IAI Journals.ASM.org

Attenuation of Intestinal Inflammation in Interleukin-10-Deficient Mice Infected with *Citrobacter rodentium*

Sara M. Dann,[a,b] Christine Le,[d] Barun K. Choudhury,[a] Houpu Liu,[b] Omar Saldarriaga,[a] Elaine M. Hanson,[d] Yingzi Cong,[b,c] Lars Eckmann[d]

Departments of Internal Medicine,[a] Microbiology and Immunology,[b] and Pathology,[c] University of Texas Medical Branch, Galveston, Texas, USA; Department of Medicine, University of California, San Diego, La Jolla, California, USA[d]

Interleukin-10 (IL-10) curtails immune responses to microbial infection and autoantigens and contributes to intestinal immune homeostasis, yet administration of IL-10 has not been effective at attenuating chronic intestinal inflammatory conditions, suggesting that its immune functions may be context dependent. To gain a broader understanding of the importance of IL-10 in controlling mucosal immune responses to infectious challenges, we employed the murine attaching and effacing pathogen *Citrobacter rodentium*, which colonizes primarily the surfaces of the cecum and colon and causes transient mucosal inflammation driven by Th17 and Th1 T helper cells. Infection induced macrophage and dendritic cell production of IL-10, which diminished antibacterial host defenses, because IL-10-deficient mice cleared infection faster than wild-type controls. In parallel, the mice had less acute infection-associated colitis and resolved it more rapidly than controls. Importantly, transient *C. rodentium* infection protected IL-10-deficient mice against the later development of spontaneous colitis that normally occurs with aging in these mice. Genome-wide expression studies revealed that IL-10 deficiency was associated with downregulation of proinflammatory pathways but increased expression of the anti-inflammatory cytokine IL-27 in response to infection. IL-27 was found to suppress *in vitro* Th17 and, to a lesser degree, Th1 differentiation independent of IL-10. Furthermore, neutralization of IL-27 resulted in more severe colitis in infected IL-10-deficient mice. Together, these findings indicate that IL-10 is dispensable for resolving *C. rodentium*-associated colitis and further suggest that IL-27 may be a critical factor for controlling intestinal inflammation and Th17 and Th1 development by IL-10-independent mechanisms.

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine essential for protecting the host against excessive inflammatory and immune responses (1, 2). Produced primarily by activated macrophages, dendritic cells, and T cells, it acts through a heterodimeric receptor complex and JAK/STAT signaling on hematopoietic and other cells (3). IL-10 signaling in macrophages and dendritic cells suppresses antigen presentation and release of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), IL-12, and granulocyte-macrophage colony-stimulating factor (3). Concomitantly, IL-10 promotes the production of anti-inflammatory factors, including interleukin-1 receptor antagonist and soluble TNF-α receptor (3). IL-10 also inhibits T cell differentiation, proliferation, and effector functions (3), thereby dampening effector responses that could be deleterious to the host.

The importance of IL-10 in immune homeostasis has been demonstrated in mice and humans. IL-10-deficient mice develop, over several months, spontaneous enterocolitis related to aberrant immune responses to commensal bacteria (4, 5). The severity of inflammation in IL-10-deficient mice is exacerbated upon increased exposure to luminal antigens or by epithelial disruption (6) and can be attenuated with antibiotics (7, 8). IL-10 production by $CD4^+$ T cells is critical in limiting disease under these conditions (9). In humans, IL-10 has a similar role in maintaining intestinal homeostasis. IL-10 polymorphisms associated with inflammatory bowel disease (IBD) have been identified in genome-wide association studies (10), and mutations in the IL-10 receptor have been reported in patients with severe forms of IBD (11). Given its importance in immunosuppression and association with IBD, the therapeutic potential of IL-10 has been investigated in animal inflammation models and in IBD patients (12, 13), although its administration has not proven efficacious (14).

Despite the detrimental long-term consequences of IL-10 deficiency, loss of IL-10 signaling can also be beneficial in the context of microbial infection (1, 3). For example, in *Salmonella*-infected mice, loss of IL-10 enhances macrophage functions and promotes activation of T cells, particularly IFN-γ-producing Th1 cells, leading to increased bacterial killing and thereby accelerated host defense (15). Abrogation of IL-10 signaling accelerates the clearance of several other intracellular bacteria, including *Klebsiella pneumoniae*, *Listeria monocytogenes*, and *Chlamydia trachomatis*, as well as certain viruses, parasites, and fungi, and thereby improves survival after infection (1, 16–18). However, heightened Th1 responses in the prolonged absence of IL-10 signaling can also result in severe pathology and lethal inflammatory responses in particular infections. Thus, IL-10-deficient mice infected with the intestinal helminth *Trichuris muris* show increased intestinal inflammation related to loss of Paneth cells and mucus production and exhibit increased mortality (19). Following persistent coloniza-

Received 14 January 2014 Returned for modification 12 February 2014
Accepted 18 February 2014
Published ahead of print 24 February 2014
Editor: J. L. Flynn
Address correspondence to Lars Eckmann, leckmann@ucsd.edu.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/IAI.00066-14.

doi:10.1128/IAI.00066-14

tion with *Helicobacter hepaticus*, IL-10-deficient mice display chronic inflammatory lesions in the cecum and colon (20). Similarly, infection of these mice with *Campylobacter jejuni* causes persistent intestinal inflammation in the colon and cecum (21). Together, these studies suggest that IL-10 has different functions under different infection conditions, depending on the particular antimicrobial host defenses and the severity and location of the infection-associated inflammatory responses.

The mechanisms of IL-10-dependent intestinal immunoregulation have primarily been investigated under chronic-stimulation conditions (i.e., persistent infections or spontaneous disease), yet many insults inflicted on the epithelial barrier are likely to be abrupt, transient, and restricted to the surface of the epithelium. One physiologically relevant model that is commonly used to investigate transient microbial challenges in the intestine is infection with *C. rodentium*. These Gram-negative bacteria form attachment and effacement lesions at the epithelial surfaces of the colon and cecum. In immunocompetent mice, infection causes self-limiting mucosal inflammation, characterized by crypt hyperplasia, goblet cell loss, epithelial cell disruption, and mucosal thickening, which begins to resolve within 10 to 14 days postinfection (22). Bacterial clearance is dependent on B cells, $CD4^+$ T cells, natural killer cells, neutrophils, and mast cells (22). Infection elicits both Th1 and Th17 responses at the site of infection (23, 24). Th1 cells are important for controlling pathology and bacterial clearance by secreting gamma interferon (IFN-γ), which promotes macrophage phagocytosis and activates antigen-specific $CD4^+$ T cells (25). Mice lacking Th17 cells or their associated cytokines are also susceptible to *C. rodentium* (26–29). Although mechanisms of Th1 and Th17 induction after *C. rodentium* infection have been reported (25, 28, 30–32), it is not clear whether these effector T cells are also counterregulated and controlled during and after infection.

Given the importance of IL-10 in attenuating inflammatory and immune responses, we set out to test the hypothesis that IL-10 would be essential in dampening the Th1 and Th17 responses and associated mucosal inflammation induced by transient infection with *C. rodentium*. Unexpectedly, however, we found that IL-10 is not required for limiting inflammation in response to *C. rodentium*. Furthermore, we discovered that infection activates an IL-27-dependent anti-inflammatory circuit in the absence of IL-10 that suppresses long-term spontaneous inflammation in aged mice.

MATERIALS AND METHODS

Mice. All mice were obtained from the Jackson Laboratory and housed under specific-pathogen-free conditions. IL-10-deficient mice (33) were back-crossed to a C57BL/6J background for at least 10 generations. C57BL/6J mice were used as controls. Males and females did not show any significant differences in the course of the infection or disease severity, so the results are reported together. All animal studies were approved by the Institutional Animal Care and Use Committees of the University of California, San Diego, and the University of Texas Medical Branch.

Bacterial infections. *C. rodentium* strain DBS100 (ATCC) was grown overnight in Luria-Bertani broth at 37°C and subcultured (1:100 dilution) in fresh broth for 4 to 5 h. Bacteria were harvested by centrifugation and resuspended in sterile phosphate-buffered saline (PBS) at 2.5×10^9/ml. Adult mice (>7 weeks old) were infected by oral gavage with 200 μl of bacterial suspension (5×10^8 bacteria). The bacterial burden in infected mice was determined by CFU assay. Briefly, fecal pellets were collected from individual mice, weighed, and homogenized in 5 ml of PBS. Selected organs were homogenized in 2 ml of PBS. Serial dilutions of the homogenates were plated onto MacConkey agar (feces) or LB agar (organs), and CFU were counted after overnight incubation. The detection limit of the assays was 10^3 CFU/g feces and $<10^1$ CFU per organ.

DSS-induced colitis. Mice were given 2% dextran sulfate sodium (DSS) (35 to 50 kDa; MP Biomedicals) in the drinking water for 5 days and returned to normal drinking water. Non-DSS-treated mice served as controls. All mice were monitored daily for weight loss, stool consistency, occult fecal blood, and rectal bleeding.

