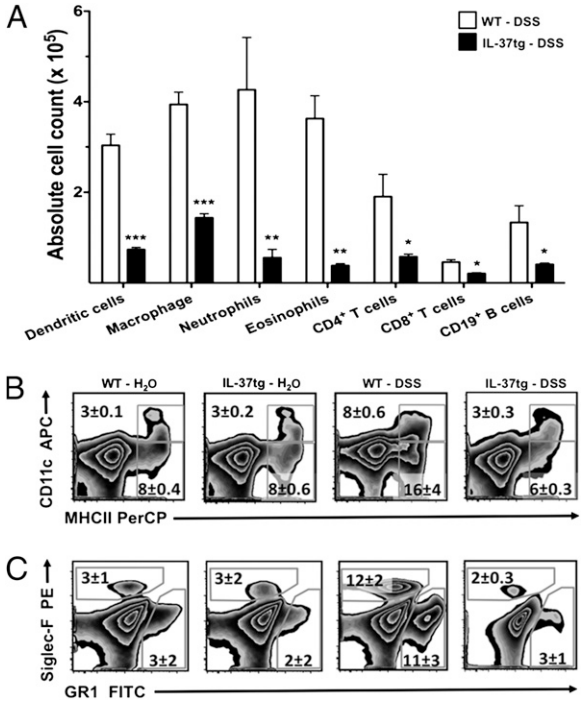

Fig. 5. Effect of hIL-37tg expression on colonic leukocyte infiltrates. Assessment of colonic leukocyte populations from WT and IL-37tg mice after DSS treatment. (*A*) Major leukocyte subsets were expressed as absolute numbers. (*B* and *C*) Representative flow cytometry plots of dendritic cells ($CD11c^{high}/MHC^{high}$), macrophages ($CD11c^{low}/MHCII^{high}$), eosinophils (Siglec $F^{high}/GR1^{neg}$), and neutrophils ($SiglecF^{neg}/GR1^{high}$). Data are expressed as mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. WT-DSS ($n = 6$ mice per strain from two independent experiments).

IL-10 signaling with a specific anti-IL10 receptor mAb (1B1.3a). DSS was administered to WT and hIL-37tg mice receiving anti–IL-10R, and a second cohort of each strain received isotype control antibody. Administration of anti–IL-10R did not alter the severity of colitis (DSS: hIL-37tg IgG vs. hIL-37tg anti–IL-10R; weight loss $P = 0.139$; DAI $P = 0.093$). Thus, the protective effect from IL-37 is not because of induction of IL-10 (Fig. S2).

Discussion

This study is a pivotal demonstration of the anti-inflammatory effect for IL-37 on a well-studied model of intestinal inflammation. We used a strain of mice transgenic for human IL-37 as previously described (19). We examined the timeline of hIL-37 induction after DSS-induced colonic injury in hIL-37tg mice because identification of the mouse ortholog through database searches or homology has been unsuccessful. The sequence of the human gene might have diverged from the mouse ortholog, or the mouse might have a functional homolog rather than a sequence ortholog. As an example of the latter, IL-8 is absent in mice and rats, which express a functional homolog: CXCL1 (14). Like other members of the IL-1 family, IL-37 shows no species specificity, and transfection of human IL-37 in mice exhibited the same properties as expression in human cells (18). Furthermore, we observed improvement of the clinical signs of colitis and amelioration of all histological indices in hIL-37tg mice. BM chimeric mice proved that IL-37 in this model is derived from hematopoietic cells and that this source was sufficient in protecting from colitis by decreasing release of TNFα and IL-1β.

Expression of IL-37 Is Inducible and Correlates with Intestinal Tissue Damage. TLR ligands and IL-1β induce IL-37 in human peripheral blood mononuclear cells (19). However, despite a constitutively active CMV promoter, basal expression of IL-37 in colonic tissues remained low and transcripts increased significantly between the third and fifth day after DSS administration, coinciding with colitic injury. Similarly, in the mouse RAW and human THP-1 macrophage cell lines transfected with the same CMV promoter vector, low or absent constitutive IL-37 protein was increased in a dose-dependent manner after LPS treatment (19). Other stimuli that increased IL-37 include cytokines such as IL-18, IFNγ, IL-1, and TNFα. Furthermore, it appears that IL-37 may amplify the anti-inflammatory properties of TGFβ because IL-37 engages SMAD3, the main intracellular effector of TGFβ (21). Because the mechanism of action of IL-37 is likely intracellular, it is unlikely that exogenous administration of IL-37 will be protective in colitis.

