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Regulation of intestinal inflammation and barrier function by IL-17C

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Abstract

In the interleukin-17 (IL-17) family cytokines, much is known about the sources and functions of IL-17, IL-17F and IL-25 in host defense against infection and in inflammatory diseases, however, the physiological function of IL-17C remains poorly understood. Using mice deficient in IL-17C, here we demonstrate that this cytokine is crucial for the regulation of an acute experimental colitis elicited by dextran sulfate sodium (DSS). In this model, mice lacking IL-17C exhibited exacerbated disease that was associated with increased IL-17 expression by γδ T cells as well as Th17 cells. Moreover, IL-17C directly regulated the expression of the tight junction molecule occludin by colonic epithelial cells. Thus, our data thus suggest that IL-17C plays a critical role in maintaining mucosal barrier integrity.

Introduction

IL-17A, also known as IL-17, is the founding member of the IL-17 cytokine family that also includes IL-17B-F (1,2). Recently, a new subset of CD4+ T lymphocytes, termed Th17, was found to be a major source of the IL-17 and IL-17F cytokines (3,4). Both IL-17 and IL-17F act on multiple cell types to induce the production of mediators crucial for the inflammatory response (1). Furthermore, IL-17 and IL-17F are critical in the host defense and are linked to the development of multiple autoimmune inflammatory disorders, including MS and arthritis (5–7). In contrast, IL-25 (IL-17E) is critical for the promotion of Th2 and Th9 responses during allergy and parasitic infection (reviewed in 8). A lot less is known about the sources and functions of the remaining IL-17 family members. IL-17B and IL-17C were originally shown to induce pro-inflammatory gene expression in THP-1 cells (9) and CD4+ T cells engineered to overexpress IL-17B and IL-17C promoted arthritis (10). Moreover, IL-17C along with IL-17 and IL-17F was enhanced in the lungs of mice infected with *Mycoplasma pneumoniae* (11). Thus, the function of IL-17B and IL-17C may in fact be pro-inflammatory.

Both IL-17 and IL-17F signal through a complex of IL-17 receptor A (IL-17RA) and IL-17RC (12,13) whereas IL-17B and IL-25 signal through IL-17RB and IL-17RA (2). The receptor specific for IL-17C remained elusive until the recent discovery by our group and others that IL-17C binds IL-17RE (14–16). IL-17RE is selectively expressed in the lymphocyte compartment by Th17 cells and disrupting this signaling pathway via IL-17C-deficient mice resulted the amelioration of experimental autoimmune encephalomyelitis (EAE) incidence and severity (14). These results indicated that IL-17C-IL-17RE could

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function in adaptive immunity to regulate T cell function; however, the potential functions of IL-17C in the innate response were not clearly defined.

IL-17 family members have also been critically linked to intestinal immunity. Commensal flora, more specifically segmented filamentous bacteria, is pivotal for the development and maintenance of gut Th17 cells (17). Furthermore, the inflammation associated with dextran sulfate sodium (DSS)-induced colitis was enhanced in mice lacking IL-17. Conversely, IL-17F-deficient animals were protected against DSS-induced colon pathology (7). Two recent reports have also demonstrated a role for IL-17C in colon inflammation. Song and colleagues found that IL-17C could promote pro-inflammatory gene expression in colonic epithelial cells and that IL-17RE-deficient mice are unable to survive *Citrobacter rodentium* infection (15). Moreover, IL-17RE-deficient mice exhibited an exacerbation of DSS-induced colitis symptoms, although the function of IL-17C in this model was not addressed (16). Here we demonstrate a critical role for IL-17C in DSS-induced colitis where IL-17C−/− mice were highly susceptible to DSS-induced inflammation. Mechanistically, the colons of diseased IL-17C−/− mice were marked by high numbers of IL-17-producing γδ+ and CD4+ T cells. In addition, IL-17C was found to promote tight junction formation in colonic epithelial cells. Overall, our results demonstrate a novel function for IL-17C in mucosal barrier maintenance.