Histological analysis. Colons were removed, opened longitudinally, and processed as Swiss rolls before overnight fixation in Bouin's solution. The fixed tissues were embedded in paraffin, and 5-μm sections were prepared and stained with hematoxylin and eosin (H&E). Histological scores (range, 0 to 14) were obtained in a blinded manner by evaluating the following parameters: (i) mucosal architecture (0, normal; 1, focally abnormal; 2, diffusely abnormal; 3, severely abnormal); (ii) inflammatory cell infiltration of mucosa (0, normal; 1, mild infiltration; 2, moderate infiltration; 3, severe infiltration), submucosa (0, normal; 1, mild infiltration; 2 moderate infiltration; 3, severe infiltration), muscle (0, normal; 1 moderate to severe), and serosa (0, absent; 1, present); (iii) Epithelial erosions and ulcerations (0, absent; 1, present); (iv) crypt abscesses (0, absent; 1, present); and (v) goblet cell loss (0, absent; 1, present). Lamina propria cell numbers were quantified per 40× visual field, an area equivalent to 0.1 mm^2. Crypt depth and submucosal thickness were measured using NIS-Elements software (Nikon). For each colon section, scoring and quantification were performed on the two most affected areas at least 10 crypts apart, and the scores were averaged.

Isolation and analysis of lamina propria cells. Colons were opened, cleaned, and cut into 5-mm pieces. To remove the epithelium, tissues were incubated in Hanks balanced salt solution (HBSS) (Ca and Mg free) with 5 mM EDTA, 5% fetal bovine serum (FBS), 10 mM HEPES, and 1 mM dithiothreitol (DTT) 2 times for 20 min each time at 37°C with shaking. The remaining tissues were collected, thoroughly rinsed in PBS, and diced into 1- to 2-mm pieces prior to digestion in RPMI 1640 medium containing 1 mg/ml collagenase D (Roche Applied Science) and 100 μl/ml DNase I (Worthington Biochemical) twice for 30 min at 37°C with shaking. The liberated cells were passed through a 40-μm strainer, pooled, and collected by centrifugation. The cells were then stimulated for 6 h with 50 ng/ml phorbol myristate acetate and 750 ng/ml ionomycin in complete RPMI 1640 medium at 37°C. GolgiStop (BD Biosciences) was added for the last 2 h of incubation. The stimulated cells were washed and stained with anti-CD4 phycoerythrin (PE)-Cy5. Following fixation, the cells were permeabilized and stained with fluorescein isothiocyanate (FITC)-labeled anti-IFN-γ and PE-labeled anti-IL-17A to detect the intracellular cytokines. All antibodies were purchased from eBioscience. The cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences), followed by data analysis with FlowJo software (TreeStar Inc.).

Microarray and quantitative reverse transcription (RT)-PCR analysis. Total RNA was extracted from whole-colon tissue using TRIzol reagent (Invitrogen). Equal amounts of RNA from four or five mice were pooled and quality assessed with an Agilent Technologies bioanalyzer. Microarray studies were performed using the Whole Mouse Gene Expression Microarray, 4 by 44,000 (Agilent Technologies), following the manufacturer's protocols. Intensities were normalized, means of array replicates were calculated, and the data were analyzed with Ingenuity Pathways Analysis (IPA) (Ingenuity Systems). Genes with a ≥2-fold expression change compared to uninfected mice were used for pathway and functional analyses. Canonical pathway analysis identified pathways from the IPA library that were most significant in the data sets. Significance was determined by the number of differentially expressed transcripts from the data set that mapped to the pathway divided by the total number of molecules that exist in the pathway and calculation of a *P* value by Fisher's exact test. Functional analysis identified biological functions most relevant to the data set by calculating Benhamini and Hochberg's false-discovery rate. Genes from the data set that met the false-discovery rate cutoff

($P < 0.05$) and were associated with particular functions were included in the analysis. Fisher's exact test was used to determine the probability that an association was due to chance.

For quantitative RT-PCR analysis, individual and pooled RNA samples were treated with Turbo DNA-free (Ambion) to remove contaminating DNA. The RNA yield and purity were determined using a NanoDrop spectrophotometer (NanoDrop Technologies). Total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) and amplified using Mesa Green 2× SYBR mix (Eurogentec). The primers and expected PCR product sizes were as follows: IL-10, 5′-GCT CTT ACT GAC TGG CAT GAG-3′ (sense) and 5′-CGC AGC TCT AGG AGC ATG TG-3′ (antisense), 108 bp; transforming growth factor β1 (TGF-β1), 5′-GCG TGC TAA TGG TGG ACC GCA-3′ (sense) and 5′-CGG GCA CTG CTT CCC GAA TGT-3′ (antisense), 108 bp; IL-6, 5′-ACA CAT GTT CTC TGG GAA ATC GTG G-3′ (sense) and 5′-TCT GCA AGT GCA TCA TCG TTG TTC A-3′ (antisense), 89 bp; IL-27, 5′-GTG CTG GCT GCA GGA TTC AAA TGT-3′ (sense) and 5′-AGG ACA CTT GGG ATG ACA CCT GAT-3′ (antisense), 133 bp; 18S rRNA, 5′-TTA GAG TGT TCA AAG CAG GCC CGA-3′ (sense) and 5′-TCT TGG CAA ATG CTT TCG CTC TGG-3′, 198 bp. Relative changes in target mRNA levels were calculated by the $2^{-\Delta\Delta CT}$ method, with 18S rRNA as the reference standard.

Isolation and stimulation of BMDMs. To generate bone marrow-derived macrophages (BMDMs), bone marrow was flushed out from the femur and tibia, and the cells were grown in RPMI-Glutamax (Gibco) supplemented with 10% heat-inactivated fetal calf serum (FCS), 200 U/ml penicillin, 200 μg/ml streptomycin, 50 μM 2-mercaptoethanol (2-ME), and 20 ng/ml of mouse recombinant macrophage colony-stimulating factor (M-CSF) (Peprotech) at 37°C in a humidified incubator containing 5% CO_2. Media were replaced once after 3 days. Differentiated macrophages were left untreated or stimulated with recombinant murine IFN-γ (1,000 U/ml; Peprotech) or lipopolysaccharide (LPS) (10 to 1,000 ng/ml; Sigma). After stimulation, the supernatants were removed, centrifuged at 1,500 rpm for 10 min, and stored at −80°C until analysis. The monolayers were covered with 500 μl of RNAlater (Qiagen) and stored at 4°C until RNA was recovered and analyzed by RT-PCR.

ELISA. To quantitate IL-27 in culture supernatants, enzyme-linked immunosorbent assays (ELISAs) were performed using a mouse IL-27 ELISA Ready-Set-Go kit from eBioscience according to the manufacturer's instructions.

***In vitro* T cell polarization.** Splenic $CD4^+$ T cells from CBir1 TCR transgenic mice were isolated and enriched using anti-mouse CD4 magnetic particles (BD Biosciences), as previously described (34). Purified cells (2×10^5 cells/well) were cultured with 2×10^5 irradiated splenic antigen-presenting cells (APC) from IL-10-deficient or control mice and CBir1 antigen (0.3 or 1 μg/ml) at 37°C in a humidified incubator containing 5% CO_2 for 5 days in the presence or absence of murine IL-27 (20 ng/ml; eBioscience) under the following T cell-polarizing conditions: Th1-polarizing conditions, murine IL-12 (mIL-12) (10 ng/ml) and anti-IL-4 (10 μg/ml); Th17-polarizing conditions, TGF-β (2 ng/ml), IL-6 (30 ng/ml), anti-IFN-γ (10 μg/ml), and anti-IL-4 (10 μg/ml); and T regulatory (Treg)-polarizing conditions, TGF-β (5 ng/ml). Five days later, cells were harvested; stimulated with phorbol myristate acetate (PMA) and ionomycin; stained with fluorescence-conjugated antibodies against CD4, IFN-γ, IL-4, IL-17A, and Foxp3; and counterstained with LIVE/DEAD dye (Invitrogen). The cells were analyzed by flow cytometry using an LSRII Fortessa instrument (BD Biosciences).

***In vivo* IL-27 neutralization.** To neutralize IL-27 *in vivo*, mice were injected with 30 μg polyclonal goat anti-mouse IL-27 p28 IgG antibody (R&D Systems) intraperitoneally (i.p.) every 3 days starting on day 2 p.i. Normal goat IgG (R&D Systems) was used as a negative control.

Data analysis. Statistical analysis was performed using SigmaPlot12 (Systat Software Inc.). Differences between groups of mice were evaluated by Student's *t* test or the Mann-Whitney rank sum test, as appropriate.