IL-37 might prevent excessive inflammatory responses without suppressing beneficial homeostatic intestinal inflammation, which is necessary to combat infection. Perhaps the role of IL-37 in vivo is to assist in detaining the progression from regulated to dysregulated inflammation, therefore limiting tissue damage. Thus, disruption of intestinal epithelial integrity in vivo with the

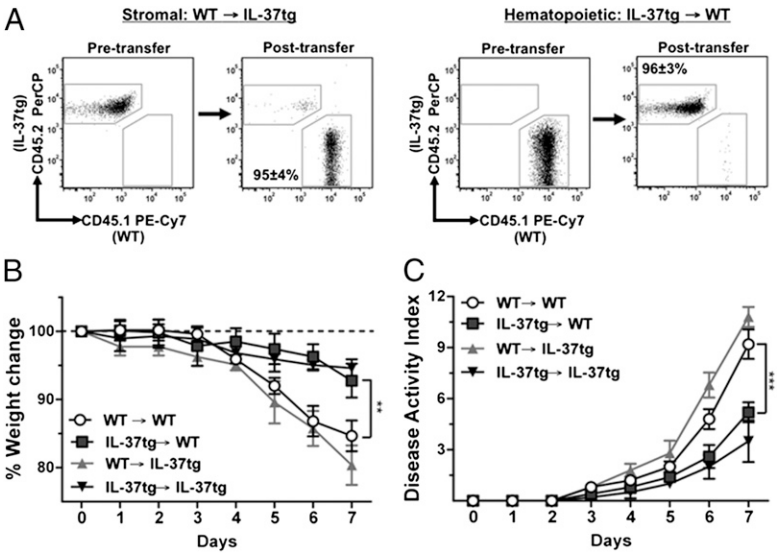

Fig. 6. Hematopoietic-derived IL-37 protects mice from colitis. BM chimeric mice were generated after irradiation of WT (CD45.1) or hIL-37tg(CD45.2) mice as described in *Methods*. (*A*) Flow cytometry analyses of donor and recipient $CD45^+$ splenocytes demonstrated >95% reconstitution. DSS or water vehicle was administered to indicated chimeric mice ad libitum in drinking water for 7 d. (*B*) Weight changes during treatment were expressed as percentage change from day 0. (*C*) Clinical DAI (*SI Methods*). Data are expressed as mean ± SEM. $**P < 0.01$, $***P < 0.001$ vs. WT→WT-DSS ($n = 6$–11 mice per strain from two independent experiments).