Materials and Methods

Mice

C57BL/6 (wild-type) mice were purchased from NCI. IL-17C−/− mice were generated and characterized as previously described (14). IL-17RE−/− mice were purchased from the Mutant Mouse Regional Resource Centers. 6–12 wk male mice were utilized for all experiments with protocols approved by the UT MD Anderson Institutional Animal Care and Use Committee.

DSS-induced colitis

DSS-induced colitis was performed as previously described (7). Briefly, male mice were administered drinking water containing 3.5% DSS for 5 d. Mice were monitored and weighed on a daily basis until experimental end point (d 8).

Colon mRNA and histological analysis

Whole colons from healthy and DSS-induced animals were equally divided into 3 sections: proximal, intermediate, and distal. For mRNA analysis, equal fractions from each section were combined and homogenized in Trizol (Invitrogen). RT-PCR reactions were performed using the MMLV system (Invitrogen). Real time PCR was performed using IQ SYBR Green (Bio-Rad Laboratories) on a CFX96 instrument (Bio-Rad). All gene quantities were normalized to the expression of the reference gene β-actin. Most primer pairs have been previously described (18). Other primers - occludin: F-ATTCCGGCCGCCAAGGTTCG and R- GCTGGCTGAGAGAGCATCGGC; claudin-1: F- ACTGCCCTGCCCCAGTGGAA and R- TCAGCCCCAGCAGGATGCCA; and claudin-4: F-TCGCGCTTGGTAGCTGGTGC and R- GATCCCCAGCCAGCCCAGGA. For histology, equal portions from each section were individually embedded in paraffin and then stained with H&E as previously described (7). Histological scores of colon tissue from proximal, intermediate, and distal sections were assessed using a scoring system according to Wirtz et. al (19).

Colon leukocyte and epithelial cell isolation and analysis

Resident epithelial cells and leukocytes infiltrating into the colon were isolated using a modified protocol previously described (20). Briefly, colons were washed 3X with HBSS/5% FBS/EDTA (Gibco) for 15 min at 37°C with vigorous mixing. For epithelial cells, healthy colon tissue was incubated with dispase (Gibco) for 1 hr and CD16/32^{-} and CD45^{-} cells were separated by AutoMACS (Miltenyi). For leukocyte isolation, colon tissue was digested for 1 hr at 37°C using 1.75 mg/ml Collagenase D (Roche) and leukocytes were purified on an LSM gradient (MP Biomedicals). Purified cells were washed and cultured with PMA, ionomycin (Sigma), and Golgistop (BD Biosciences) prior to staining and flow cytometric analysis using antibodies from BD. Occludin protein was analyzed by Western blotting of whole colon lysates following 8 d DSS treatment using antibodies from Invitrogen.

In vitro epithelial cell line analysis

The young adult mouse colon (YAMC) epithelial cell line was previously described (21). YAMC cells were maintained at 33°C in RPMI with 5 U/ml IFNγ (R & D Systems) and ITS (insulin-transferrin-selenium, Gibco). For induction experiments, YAMC cells were cultured in RPMI media without IFNγ and ITS at 37°C. YAMC cells were cultured with media alone or with a titration of IL-17C (R & D Systems) for 6 h prior to mRNA analysis as described above.

Results and Discussion

Our previous observation that IL-17C was induced in inflamed central nervous system tissue (14) led us to examine additional inflammatory disease models. We observed a significant increase of IL-17C mRNA in colons derived from DSS-treated animals (Fig. 1*A*), suggesting that IL-17C is a determinant for DSS-induced colon inflammation. Treatment of IL-17C-deficient mice with DSS led to substantial weight loss compared to WT controls (Fig. 1*B*). Interestingly, IL-17C−/− mice exhibited weight loss earlier than control animals (d 4 versus d 6, respectively) and retained a more pronounced decrease of starting weight until experimental endpoint (d 8). Colon shortening was observed in both groups; however, colons derived from IL-17C-deficient mice were considerably shorter (Fig. 1*C*). Analysis of H&E sections revealed severe wall thickening, extensive leukocyte infiltration, and loss of intestinal crypts and goblet cells in IL-17C−/− colons compared to WT controls (Fig. 1D and 1*E*). These results indicated that IL-17C along with IL-17RE (16) expression is protective against DSS colitis development.