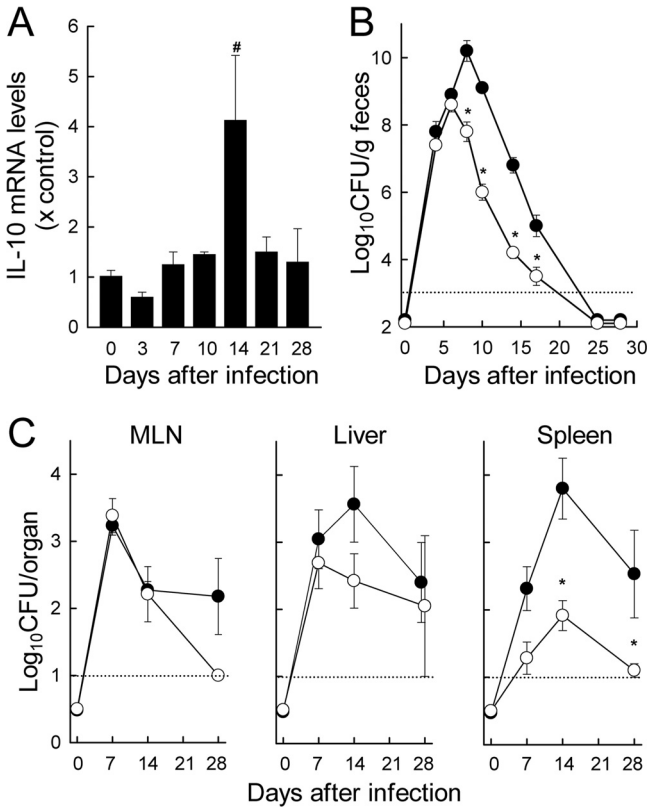

FIG 1 Function of IL-10 in *C. rodentium* clearance. (A) C57BL/6J mice were orally infected with *C. rodentium*, and tissues were collected at the indicated times. IL-10 mRNA levels were determined by qPCR and are shown relative to uninfected controls. The data are means + SEM ($n = 5$ or 6 mice/group). #, $P < 0.05$ versus uninfected controls (rank sum test). The pathology scores for these experiments are shown in Fig. 2B. (B and C) IL-10-deficient (○) and wild-type (●) mice were infected with *C. rodentium*. Bacterial numbers in feces (B) and homogenates of mesenteric lymph nodes (MLN), liver, and spleen (C) were determined by CFU assay. The data are means ± SEM ($n \geq 6$ mice/group). *, $P < 0.05$ versus the wild type at the same time point (rank sum test). The dashed lines represent the CFU assay sensitivities.

Kaplan-Meier analysis was used to compare survival curves. Differences were considered statistically significant at a *P* value of <0.05.

Microarray data accession number. The microarray data were deposited in the Gene Expression Omnibus (GEO) database under accession number GSE55812.

RESULTS

IL-10 is not required for resolving *C. rodentium*-induced acute intestinal inflammation. *C. rodentium* infection induces IFN-γ- and IL-17-expressing $CD4^+$ T cells and causes mucosal inflammation (22–24), but the inflammation resolves rapidly after the peak of infection. To determine whether the anti-inflammatory cytokine IL-10 is important for controlling inflammatory resolution, we first examined its expression during the course of the infection in wild-type mice. Analysis of colonic IL-10 mRNA levels by quantitative PCR (qPCR) revealed a transient response, with a significant increase on day 14 followed by a return to preinfection levels by day 21 (Fig. 1A). Similar patterns of expression were observed for cytokines that promote IL-10 production, including TGF-β and IL-6 (2.3- and 4.1-fold, respectively). Expression of ICOS (*i*nducible T-cell *co*stimulator; CD278), a critical factor involved

in IL-10 production by antigen-specific T cells, was also increased by day 10 and remained elevated (4.0-fold) through day 28. The time course data suggested that adaptive immune cells were the major source of IL-10, since its increased expression coincided with the steepest decline in the bacterial load around day 14, when strong adaptive immune responses develop (22). However, analysis of isolated subsets of colon lamina propria cells after 14 days showed that IL-10 was most highly expressed in $CD11b^+$ $CD11c^+$ dendritic cells (3.6-fold) and $CD11b^+$ $CD11c^-$ macrophages (3.5-fold), but not in $CD11b^-$ $CD11c^+$ dendritic cells (0.3-fold) or other $CD45^+$ leukocytes, including T cells (0.4-fold). Thus, $CD11b^+$ dendritic cells and macrophages were the major IL-10 producers during the clearance phase of *C. rodentium* infection.

To define the physiological function of IL-10 in host defense against *C. rodentium* and the resolution of the infection-associated inflammation, we infected IL-10-deficient mice and wild-type littermate controls. Infection-related mortality was low in both groups of mice (6 versus 7%). Similarly, no significant differences in maximal body weight loss, a clinical marker of disease severity, were observed between the groups (maximal weight loss was observed on day 3 for both groups, with 4.0% ± 2.2% in wild-type mice versus 10.7% ± 2.9% in IL-10-deficent mice [mean ± standard error of the mean {SEM}; $n \geq 25$; P, not significant]; both groups had returned to baseline weights by day 6 and maintained the weights throughout the remainder of the infection). Analysis of the bacterial burden revealed that the two groups had similar fecal shedding during the first 6 days after infection (Fig. 1B), although IL-10-deficient mice showed an enhanced and highly reproducible ability to subsequently control the infection, with >100-fold-lower bacterial numbers than wild-type mice on days 8 to 17 and accelerated bacterial eradication thereafter. Reduced bacterial numbers were also found in the spleen, but not in mesenteric lymph nodes or the liver, in IL-10-deficent compared to wild-type mice at 1 to 2 weeks (Fig. 1C).

Aged IL-10-deficient mice develop spontaneous colitis over time (33) and are more susceptible to chemically induced colitis (35), which we confirmed in our mice (see Fig. S1 in the supplemental material), so we anticipated that the mice would also show greater infection-induced colon inflammation. Surprisingly, histological examination of the colons of infected IL-10-deficient mice revealed that they had less inflammation than those of infected wild-type mice on day 14, the peak of the histologically observable host response, as demonstrated by reduced epithelial hyperplasia, fewer infiltrating lamina propria leukocytes, and less submucosal edema (Fig. 2A and B). Consistent with this, we found by flow cytometric analysis of isolated lamina propria T cells that infected IL-10-deficient mice had attenuated induction of Th1 and Th17 cells compared to wild-type controls (Fig. 2C). Interestingly, on day 7, IL-10-deficient mice displayed slight increases in epithelial hyperplasia and submucosal edema compared to wild-type mice (Fig. 2B), suggesting that their mucosal responses occurred more rapidly but were overall less severe. Inflammation had resolved in both groups by 28 days, indicating that IL-10 is not required for resolution of *C. rodentium*-induced inflammation.

***C. rodentium* infection protects against chronic colitis in aged IL-10-deficient mice.** Our observation of reduced and shortened inflammation in *C. rodentium*-infected IL-10-deficient mice not only demonstrated that IL-10 was dispensable for resolution of inflammation, but also suggested that other anti-inflammatory mechanisms must be active. We speculated that these effects may be sustained and could potentially impact the spontaneous development of chronic colitis that occurs in aged IL-10-deficient mice (33). To test this notion, 7- to 8-week-old, disease-free, IL-10-deficient mice were infected with *C. rodentium* and rechallenged every 4 weeks for a total of four exposures. The mice were closely monitored for clinical signs of colitis, weight loss, and mortality up to 5 months of age. Uninfected IL-10-deficient mice, which were littermates of the infected IL-10 knockout animals but were separated just before infection, and infected wild-type mice served as controls. Analysis of disease-free survival showed significant improvement in *C. rodentium*-exposed compared to uninfected IL-10-deficient mice (Fig. 3A). Histological examination of the colons of uninfected IL-10-deficient mice revealed moderate to severe inflammation, characterized by mucosal thickening, epithelial hyperplasia and erosions, crypt abscesses, and mixed leukocytic infiltrates (Fig. 3B to D). In contrast, IL-10-deficient mice that had been repeatedly infected with *C. rodentium* showed significantly less mucosal inflammation with reduced mucosal thickening and crypt hyperplasia and fewer leukocytic infiltrates. Together, these results indicate that repeated *C. rodentium* infection can protect against the development of spontaneous chronic colitis in the absence of IL-10.

Differential cytokine expression in *C. rodentium*-infected IL-10-deficient mice. To identify potential mechanisms of anti-inflammatory protection observed in IL-10-deficient mice, we used microarrays to determine genome-wide gene expression profiles of the colonic response to infection. Two weeks after *C. rodentium* challenge, total RNAs from the colons of infected IL-10-deficient and wild-type mice were analyzed. Using changes of ≥2-fold as the cutoff, we found 3,853 gene transcripts with differential expression in the colons of infected IL-10-deficient versus infected wild-type mice. Of these, 46% were decreased and 53% were increased. Analysis of the data with Ingenuity Pathways Analysis software revealed significant downregulation of IL-17A and granzyme signaling (see Table S1 in the supplemental material) and decreased chemotaxis and activation of neutrophils and granulocytes (see Table S2 in the supplemental material). Consistent with the histological and cellular analyses, the expression levels of many chemokines and cytokines involved in recruiting leukocytes and promoting inflammation were, on average, lower in IL-10-deficient than in wild-type mice after infection (Table 1). An exception to this general trend was IL-27, a cytokine with both pro- and anti-inflammatory properties (36), which was significantly increased in the colons of infected IL-10-deficient mice.