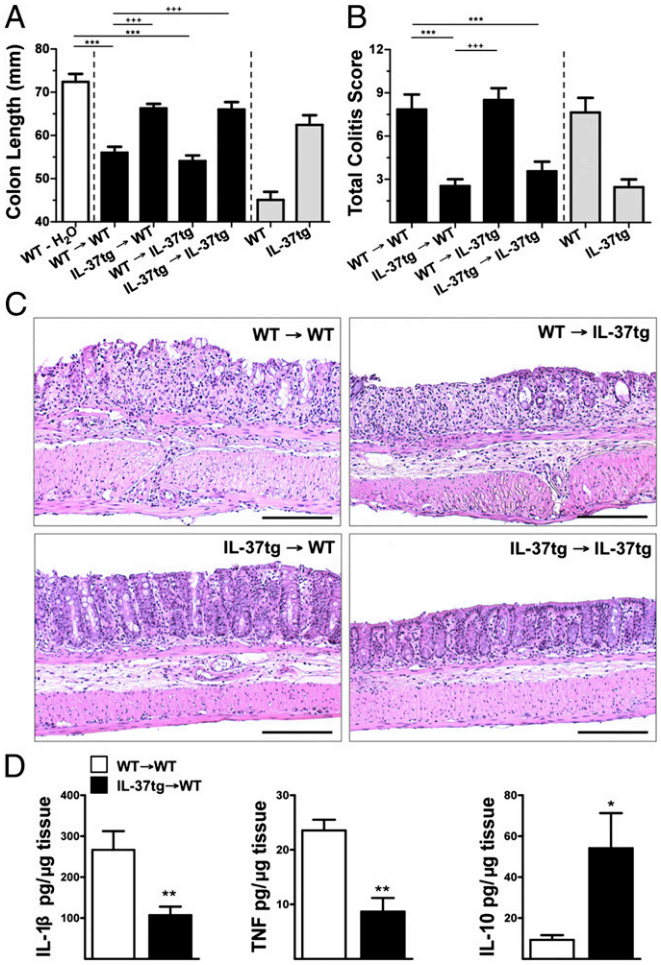

Fig. 7. Effect of hematopoietic-derived IL-37 on colitis and cytokine production. DSS or water vehicle was administered ad libitum in drinking water for 7 d to WT, IL-37tg, and indicated BM chimeric mice. (*A*) Colon lengths were assessed at necropsy on day 7. (*B*) Histological evaluation of colons was performed as described. (*C*) Representative micrographs of colons of indicated BM chimeric mice. (Scale bars: 200 μm.) (*D*) Ex vivo colonic tissue explants of indicated BM chimeric mice treated with DSS were cultured at 37 °C for 24 h. Data are expressed as mean ± SEM. (*A*) $^{***}P < 0.001$ vs. WT-H_2O, $^{+++}P < 0.001$ vs. WT→WT-DSS. (*B*) $^{***}P < 0.001$ vs. WT→WT-DSS, $^{+++}P < 0.001$ vs. IL-37tg→WT-DSS. (*D*) $^{*}P < 0.05$, $^{**}P < 0.001$, IL-37tg→WT vs. WT→WT-DSS (n = 6–11 mice per cohort from two independent experiments).

resultant infiltration of bacterial antigens was necessary to trigger an increase of IL-37 transcripts.

Hematopoietic-Derived hIL-37 Is Critical for Protection During Experimental Colitis. Because the insertion of the IL-37 transgene occurs at random, it was unknown whether protection from colitis originates from BM-derived or stromal cells. This distinction might have clinical implications: As with the development of nonmyeloablative approaches to achieve partial BM reconstitution (22), subsets of cells engineered to overproduce IL-37 under innocuous promoters could be exploited to control relapses of IBD. Because leukocytes are naturally attracted to sites of inflammation, delivery of IL-37 to effector sites could be greatly facilitated if leukocytes were its main source. Although leukocytes are not the sole source of IL-37, which is also expressed by colonic epithelium (10, 14), in the present study, only BM-derived cells were sufficient to afford protection and most likely by reducing the production of proinflammatory cytokines. Whether this will be the case with physiologic production in a nontransgenic, nonmanipulated system remains to be determined. The expression of several endothelial adhesion molecules [e.g., intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and mucosal addressin cell adhesion molecule (MAdCAM-1)] that mediate firm adhesion and transmigration of leukocytes to sites of inflammation are regulated by proinflammatory cytokines such as TNFα and IL-1β (23). Thus, down-regulation of these cytokines might result in decreased recruitment of leukocyte subsets into the inflamed colon. In concert with this effect, hIL-37 expression resulted in decreased recruitment of leukocytes of myeloid and lymphoid lineages.

IL-10 Receptor Blockade Fails to Attenuate Protective Effects of hIL-37 in DSS Colitis. A key finding from this study was the marked induction of IL-10 in hIL-37tg mice. IL-10 is protective in colitis: IL-10-deficient mice develop enterocolitis and colon cancer (24), whereas reconstitution of $IL\text{-}10^{-/-}$ mice with WT BM prevents and treats colitis (25). The observed induction of IL-10 by IL-37 appears to be a unique component of its effect in experimental colitis, and it was likely that IL-37–induced IL-10 produced by infiltrating leukocytes could be the critical mediator of protection from colitis. Nevertheless, this does not appear to be the case because blockade of IL-10R did not reverse the protective effect in hIL-37tg mice. Thus, although the induction of IL-10 does not appear to mediate the protective effect of IL-37, our data agree with the existing data from clinical trials in IBD in which dampening TNFα levels is more effective than supplementing IL-10. Although DSS-induced colitis is an acute chemically induced model mediated by innate immune responses, its pathogenesis is markedly different from human IBD, in which adaptive immune responses predominate. Ongoing studies will examine whether IL-37 plays a protective role in both the $CD45Rb^{high}$ transfer model of colitis and the TNFΔARE model of chronic ileitis.

An imbalance of pro- and anti-inflammatory mechanisms has been demonstrated in IBD (26), and the therapeutic effect of blocking proinflammatory or increasing anti-inflammatory cytokines has been formally evaluated clinically. However, only the former strategy has become standard of care for patients, after reproducibly inducing and maintaining remission in both ulcerative colitis and Crohn's disease. It remains unclear whether the failure of boosting anti-inflammatory mechanisms to restore homeostasis could be at least in part because of suboptimal delivery to the intestine. The transient induction of IL-37 suggests that it may be an early checkpoint to prevent the perpetuation of inflammation that results in chronic inflammatory diseases. Thus, for its further development as a therapeutic agent, a mechanism that triggers and maintains its expression might be required. Given its broad effect on multiple proinflammatory mechanisms, induction of IL-37 might be that ideal therapeutic. Fiocchi proposed a decade ago that manipulating physiologic regulatory mechanisms through "an endogenous approach" might be the next therapeutic frontier in IBD (27). Further understanding the mechanisms of action of IL-37 and ways to up-regulate its expression during a disease flare might represent such novel strategy.