Next, we investigated potential mechanisms involved in the aggravated colitis observed in IL-17C-deficient animals. We have reported that IL-17C can potentiate Th17 responses and that the loss of this cytokine leads to decreased pro-inflammatory T cell effector function in EAE (14). However, DSS-induced colitis is largely an innate disease model involving pro-inflammatory cytokine production from cells of both hematopoietic and non-hematopoietic origins (19,22). Colon analysis revealed increased mRNA expression of pro-inflammatory cytokines and chemokines in DSS IL-17C−/− mice compared to WT controls (Fig. 2*A*). Of note, IL-17, IL-6, RANTES, and CCL20 were increased where expression of other pro-inflammatory mediators was enhanced but failed to reach the level of statistical significance. No differences were observed for the expression of IL-10, IL-21, and IL-17F between the two groups (not shown). Surprisingly, we found an increase of the Th17-related mediators IL-17 and CCL20. IL-17, conversely, has been shown to be protective against the development DSS-induced colitis (7). Thus, IL-17C−/− mice display a similar DSS colitis phenotype as IL-17−/− animals, suggesting that increased DSS severity in IL-17C−/− is unrelated to elevated IL-17 expression.

To further characterize the intestinal T cell responses in IL-17C−/− animals, we directly isolated infiltrating leukocytes from the colons of diseased animals. We found substantial increases in total numbers of CD4+ T lymphocytes and CD11b+ monocytes infiltrating into the colons of IL-17C-deficient animals (Fig. 2*B* and *C*). The number of γδ T cells, however, remained unchanged between groups. Intracellular cytokine analysis revealed a strikingly high number of IL-17-producing cells in both the γδ and CD4+ T cell fractions in colons isolated from IL-17C−/− animals (Fig. 2*B* and *C*). The production of IFNγ was similar between groups and was only slightly enhanced compared to healthy controls, suggesting an influx of Th17 rather than Th1 cells in our system. It is important to note that the CD4+ cells infiltrating the colons of DSS mice were also CD3+ and not characteristic of lymphoid tissue inducer-like cells, which also have the ability to produce IL-17 (23) (Fig. 2*D*). Our previous results demonstrated that IL-17C promotes IL-17 production by T cells (14). Thus, in the DSS model, we believe that the observed enhancement of IL-17 responses in IL-17C −/− mice is a byproduct of exacerbated intestinal inflammation rather than a direct effect of IL-17C on T cells.

To investigate the mechanisms initiating inflammation and downstream IL-17 responses, we considered the possibility that IL-17C may be involved in barrier stability through tight junction formation. We examined the effect of IL-17C treatment on a colonic epithelial cell line, YAMC, which was found to express IL-17RE mRNA (Fig. 3*A*). IL-17C treatment of YAMC cells enhanced mRNA expression of occludin, claudin-1, and claudin-4 (Fig. 3*B*), which are involved in tight junction formation (24,25). Interestingly, IL-17, but not IL-17F, could induce the expression of the same tight junction mRNAs in YAMC cells, suggesting redundancy among IL-17 family cytokines in promoting epithelial stability (Supplemental Fig. 1). In addition to IL-17RE, YAMC cells also expressed IL-17RA and IL-17RC mRNA, further supporting the idea of redundancy (Supplemental Fig. 2A). In regard to IL-17C, in vitro treatment of primary colon epithelial cells resulted in enhanced occludin mRNA expression as well (Fig. 3*C*). These results indicated IL-17C-IL-17RE signaling indeed could play a role in mediating mucosal barrier stability.