Intrigued by the increased expression of IL-27 after 2 weeks, we questioned whether the cytokine might have a role in mucosal protection after *C. rodentium* infection. We first determined its time course of expression in the colons of IL-10-deficient and wild-type mice. In wild-type mice, levels of IL-27 mRNA were constant and showed only minor induction (<2-fold) by day 14 (Fig. 4A). In contrast, IL-10-deficient mice had lower baseline expression of IL-27 than wild-type mice but displayed a significant (>3-fold) increase by 10 to 14 days after infection. Because IL-27 is produced by activated APC (37), we tested whether IL-10-deficient macrophages express more IL-27 than wild-type cells. Relative to wild-type cells, IL-10-deficient macrophages had significantly higher IL-27 mRNA levels and secreted more IL-27 after stimulation with LPS and IFN-γ (Fig. 4B), suggesting that increased IL-27 expression after *C. rodentium* infection of IL-10-deficient mice is related to a cell-autonomous mechanism.

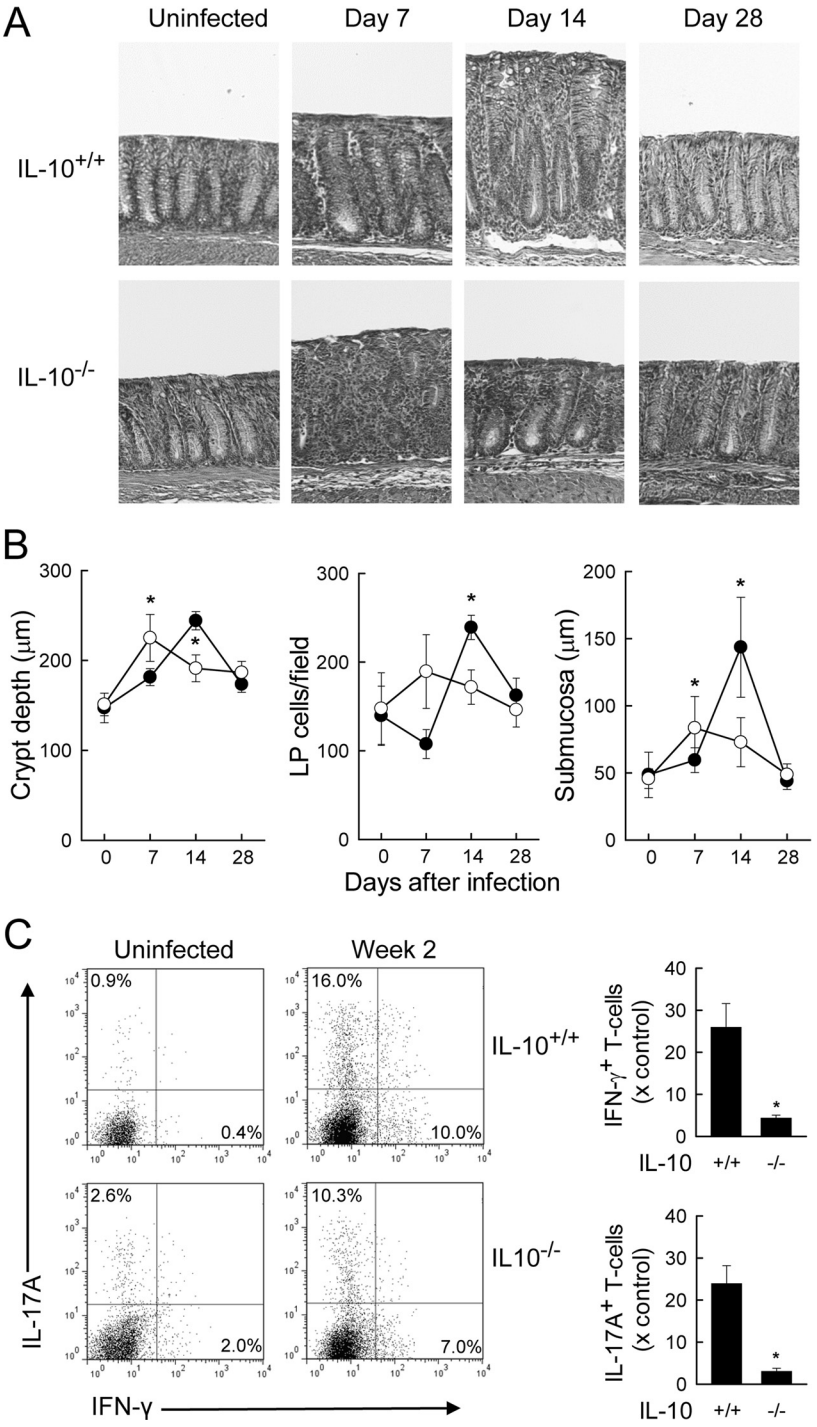

FIG 2 Inflammatory responses in *C. rodentium*-infected IL-10-deficient mice. IL-10-deficient mice (IL-10$^{-/-}$; ○) and wild-type controls (IL-10$^{+/+}$; ●) were infected with *C. rodentium* or left uninfected as controls. (A) H&E-stained paraffin sections of the colon were assessed for epithelial damage and inflammatory cell infiltration. (B) Crypt depths, lamina propria cell density, and submucosal thickness were quantified morphometrically. (C) Lamina propria (LP) T cells were isolated from the colon and assessed for IL-17A- and IFN-γ-producing cells. The bar graphs show the fold induction compared to uninfected controls. The data are means ± SEM ($n = 5$ or 6 mice/group). *, $P < 0.05$ versus the wild type (rank sum test).

IL-27 regulates T cell differentiation in the absence of IL-10. IL-27 has been shown to antagonize inflammation and T effector cells through induction of IL-10 (36), yet our *in vivo* data suggested IL-10 is not required for attenuation of inflammation. To determine whether IL-27 could suppress Th induction in the absence of IL-10, we isolated naive CD4$^+$ T cells from CBir1 T cell receptor-transgenic (Cbir1 Tg) mice, whose T cells are specific for bacterial flagellin (38), and polarized them *in vitro* with antigen-loaded IL-10-deficient or wild-type APC under standard Th differentiation (or control) conditions in the absence or presence of

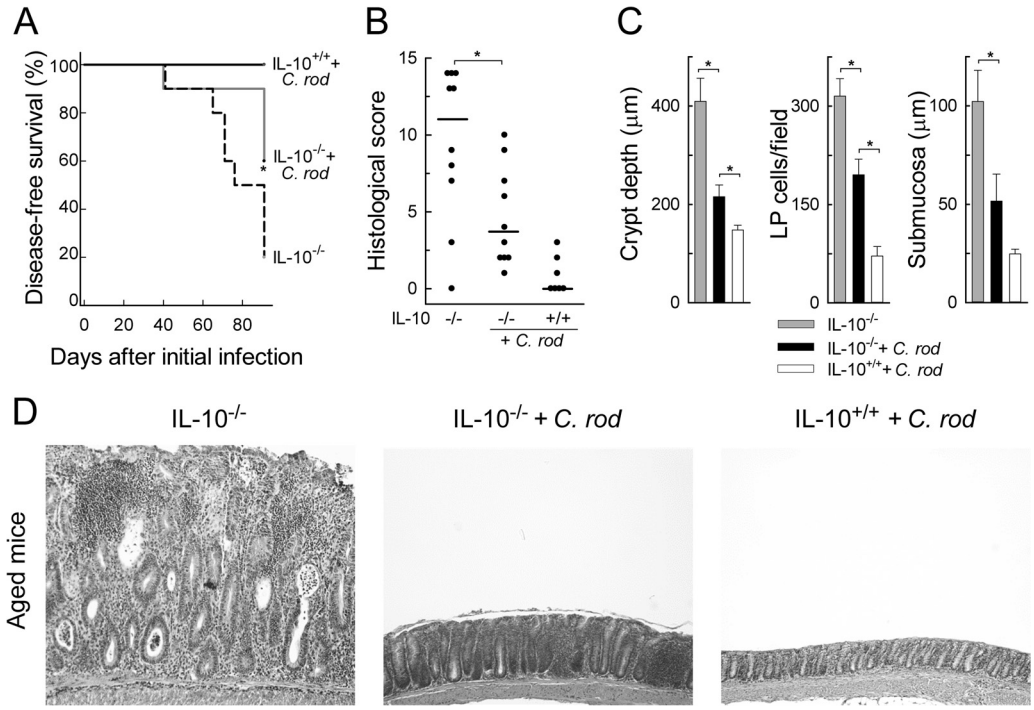

FIG 3 Protection against spontaneous colitis after infection of IL-10-deficent mice with *C. rodentium*. Littermates of age-matched (7- to 8-week-old) disease-free IL-10-deficent and wild-type mice were infected with *C. rodentium* (*C. rod*) or left uninfected as controls and followed for 12 weeks. (A) Occurrence of disease, defined as >20% weight loss, was recorded in groups of 10 to 15 mice. The significance relative to wild-type mice was determined by Kaplan-Meier survival statistics (*, $P < 0.05$). (B) A histological damage score was determined on H&E-stained paraffin sections of the colon. Each data point represents one animal. Horizontal lines represent median values for each group. *, $P < 0.05$ (rank sum test). (C) Crypt depths, lamina propria cell numbers, and submucosal thickness were quantified morphometrically. The results are means + SEM ($n = 7$ to 10 mice/group); *, $P < 0.05$ (rank sum test). (D) Representative colon sections from mice 12 weeks postinfection and an uninfected IL-10-deficient littermate control of the same age.