Materials and Methods

Mice. Transgenic mice expressing human IL-37 (i.e., hIL-37tg) have been previously described (19). C57BL/6J (WT) and CD45.1 (B6.SJL-Ptprc^a Pepc^b/BoyJ) mice were purchased from the Jackson Laboratory and maintained under specific-pathogen free conditions. Fecal samples were negative for murine *Helicobacter* species, protozoa, and helminthes. The Institutional Animal Care and Use Committee at the University of Colorado approved all animal procedures.

Generation of BM Chimeric Mice. Recipient mice (6 wk old) were irradiated with 1,100 rad, and BM cells (isolated from femur and tibia) were injected in 0.1-mL 0.9% sodium chloride i.v. via the retro-orbital plexus (50×10^6 BM cells per mouse). Tetracycline (100 mg/L) was administered (i.p.) for 2 wk after BM transplantation. Mice were housed for 8 wk before induction of DSS colitis. To assess BM reconstitution, spleens were excised at necropsy followed by flow cytometry expressing either CD45.1(A20) or CD45.2(104) (eBioscience).

Induction of DSS Colitis. DSS (3% wt/vol, 36,000–50,000 kDa; MP Biomedicals) was administered in drinking water ad libitum for 7 d. DSS solution was replaced on day 3. Clinical signs of colitis (i.e., weight loss, stool consistency, and fecal blood) were recorded daily. Upon necropsy, colonic lengths were measured.

Tissue Processing and Assessment of Colitis. Colons were excised, opened longitudinally, and fixed in 10% buffered formalin. Then they were embedded in paraffin, sectioned (3–5 μm), and stained with hematoxylin/eosin. The severity of colitis was assessed by two pathologists (M.M. and P.J.) blinded to the time point, strain, and treatment groups, as per published methods (28, 29).

Real-Time RT-PCR. Total RNA was isolated from colon with the RNeasy Mini Kit (Qiagen). RNA samples (0.5 μg) were reversed-transcribed into cDNA with a high-capacity cDNA archive kit (Applied Biosystems), and real-time RT-PCR was performed with an ABI Prism 7300. IL-37 and 18s TaqMan Gene Expression Assays (Applied Biosystems) containing primers, and FAM dye-labeled TaqMan MGB probes were used to quantify mRNA transcripts of interest. A relative quantity (RQ) value (2^{-DDCt}, where *Ct* is the threshold cycle) was calculated for each sample by using ABI RQ software (Applied Biosystems). RQ values were calculated as fold change in gene expression relative to the control group.

Leukocyte Isolation. Lamina propria mononuclear cells were isolated as previously described (30).

Flow Cytometry. Colonic lamina propria mononuclear cells were incubated with fluorescently labeled antibodies against the following: Siglec-F(E50-2440) (BD Biosciences); GR-1(RB6-8C5) or CD19(6D5) (Biolegend); MHCII (M5/114.15.2), CD4(RM4-5), CD11b(M1/70), CD11c(N418), or F4/80(BM8) (eBioscience); CD8(53-6.7) (Biolegend); or corresponding isotype controls. Cells were fixed with 1% paraformaldehyde and assayed with a BD FACSCanto II (BD Biosciences). Further analyses were performed with FlowJo software (Tree Star Inc.).

Colonic Explant Cultures and Assessment of Cytokine Production. Colons were excised, placed in cold PBS, and opened longitudinally. Then they were washed in complete RPMI medium 1640 [10% FBS (vol/vol), 100 IU penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine], and a 5-mm^2 piece of tissue from midcolon was placed in 1 mL of complete RPMI medium 1640 (5% FBS). Explants were cultured at 37 °C for 24 h. The supernatants were aspirated, centrifuged at 18,000 × *g* for 10 min, and stored at −70 °C for subsequent cytokine analysis with Multiplex mouse cytokine assay (Quansys). The remaining colonic explant tissue was homogenized, and a Bradford protein assay was performed. The concentration of secreted cytokines in the supernatant was subsequently normalized to total tissue protein and expressed as picogram of cytokine per microgram of tissue.

IL-10 Receptor Immunoblockade. DSS was administered to 8-wk-old WT or hIL-37tg mice for 7 d as described in *Induction of DSS Colitis*. One experimental group from each strain simultaneously received 200 μg of anti–IL-10 receptor mAb (1B1.3a; American Type Culture Collection) or isotype mAb i.p. on days 0, 3, and 5 during the DSS administration period. Clinical signs of colitis were recorded daily. Then mice were killed, and tissues were harvested at day 7, as described in *Tissue Processing and Assessment of Colitis*.

Statistical Analysis. Statistical analyses were performed with the two-tailed Student's *t* test or a one-way ANOVA followed by a Newman–Keuls post hoc test. Data were expressed as mean ± SEM.

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