To examine in vivo, we tested tight junction expression in colons isolated from DSS WT and IL-17C−/− mice. As expected, colon tissue expressed high levels of IL-17RA, IL-17RC, and IL-17RE; however, DSS treatment did not drastically change IL-17RE expression (Supplemental Fig. 2B). The expression of various claudins remained unchanged between healthy and sick animals (not shown). Conversely, the expression of occludin, a tight junction protein degraded as a result of DSS treatment (25), was greatly reduced in IL-17C−/− DSS colons compared to WT controls (Fig. 3*D*–*F*). These results collectively suggest that one function of IL-17C is to promote the formation of tight junctions under inflammatory conditions. Presumably, IL-17C is produced by colon epithelial cells and acts in an autocrine manner (15,16) to mediate barrier stability. The loss of IL-17C renders mice more susceptible to mucosal barrier breakage and the development of colitis. At this time, we cannot rule out a role of IL-17C in mediating pro-inflammatory mediator expression by epithelial cells as was recently described (15,16). However, the loss of IL-17C and the subsequent decreased expression of inflammatory cytokines could potentially lead to DSS protection rather than increased susceptibility. Thus, future studies will be required to not only investigate the role IL-17C in regulating intestinal inflammation but also to further characterize the mechanisms promoting mucosal barrier integrity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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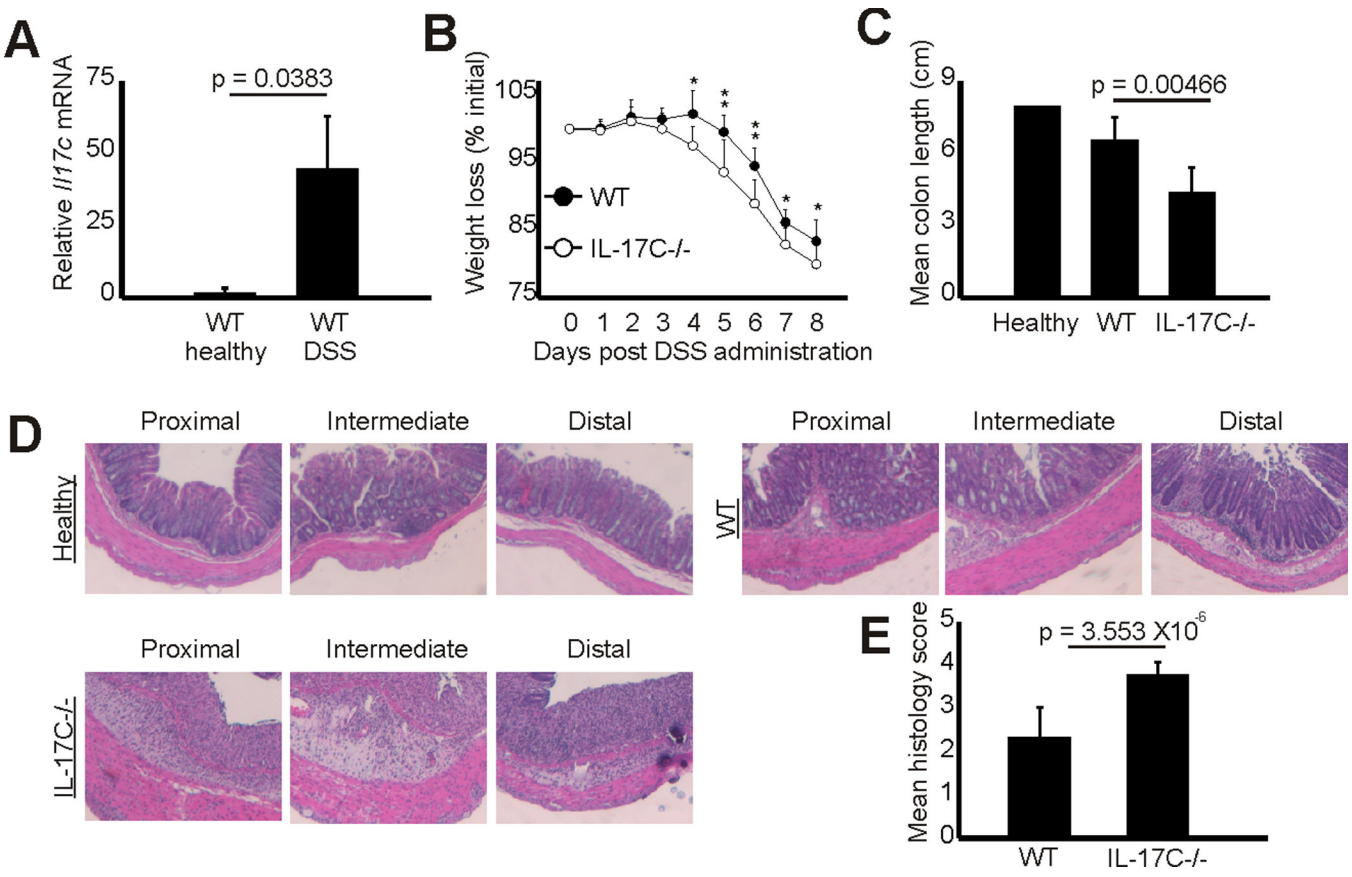