exogenous IL-27. One week later, cytokine production was analyzed by flow cytometry. Activation of naive CBir1 Tg $CD4^+$ T cells under Th1 conditions resulted in a strong Th1 phenotype (>80% of the cells expressed IFN-γ), which was only minimally affected by IL-27 in cultures with either wild-type or IL-10-deficient APC (Fig. 4C). In comparison, IL-27 moderately inhibited the spontaneous development of Th1 cells under neutral control conditions in both genotypes, suggesting that it was more Th1 suppressive under less stringent Th1 induction conditions. Furthermore, IL-27 strongly suppressed the development of Th17 effector and $Foxp3^+$ Treg cells in culture with wild-type and IL-10-deficient APC (Fig. 4C). Collectively, these results indicate that IL-27, even in the absence of IL-10, profoundly suppresses the induction of antigen-specific Th17 cells and Tregs under strong induction conditions and that of Th1 under suboptimal induction conditions.

TABLE 1 Differential gene expression in *C. rodentium*-infected $IL\text{-}10^{-/-}$ vs $IL\text{-}10^{+/+}$ mice[a]

Gene product	mRNA ratio ($IL\text{-}10^{-/-}/IL\text{-}10^{+/+}$)
CXCL5	0.02
IL-6	0.11
IL-22	0.12
IL-23α	0.15
IL-17A	0.23
IL-18	0.26
CCL3	0.29
CCL2	0.39
IL-27	2.18

[a] Colonic gene expression was determined by whole-genome microarrays in IL-10-deficient ($IL\text{-}10^{-/-}$) and wild-type ($IL\text{-}10^{+/+}$) mice 14 days after *C. rodentium* infection. Cytokine and chemokine genes with a >2-fold increase or decrease in mRNA expression in $IL\text{-}10^{-/-}$ compared to $IL\text{-}10^{+/+}$ mice are shown.

Neutralization of IL-27 exacerbates colitis in IL-10-deficient mice. Given the increase in IL-27 expression in IL-10-deficient mice and the ability of IL-27 to suppress the development of potentially colitogenic Th17 cells, we sought to determine the physiological importance of IL-27 in IL-10-deficient mice infected with *C. rodentium*. Mice were infected and treated with neutralizing anti-IL-27 antibodies or an IgG control. Antibody treatment had little impact on the bacterial burden at day 7 (mean log CFU/g of feces ± SEM, 8.1 ± 0.2 in IgG control-treated mice versus 8.7 ± 0.3 in anti-IL-27-treated mice; $P = 0.2$). However, histological examination revealed clear differences. Animals given IgG control antibodies exhibited moderate colon inflammation with epithelial hyperplasia, modest leukocytic infiltration of the mucosa and submucosa, and increased submucosal thickening after infection (Fig. 5). In contrast, IL-10-deficient mice treated with anti-IL-27 antibodies exhibited a similar degree of epithelial hyperplasia but had marked mucosal infiltration with inflammatory cells and extensive submucosal swelling, which was accompanied by increased expression of the proinflammatory cytokine IL-6 compared to untreated controls (3.4-fold versus 1.8-fold, respectively). Thus, neutralization of IL-27 enhanced the mucosal inflammatory response of IL-10-deficient mice to *C. rodentium*, which is consistent with the

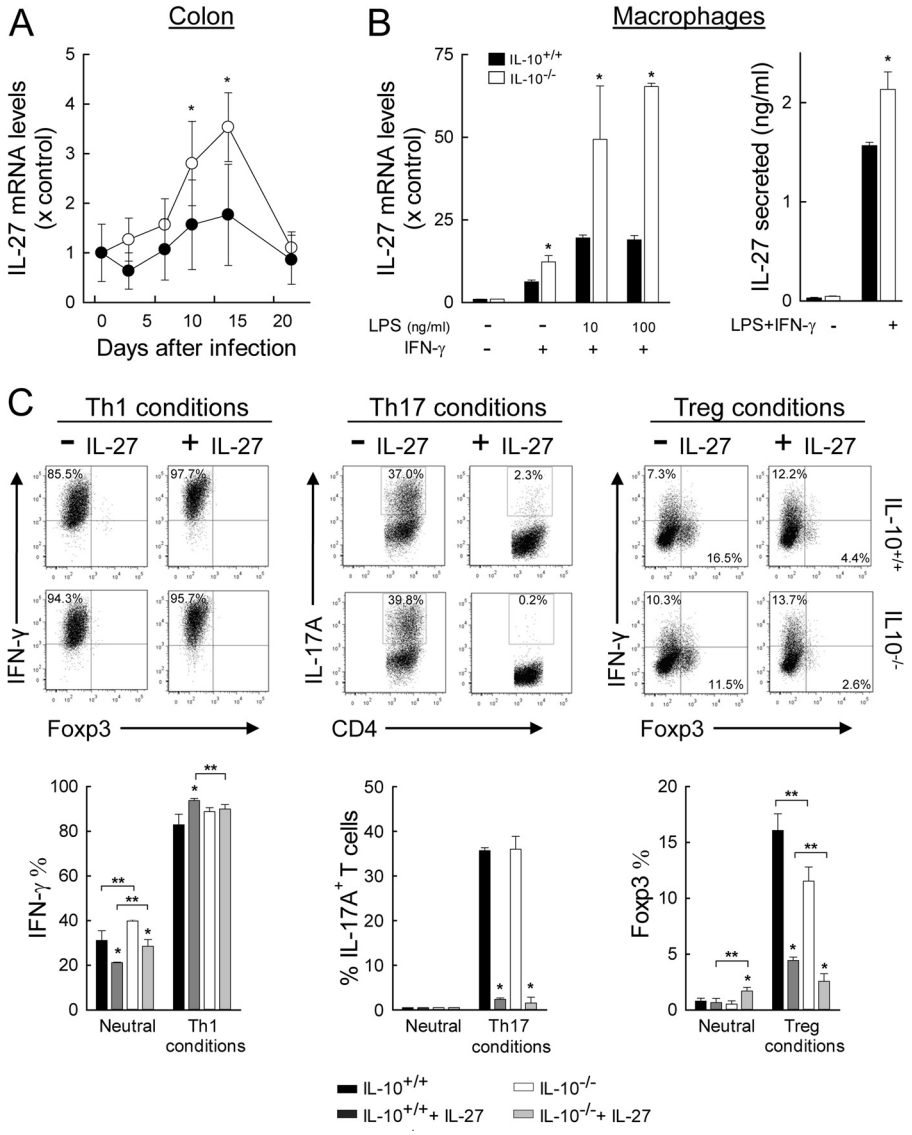

FIG 4 IL-10-independent suppression of Th17 differentiation by IL-27. (A) Total RNA from the colons of *C. rodentium*-infected IL-10-deficient ($IL\text{-}10^{-/-}$; ○) and wild-type ($IL\text{-}10^{+/+}$; ●) mice was analyzed for IL-27 mRNA expression by qPCR. The data are means ± SEM ($n = 6$ or 7 mice/group) of mRNA changes relative to uninfected controls; *, $P < 0.05$ versus uninfected controls (rank sum test). (B) Bone marrow-derived macrophages obtained from $IL\text{-}10^{-/-}$ and $IL\text{-}10^{+/+}$ mice were treated with LPS and IFN-γ, and 24 h later, cells and supernatants were harvested. IL-27 mRNA expression was determined by qPCR, and protein levels in supernatants were measured by ELISA. The results are means + standard deviations (SD) of 3 replicates. *, $P < 0.05$ versus wild-type cells (*t* test). (C) CBir1-Tg CD4 T^+ cells were cultured with CBir1-peptide-pulsed $IL\text{-}10^{-/-}$ and $IL\text{-}10^{+/+}$ splenic APC in the presence of various cytokines or antibodies with and without IL-27. Five days later, Foxp3, IFN-γ, and IL-17A expression by $CD4^+$ T cells was determined by flow cytometry. Shown are representative dot plots and bar charts of cells cultured under Th1 (IL-12 plus αIL-4), Th17 (TGF-β plus IL-6 plus αIL-4 plus αIFN-γ), Treg (TGF-β), or neutral (no cytokines or antibodies) conditions. The bars represent mean cell percentages + SD ($n = 5$/group). *, $P < 0.05$ versus wild-type cells (*t* test); **, $P < 0.05$ for the indicated pairs of $IL\text{-}10^{+/+}$ versus $IL\text{-}10^{-/-}$ groups (*t* test). The data are representative of two separate experiments.

notion that increased IL-27 expression in these mice may be responsible for the diminished Th17 responses and reduced inflammation that we had observed in the absence of IL-10 (Fig. 2).

DISCUSSION

Our study demonstrates that IL-10 is not required for early or late host defense against *C. rodentium*. Thus, during the first week after infection, IL-10 loss had no impact on the bacterial burden, suggesting that the functions of innate immune cells, such as macrophages and granulocytes, which limit infection early after infection, are IL-10 independent. In the second week, which is characterized by the development of effective adaptive immunity, IL-10 deficiency even accelerated antibacterial host defense by several days. A possible explanation may be that the absence of IL-10 can lead to increased production of IFN-γ and TNF-α by antigen-specific T cells (19, 39), and both of these cytokines are important in host defense against *C. rodentium* (22). However, we did not find elevated expression of IFN-γ or IL-17 after infection of IL-10-deficient mice, nor were Th1 and Th17 responses obvi-

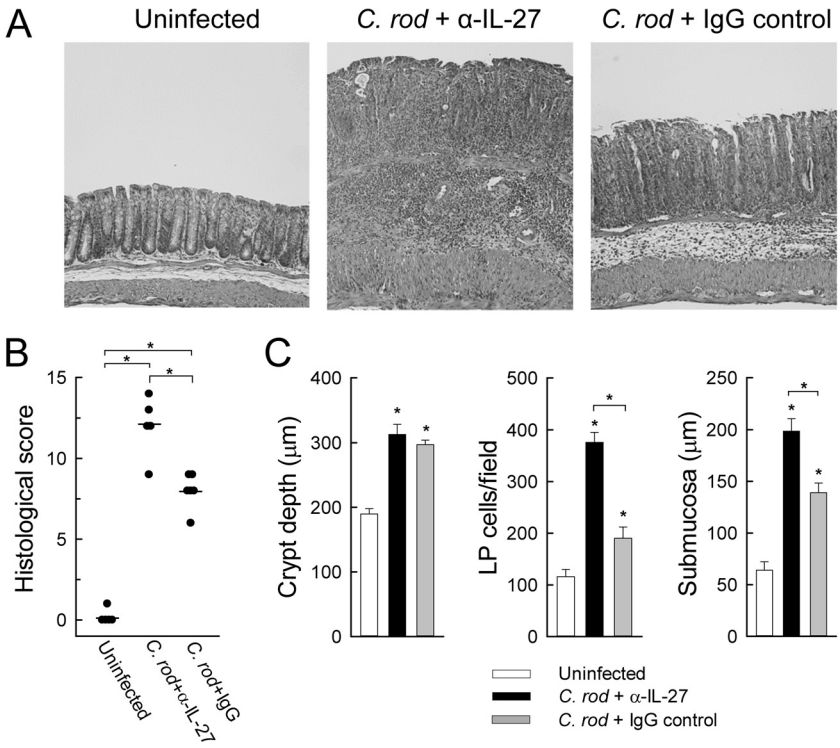

FIG 5 Importance of IL-27 in controlling *C. rodentium*-induced inflammation in IL-10-deficient mice. IL-10-deficient mice treated with anti-IL-27 antibody or IgG control antibodies were infected with *C. rodentium* (*C. rod*). Uninfected IL-10-deficient littermates served as an additional control. The colons were collected at day 12 and examined histologically. (A) Representative colon sections showing greater inflammatory infiltration in IL-10-deficient animals treated with anti-IL-27 antibody than in controls. (B) A histological inflammation score was determined on H&E-stained paraffin colon sections. Each data point represents one animal. *, $P < 0.05$ (rank sum test). (C) Crypt depths, lamina propria cell numbers, and submucosal thickness were quantified morphometrically. The results are means + SEM ($n = 5$ or 6 mice/group); *, $P < 0.05$ (rank sum test).

ously enhanced in these mice. Nonetheless, it is possible that the absence of IL-10 allowed the accelerated development of specific T cells that orchestrate effective early antibacterial defenses and reduce the bacterial burden (and thus antigenic stimulus) before the substantial expansion of Th1 and Th17 that normally occurs in infected wild-type mice.

Beyond accelerated host defense, the loss of IL-10 was unexpectedly associated with diminished mucosal inflammation during *C. rodentium* infection. This finding indicates that IL-10 is dispensable for controlling and resolving inflammation in this situation, which contrasts with the observations in other intestinal infection models, such as *H. hepaticus* or *T. muris* (19, 20). Nonetheless, consistent with our results, other data also support the notion that IL-10 has a limited role in controlling intestinal inflammation under certain conditions. For example, prolonged administration of IL-10 was shown to reduce IFN-γ expression in the colon but failed to improve colitis in genetically susceptible rats (40), and treatment of Crohn's disease patients with high doses of IL-10 has failed to ameliorate intestinal inflammation (13). These and our findings suggest that IL-10-independent mechanisms of immunoregulation exist in the intestine to curtail inflammatory responses to infectious agents, such as *C. rodentium*, and to other inflammatory stimuli. While the exact mechanism of IL-10-independent control of inflammation in our model is not known, recent studies have identified potential immunoregulatory cells and factors that may be involved (41). For example, in a murine malaria model, in which IL-10 treatment failed to ameliorate liver damage, IL-27 was shown to be essential for limiting the migration and function of Th1 cells through IL-10-independent processes (42, 43). Furthermore, IL-27Rα-deficient mice develop more severe chemically induced colitis than wild-type controls (44). Our data also suggest that IL-27 is important in controlling mucosal inflammation in response to *C. rodentium* infection.

The regulatory functions of IL-27 are generally thought to be mediated by induction of IL-10 (37), particularly in $CD4^+$ T cells during intestinal inflammation (45). However, our data indicate that IL-27 can regulate immune responses by other mechanisms. Consistent with previous studies (37, 46, 47), we observed that IL-27 strongly inhibited the *in vitro* development of Th17 and Tregs, as well as modestly suppressing Th1 development under suboptimal induction conditions in wild-type cells. Importantly, we discovered that this inhibition was independent of IL-10 in our model. Consistent with this finding, IL-27 was found to suppress Th17 cell-driven autoimmune encephalomyelitis independently of IL-10 (48). Several mechanisms may be responsible for the IL-10-independent functions of IL-27. For example, IL-27 can antagonize Th17 cell differentiation through direct inhibition of RORc (47). IL-27 promotes programmed death ligand 1 upregulation on naive T cells, which inhibits the differentiation of Th17 cells *in vivo* and can limit the severity of autoimmune disease (49). In addition to T cell effects, IL-27 also stimulates differential STAT signaling in intestinal epithelial cells and promotes epithelial restitution and expression of antimicrobial genes (50), which could contribute to

reducing microbial stimulation of innate immune cells in the mucosa. It must be noted that IL-27 can also promote inflammation under certain conditions. For example, mice lacking IL-27 receptors are protected against mucosal inflammation in response to the gastrointestinal helminth *T. muris* (51), indicating that the pro- and anti-inflammatory effects of IL-27 may depend on the immune context.

Activation of macrophages from IL-10-deficient mice induced more IL-27 production than in wild-type mice, whereas stimulated dendritic cells were reported to be unable to produce IL-27 without IL-10 (52). These results indicate that IL-10 normally acts in a cell-autonomous fashion to limit IL-27 production in macrophages but is a critical autocrine inducer of IL-27 in dendritic cells. These data also indirectly suggest that macrophages, rather than dendritic cells, are important for attenuating *C. rodentium*-induced mucosal inflammation in the absence of IL-10. This general concept of a regulatory role of macrophages is supported by the observation that their activation can induce other anti-inflammatory mediators, as exemplified by TGF-β (53).

An important and unexpected observation in our studies was the apparently long-lasting effect of *C. rodentium* infection on protection against the development of spontaneous colitis in aged IL-10-deficient mice. The underlying mechanisms are presently unclear, but one could speculate that enhanced IL-27 production and the accompanying suppression of Th17 responses may be involved. It also remains to be established what the optimal duration and frequency of *C. rodentium* infections are to achieve maximal protection against spontaneous colitis. Regardless of the exact conditions and mechanisms, our finding that IL-10-independent processes are involved in attenuating T effector responses points to novel opportunities in identifying therapeutic strategies for resolving inflammatory processes in the intestinal tract.