Figure 1.

IL-17C deficiency results in exacerbated DSS-induced colitis. (*A*) WT colon tissue samples were harvested from healthy animals (n = 4) or animals after 8 d DSS treatment (n = 16) and then analyzed for the expression of IL-17C mRNA by real time PCR. (*B*) Pooled weight loss data from WT (n = 15) and IL-17C−/− (n = 14) mice following 3.5% DSS administration. (*C*) Representative colon length data from WT (n = 6) and IL-17C−/− (n = 5) animals presented in (*B*). (*D*) Representative H&E staining from healthy colons and those following DSS treatment. (*E*) Histological scoring compilation from the mice presented in (*D*). n = 5 mice per group. Data are presented as mean + SD and are representative of at least 5 independent experiments. p = Students t test, * $p < 0.02$, ** $p < 0.0002$.

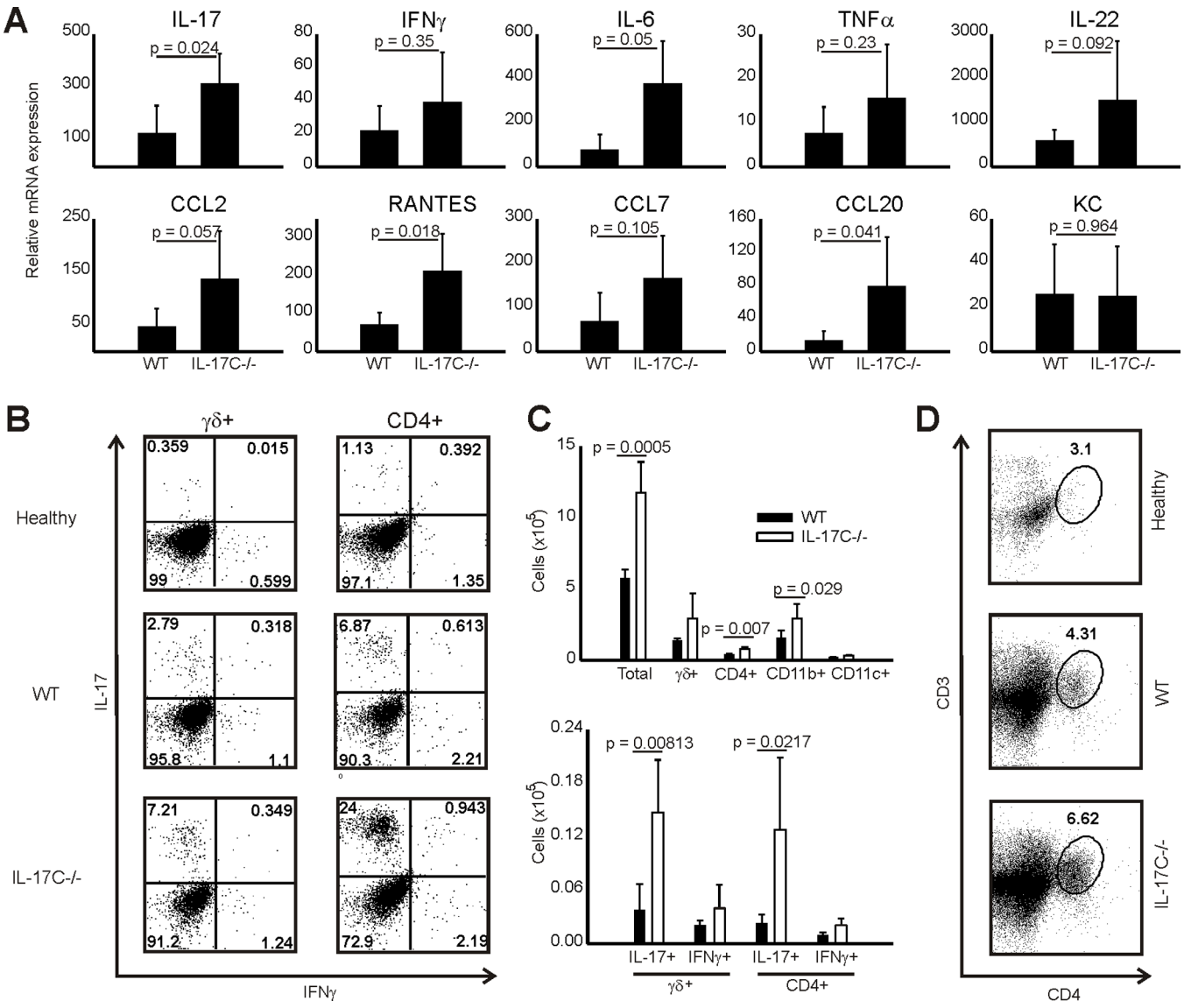

Figure 2.

Increased pro-inflammatory mediator production in IL-17C−/− colons. (*A*) Proximal, intermediate, and distal colon sections from DSS animals were pooled prior to mRNA isolation and gene quantification by real time PCR. n = 5 animals per group. (*B*). Representative staining of colon-derived lymphocytes isolated from DSS-induced colitis animals in (*A*). For cytokine analysis, colon cells were stimulated 5 h with PMA, ionomycin, and brefeldin A prior to intracellular staining. (*C*) Summary of the infiltration and cytokine staining results from colon tissues presented in (*B*). Data are presented as total cell numbers. (*D*) Colon-derived cells from DSS animals were gated as CD11c- before the analysis of CD3 and CD4 expression. Data are presented as mean + SD and are representative of 3 independent experiments. p = Students t test.

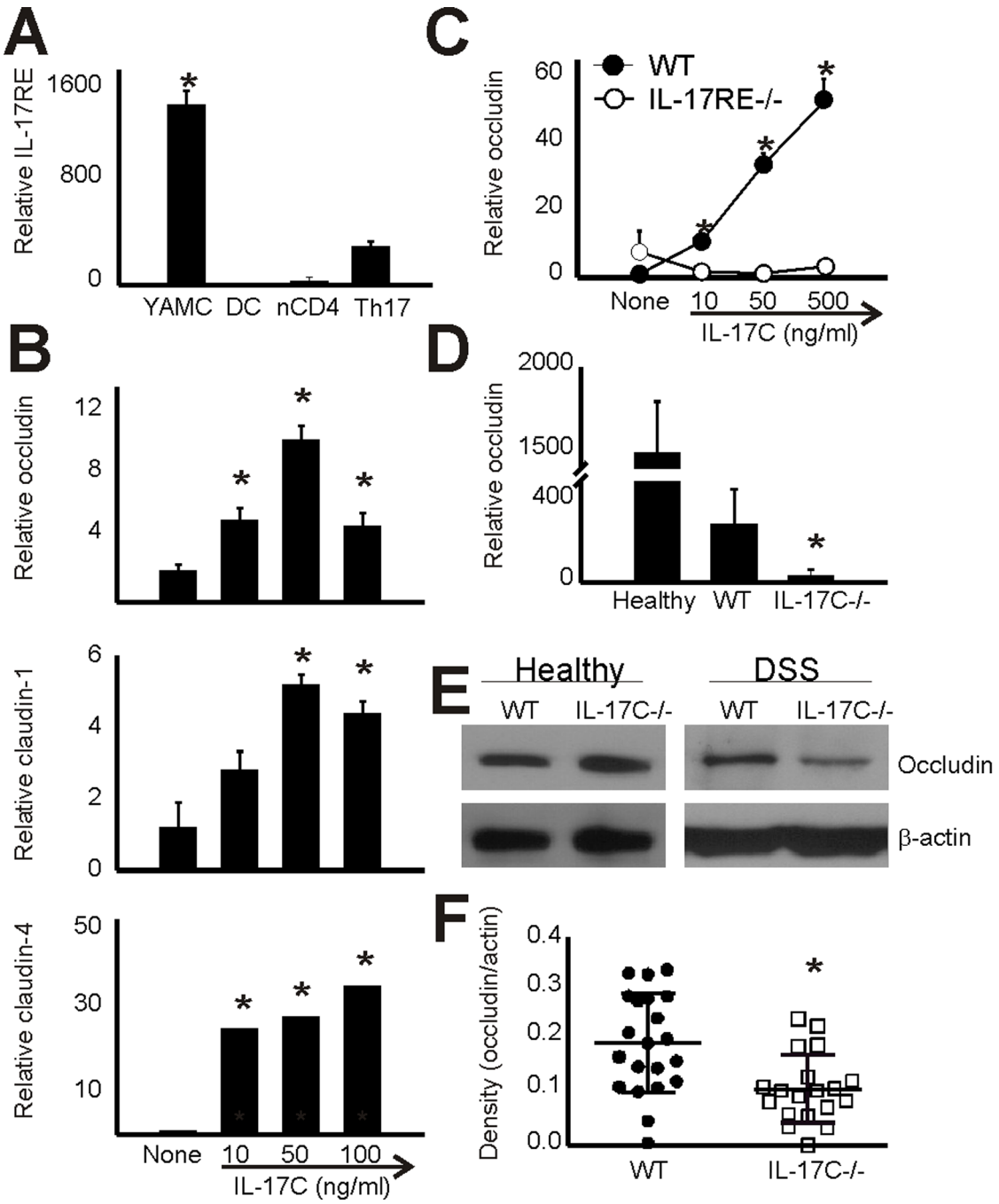

Figure 3.

IL-17C-IL-17RE signaling promotes tight junction protein expression. (*A*) YAMC cells were examined for IL-17RE expression by real-time RT-PCR. Bone marrow-derived dendritic cells (DC), naïve CD4+ T cells (nCD4), and Th17 cells were utilized as expression controls. (*B*) YAMC cells were analyzed for mRNA expression of various tight junction proteins by real-time RT-PCR. Cells were stimulated with media alone or a titration of IL-17C for 6 h before mRNA quantification. (*C*) Primary colon epithelial cells were isolated and stimulated +/− IL-17C 16 h before mRNA analysis. (*D*) Colon tissue samples from DSS WT (n = 5) and IL-17C−/− (n = 5) animals were analyzed for the expression of occludin by real-time RT-PCR. Data are presented as mean + SD for duplicate determinations. (*E*)

Representative blots for occludin protein from total colons derived from healthy and DSS WT and IL-17C−/− animals. (*F*) Summary of occludin blots from DSS animals presented as the ratio of occludin:β-actin densities. n = 19–21 colon samples isolated from 4 independent experiments per group. * = Students t test, $p < 0.05$ compared to DC control (*A*), unstimulated controls (*B* + *C*), WT control (*D* + *F*).