ACKNOWLEDGMENTS

We thank James Sprague, Garry Hardiman, and Lucia Hall for technical assistance.

This work was supported by NIH grants DK035108 and DK080508. S.M.D. was supported by a Research Fellowship Award from the Crohn's and Colitis Foundation of America and by the Institute for Translational Sciences at the University of Texas Medical Branch, supported in part by an NIH Clinical and Translational Science Award (UL1TR000071 and KL2TR000072).

REFERENCES

1. **Cyktor JC, Turner J.** 2011. Interleukin-10 and immunity against prokaryotic and eukaryotic intracellular pathogens. Infect. Immun. **79:**2964–2973. http://dx.doi.org/10.1128/IAI.00047-11.
2. **Iyer SS, Cheng G.** 2012. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit. Rev. Immunol. **32:**23–63. http://dx.doi.org/10.1615/CritRevImmunol.v32.i1.30.
3. **Sabat R, Grutz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J.** 2010. Biology of interleukin-10. Cytokine Growth Factor Rev. **21:**331–344. http://dx.doi.org/10.1016/j.cytogfr.2010.09.002.
4. **Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB.** 1998. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect. Immun. **66:**5224–5231.
5. **Kim SC, Tonkonogy SL, Karrasch T, Jobin C, Sartor RB.** 2007. Dual-association of gnotobiotic IL-10-/- mice with 2 nonpathogenic commensal bacteria induces aggressive pancolitis. Inflamm. Bowel Dis. **13:**1457–1466. http://dx.doi.org/10.1002/ibd.20246.
6. **Hansen JJ, Holt L, Sartor RB.** 2009. Gene expression patterns in experimental colitis in IL-10-deficient mice. Inflamm. Bowel Dis. **15:**890–899. http://dx.doi.org/10.1002/ibd.20850.
7. **Madsen KL, Doyle JS, Tavernini MM, Jewell LD, Rennie RP, Fedorak RN.** 2000. Antibiotic therapy attenuates colitis in interleukin 10 gene-deficient mice. Gastroenterology **118:**1094–1105. http://dx.doi.org/10.1016/S0016-5085(00)70362-3.
8. **Hoentjen F, Harmsen HJ, Braat H, Torrice CD, Mann BA, Sartor RB, Dieleman LA.** 2003. Antibiotics with a selective aerobic or anaerobic spectrum have different therapeutic activities in various regions of the colon in interleukin 10 gene deficient mice. Gut **52:**1721–1727. http://dx.doi.org/10.1136/gut.52.12.1721.
9. **Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F.** 1999. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J. Exp. Med. **190:**995–1004. http://dx.doi.org/10.1084/jem.190.7.995.
10. **Franke A, Balschun T, Karlsen TH, Sventoraityte J, Nikolaus S, Mayr G, Domingues FS, Albrecht M, Nothnagel M, Ellinghaus D, Sina C, Onnie CM, Weersma RK, Stokkers PC, Wijmenga C, Gazouli M, Strachan D, McArdle WL, Vermeire S, Rutgeerts P, Rosenstiel P, Krawczak M, Vatn MH, Mathew CG, Schreiber S.** 2008. Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. Nat. Genet. **40:**1319–1323. http://dx.doi.org/10.1038/ng.221.
11. **Moran CJ, Walters TD, Guo CH, Kugathasan S, Klein C, Turner D, Wolters VM, Bandsma RH, Mouzaki M, Zachos M, Langer JC, Cutz E, Benseler SM, Roifman CM, Silverberg MS, Griffiths AM, Snapper SB, Muise AM.** 2013. IL-10R polymorphisms are associated with very-early-onset ulcerative colitis. Inflamm. Bowel Dis. **19:**115–123. http://dx.doi.org/10.1002/ibd.22974.
12. **Duchmann R, Schmitt E, Knolle P, Meyer zum Buschenfelde KH, Neurath M.** 1996. Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. Eur. J. Immunol. **26:**934–938. http://dx.doi.org/10.1002/eji.1830260432.
13. **Tilg H, van Montfrans C, van den Ende A, Kaser A, van Deventer SJ, Schreiber S, Gregor M, Ludwiczek O, Rutgeerts P, Gasche C, Koningsberger JC, Abreu L, Kuhn I, Cohard M, LeBeaut A, Grint P, Weiss G.** 2002. Treatment of Crohn's disease with recombinant human interleukin 10 induces the proinflammatory cytokine interferon gamma. Gut **50:**191–195. http://dx.doi.org/10.1136/gut.50.2.191.
14. **Buruiana FE, Sola I, Alonso-Coello P.** 2010. Recombinant human interleukin 10 for induction of remission in Crohn's disease. Cochrane Database Syst. Rev. CD005109. http://dx.doi.org/10.1002/14651858.CD005109.pub3.
15. **Arai T, Hiromatsu K, Nishimura H, Kimura Y, Kobayashi N, Ishida H, Nimura Y, Yoshikai Y.** 1995. Effects of in vivo administration of anti-IL-10 monoclonal antibody on the host defence mechanism against murine Salmonella infection. Immunology **85:**381–388.
16. **Greenberger MJ, Strieter RM, Kunkel SL, Danforth JM, Goodman RE, Standiford TJ.** 1995. Neutralization of IL-10 increases survival in a murine model of Klebsiella pneumonia. J. Immunol. **155:**722–729.
17. **Dai WJ, Kohler G, Brombacher F.** 1997. Both innate and acquired immunity to Listeria monocytogenes infection are increased in IL-10-deficient mice. J. Immunol. **158:**2259–2267.
18. **Igietseme JU, Ananaba GA, Bolier J, Bowers S, Moore T, Belay T, Eko FO, Lyn D, Black CM.** 2000. Suppression of endogenous IL-10 gene expression in dendritic cells enhances antigen presentation for specific Th1 induction: potential for cellular vaccine development. J. Immunol. **164:**4212–4219.
19. **Schopf LR, Hoffmann KF, Cheever AW, Urban JF, Jr, Wynn TA.** 2002. IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. J. Immunol. **168:**2383–2392.
20. **Kullberg MC, Andersen JF, Gorelick PL, Caspar P, Suerbaum S, Fox JG, Cheever AW, Jankovic D, Sher A.** 2003. Induction of colitis by a CD4+ T cell clone specific for a bacterial epitope. Proc. Natl. Acad. Sci. U. S. A. **100:**15830–15835. http://dx.doi.org/10.1073/pnas.2534546100.
21. **Mansfield LS, Bell JA, Wilson DL, Murphy AJ, Elsheikha HM, Rathinam VA, Fierro BR, Linz JE, Young VB.** 2007. C57BL/6 and congenic interleukin-10-deficient mice can serve as models of Campylobacter jejuni colonization and enteritis. Infect. Immun. **75:**1099–1115. http://dx.doi.org/10.1128/IAI.00833-06.
22. **Borenshtein D, McBee ME, Schauer DB.** 2008. Utility of the Citrobacter rodentium infection model in laboratory mice. Curr. Opin. Gastroenterol. **24:**32–37. http://dx.doi.org/10.1097/MOG.0b013e3282f2b0fb.
23. **Higgins LM, Frankel G, Douce G, Dougan G, MacDonald TT.** 1999. Citrobacter rodentium infection in mice elicits a mucosal Th1 cytokine

response and lesions similar to those in murine inflammatory bowel disease. Infect. Immun. **67:**3031–3039.
24. **Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT.** 2006. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature **441:**231–234. http://dx.doi.org/10.1038/nature04754.
25. **Shiomi H, Masuda A, Nishiumi S, Nishida M, Takagawa T, Shiomi Y, Kutsumi H, Blumberg RS, Azuma T, Yoshida M.** 2010. Gamma interferon produced by antigen-specific CD4+ T cells regulates the mucosal immune responses to Citrobacter rodentium infection. Infect. Immun. **78:**2653–2666. http://dx.doi.org/10.1128/IAI.01343-09.
26. **Basu R, O'Quinn DB, Silberger DJ, Schoeb TR, Fouser L, Ouyang W, Hatton RD, Weaver CT.** 2012. Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria. Immunity **37:**1061–1075. http://dx.doi.org/10.1016/j.immuni.2012.08.024.
27. **Satoh-Takayama N, Dumoutier L, Lesjean-Pottier S, Ribeiro VS, Mandelboim O, Renauld JC, Vosshenrich CA, Di Santo JP.** 2009. The natural cytotoxicity receptor NKp46 is dispensable for IL-22-mediated innate intestinal immune defense against Citrobacter rodentium. J. Immunol. **183:**6579–6587. http://dx.doi.org/10.4049/jimmunol.0901935.
28. **Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, Fujikado N, Tanahashi Y, Akitsu A, Kotaki H, Sudo K, Nakae S, Sasakawa C, Iwakura Y.** 2009. Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity **30:**108–119. http://dx.doi.org/10.1016/j.immuni.2008.11.009.
29. **Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR.** 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell **139:**485–498. http://dx.doi.org/10.1016/j.cell.2009.09.033.
30. **Osborne LC, Joyce KL, Alenghat T, Sonnenberg GF, Giacomin PR, Du Y, Bergstrom KS, Vallance BA, Nair MG.** 2013. Resistin-like molecule alpha promotes pathogenic Th17 cell responses and bacterial-induced intestinal inflammation. J. Immunol. **190:**2292–2300. http://dx.doi.org/10.4049/jimmunol.1200706.
31. **Ryz NR, Patterson SJ, Zhang Y, Ma C, Huang T, Bhinder G, Wu X, Chan J, Glesby A, Sham HP, Dutz JP, Levings MK, Jacobson K, Vallance BA.** 2012. Active vitamin D (1,25-dihydroxyvitamin D3) increases host susceptibility to Citrobacter rodentium by suppressing mucosal Th17 responses. Am. J. Physiol. Gastrointest. Liver Physiol. **303:**G1299–G1311. http://dx.doi.org/10.1152/ajpgi.00320.2012.
32. **Rubino SJ, Geddes K, Magalhaes JG, Streutker C, Philpott DJ, Girardin SE.** 2013. Constitutive induction of intestinal Tc17 cells in the absence of hematopoietic cell-specific MHC class II expression. Eur. J. Immunol. **43:**2896–2906. http://dx.doi.org/10.1002/eji.201243028.
33. **Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W.** 1993. Interleukin-10-deficient mice develop chronic enterocolitis. Cell **75:**263–274. http://dx.doi.org/10.1016/0092-8674(93)80068-P.
34. **Liu H, Yao S, Dann SM, Qin H, Elson CO, Cong Y.** 2013. ERK differentially regulates Th17- and Treg-cell development and contributes to the pathogenesis of colitis. Eur. J. Immunol. **43:**1716–1726. http://dx.doi.org/10.1002/eji.201242889.
35. **Pils MC, Bleich A, Prinz I, Fasnacht N, Bollati-Fogolin M, Schippers A, Rozell B, Muller W.** 2011. Commensal gut flora reduces susceptibility to experimentally induced colitis via T-cell-derived interleukin-10. Inflamm. Bowel Dis. **17:**2038–2046. http://dx.doi.org/10.1002/ibd.21587.
36. **Hunter CA, Kastelein R.** 2012. Interleukin-27: balancing protective and pathological immunity. Immunity **37:**960–969. http://dx.doi.org/10.1016/j.immuni.2012.11.003.
37. **Pot C, Apetoh L, Awasthi A, Kuchroo VK.** 2011. Induction of regulatory Tr1 cells and inhibition of T(H)17 cells by IL-27. Semin. Immunol. **23:**438–445. http://dx.doi.org/10.1016/j.smim.2011.08.003.
38. **Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO.** 2009. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. Proc. Natl. Acad. Sci. U. S. A. **106:**19256–19261. http://dx.doi.org/10.1073/pnas.0812681106.
39. **Gazzinelli RT, Wysocka M, Hieny S, Scharton-Kersten T, Cheever A, Kuhn R, Muller W, Trinchieri G, Sher A.** 1996. In the absence of endogenous IL-10, mice acutely infected with Toxoplasma gondii succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. J. Immunol. **157:**798–805.
40. **Bertrand V, Quere S, Guimbaud R, Sogni P, Chauvelot-Moachon L, Tulliez M, Lamarque D, Charreire J, Giroud JP, Couturier D, Chaussade S, Breban M.** 1998. Effects of murine recombinant interleukin-10 on the inflammatory disease of rats transgenic for HLA-B27 and human beta 2-microglobulin. Eur. Cytokine Netw. **9:**161–170.
41. **Sarangi PP, Sehrawat S, Suvas S, Rouse BT.** 2008. IL-10 and natural regulatory T cells: two independent anti-inflammatory mechanisms in herpes simplex virus-induced ocular immunopathology. J. Immunol. **180:**6297–6306.
42. **Findlay EG, Greig R, Stumhofer JS, Hafalla JC, de Souza JB, Saris CJ, Hunter CA, Riley EM, Couper KN.** 2010. Essential role for IL-27 receptor signaling in prevention of Th1-mediated immunopathology during malaria infection. J. Immunol. **185:**2482–2492. http://dx.doi.org/10.4049/jimmunol.0904019.
43. **Villegas-Mendez A, de Souza JB, Lavelle SW, Gwyer Findlay E, Shaw TN, van Rooijen N, Saris CJ, Hunter CA, Riley EM, Couper KN.** 2013. IL-27 receptor signalling restricts the formation of pathogenic, terminally differentiated Th1 cells during malaria infection by repressing IL-12 dependent signals. PLoS Pathog. **9:**e1003293. http://dx.doi.org/10.1371/journal.ppat.1003293.
44. **Troy AE, Zaph C, Du Y, Taylor BC, Guild KJ, Hunter CA, Saris CJ, Artis D.** 2009. IL-27 regulates homeostasis of the intestinal CD4+ effector T cell pool and limits intestinal inflammation in a murine model of colitis. J. Immunol. **183:**2037–2044. http://dx.doi.org/10.4049/jimmunol.0802918.
45. **Hall AO, Beiting DP, Tato C, John B, Oldenhove G, Lombana CG, Pritchard GH, Silver JS, Bouladoux N, Stumhofer JS, Harris TH, Grainger J, Wojno ED, Wagage S, Roos DS, Scott P, Turka LA, Cherry S, Reiner SL, Cua D, Belkaid Y, Elloso MM, Hunter CA.** 2012. The cytokines interleukin 27 and interferon-gamma promote distinct Treg cell populations required to limit infection-induced pathology. Immunity **37:**511–523. http://dx.doi.org/10.1016/j.immuni.2012.06.014.
46. **El-behi M, Ciric B, Yu S, Zhang GX, Fitzgerald DC, Rostami A.** 2009. Differential effect of IL-27 on developing versus committed Th17 cells. J. Immunol. **183:**4957–4967. http://dx.doi.org/10.4049/jimmunol.0900735.
47. **Diveu C, McGeachy MJ, Boniface K, Stumhofer JS, Sathe M, Joyce-Shaikh B, Chen Y, Tato CM, McClanahan TK, de Waal Malefyt R, Hunter CA, Cua DJ, Kastelein RA.** 2009. IL-27 blocks RORc expression to inhibit lineage commitment of Th17 cells. J. Immunol. **182:**5748–5756. http://dx.doi.org/10.4049/jimmunol.0801162.
48. **Fitzgerald DC, Fonseca-Kelly Z, Cullimore ML, Safabakhsh P, Saris CJ, Zhang GX, Rostami A.** 2013. Independent and interdependent immunoregulatory effects of IL-27, IFN-beta, and IL-10 in the suppression of human Th17 cells and murine experimental autoimmune encephalomyelitis. J. Immunol. **190:**3225–3234. http://dx.doi.org/10.4049/jimmunol.1200141.
49. **Hirahara K, Ghoreschi K, Yang XP, Takahashi H, Laurence A, Vahedi G, Sciume G, Hall AO, Dupont CD, Francisco LM, Chen Q, Tanaka M, Kanno Y, Sun HW, Sharpe AH, Hunter CA, O'Shea JJ.** 2012. Interleukin-27 priming of T cells controls IL-17 production in trans via induction of the ligand PD-L1. Immunity **36:**1017–1030. http://dx.doi.org/10.1016/j.immuni.2012.03.024.
50. **Diegelmann J, Olszak T, Goke B, Blumberg RS, Brand S.** 2012. A novel role for interleukin-27 (IL-27) as mediator of intestinal epithelial barrier protection mediated via differential signal transducer and activator of transcription (STAT) protein signaling and induction of antibacterial and anti-inflammatory proteins. J. Biol. Chem. **287:**286–298. http://dx.doi.org/10.1074/jbc.M111.294355.
51. **Artis D, Villarino A, Silverman M, He W, Thornton EM, Mu S, Summer S, Covey TM, Huang E, Yoshida H, Koretzky G, Goldschmidt M, Wu GD, de Sauvage F, Miller HR, Saris CJ, Scott P, Hunter CA.** 2004. The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. J. Immunol. **173:**5626–5634.
52. **Zhou F, Ciric B, Li H, Yan Y, Li K, Cullimore M, Lauretti E, Gonnella P, Zhang GX, Rostami A.** 2012. IL-10 deficiency blocks the ability of LPS to regulate expression of tolerance-related molecules on dendritic cells. Eur. J. Immunol. **42:**1449–1458. http://dx.doi.org/10.1002/eji.201141733.
53. **Soehnlein O, Lindbom L.** 2010. Phagocyte partnership during the onset and resolution of inflammation. Nat. Rev. Immunol. **10:**427–439. http://dx.doi.org/10.1038/nri2779.