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# IL-33 signaling protects from murine oxazolone colitis by supporting intestinal epithelial function

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## Abstract

**Background—**IL-33, a member of the IL-1 cytokine family that signals through ST2, is upregulated in ulcerative colitis (UC); however, the role of IL-33 in colitis remains unclear. IL-33 augments type 2 immune responses, which have been implicated in UC pathogenesis. We sought to determine the role of IL-33 signaling in oxazolone (OXA) colitis, a type 2 cytokine-mediated murine model of UC.

**Methods—**Colon mucosal IL-33 expression was compared between pediatric and adult UC and non-IBD patients using immunohistochemistry and real-time PCR. OXA colitis was induced in

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WT, IL-33$^{-/-}$ and ST2$^{-/-}$ mice, and histopathology, cytokine levels, and goblet cells were assessed. Transepithelial resistance (TER) was measured across IL-33-treated T84 cell monolayers.

**Results—**Colon mucosal IL-33 was increased in pediatric patients with active UC and in OXA colitis. IL-33$^{-/-}$ and ST2$^{-/-}$ OXA mice exhibited increased disease severity compared to WT OXA mice. OXA induced a mixed mucosal cytokine response, but few differences were observed between OXA WT and IL-33$^{-/-}$ or ST2$^{-/-}$ mice. Goblet cells were significantly decreased in IL-33$^{-/-}$ and ST2$^{-/-}$ OXA compared to WT OXA mice. IL-33 augmented TER in T84 cells, and this effect was blocked by the ERK1/2 inhibitor PD98,059.

**Conclusions—**OXA colitis is exacerbated in IL-33$^{-/-}$ and ST2$^{-/-}$ mice. Increased mucosal IL-33 in human UC and murine colitis may be a homeostatic response to limit inflammation, potentially through effects on epithelial barrier function. Further investigation of IL-33 protective mechanisms would inform the development of novel therapeutic approaches.

**Keywords**

ulcerative colitis; inflammatory bowel disease; interleukin-33; epithelial cells; oxazolone

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Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is marked by chronic inflammation of the intestine caused by dysregulation of the mucosal immune response and epithelial barrier dysfunction. While advances in our understanding of cytokine biology have directly led to effective therapies for IBD, only 25% of patients with moderate to severe UC achieve sustained disease remission with the currently available anti-TNF biologics and 30% of patients with severe UC ultimately require total colectomy.[1,2] Therefore, further investigations to elucidate the roles of newly emerging cytokines are warranted to develop novel therapeutic strategies.

IL-33 is a member of the IL-1 cytokine family that signals through the IL-1 receptor related protein ST2[3,4] leading to downstream activation of MAP kinase and NF-κB pathways.[3,4] IL-33 is often described as an alarmin released by epithelial and endothelial cells in the setting of injury, but it is also expressed by innate immune cells.[5–7] The IL-33 receptor, ST2 is ubiquitously expressed by myeloid and lymphoid cells, granulocytes, epithelial and endothelial cells, and fibroblasts.[5] The diverse expression of the IL-33 receptor may explain the reported pleiotropic effects of IL-33 across multiple cell types and disease models.[3] IL-33 potently induces type 2 cytokines from T helper and innate lymphoid cells (ILCs),[3,8] which while pathogenic in allergic airway disease,[9–11] is critical for explusion of intestinal parasites.[8,12,13] IL-33 has also been reported to promote anti-viral responses by acting on cytotoxic effector T cells,[14] enhance allograft survival by directly inducing proliferation of regulatory T cells,[15] worsen allergic airway inflammation by inducing alternative activation of macrophages,[9] and exacerbate antigen-induced arthritis by activating mast cells.[16]

Multiple studies have now demonstrated that IL-33 is induced in the mucosa of adult patients with UC and to a lesser extent in CD, and an *IL33* polymorphism has been associated with UC and extensive colitis phenotype.[17–21] However, the primary role of IL-33 in colitis remains elusive as mechanistic studies in various animal models have

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indicated both protective and pathogenic effects.[19,22–25] Given that type 2 cytokines have been implicated in the pathogenesis of UC, and IL-33 has an established role in inducing type 2 inflammation, we sought to determine the role of IL-33 in oxazolone (OXA) colitis, a type 2 cytokine-mediated murine model of colitis with pathologic and immunologic features similar to UC.[26] We previously demonstrated a STAT6-dependent increase in IL-33 gene expression in OXA colitis correlating with histopathologic severity and hypothesized that IL-33 would have a pathogenic role in OXA colitis by promoting Th2 inflammatory responses.[27] In the present study, we observed increased mucosal IL-33 expression in both pediatric and adult UC. Surprisingly, we demonstrated increased severity of OXA colitis in both IL-$33^{-/-}$ and $ST2^{-/-}$ mice compared to WT mice indicating a primary protective role for IL-33 in this model. We observed no influence of IL-33 or ST2 genetic deletion on tissue cytokine levels, suggesting the protective role of IL-33 may be related to effects on the epithelium. In fact, in the setting of colitis, IL-$33^{-/-}$ and $ST2^{-/-}$ genetic deletion resulted in a substantial loss of goblet cells. Furthermore, we observed that IL-33 has a direct effect on augmenting epithelial barrier function *in vitro*, providing insight into the mechanism of the protective functions of IL-33.

## Materials and Methods

### Human Samples

Archived paraffin-embedded tissue sections of endoscopic rectal biopsies from pediatric patients with active UC and non-IBD controls were accessed from the Cincinnati Children's Hospital Medical Center (CCHMC) Pathology Research Core. Prospectively collected endoscopic rectal biopsy tissue from pediatric with UC and non-IBD controls enrolled at CCHMC were preserved RNALater at −80°C. The pediatric patients studied ranged in age from 6 to 18 years, with a mean age of 14 years. Prospectively collected endoscopic biopsy tissue from adult patients with UC and non-IBD controls at Vanderbilt University Medical Center (VUMC) were snap frozen at endoscopy and stored at −80°C. The adult patients ranged in age from 22 to 66 years, with a mean age of 45 years.

### Immunohistochemical Staining

Briefly, sections were deparaffinized, rehydrated, and antigen was unmasked in a high pH buffer (Vector Labs, Burlingame, CA). Sections were stained with goat anti-human or anti-mouse IL-33 antibodies (AF3625 and AF3626, R&D Systems, Minneapolis, MN). An isotype goat polyclonal IgG negative control was used to test for the specificity of the involved antibody at matched concentration and incubation conditions (Jackson Immunoresearch, West Grove, PA). Sections were counterstained with Mayer's hematoxylin.

Sections were also stained with anti-Ki67 (790-4286, Ventana, Tuscon, AZ) or phospho-histone H3 (9701S, Cell signaling, Danvers, MA) to quantify proliferation. Apoptotic epithelial cells were identified based on positive TUNEL staining (*In Situ* Cell Death Detection Kit, Roche, Indianapolis, IN).

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## Immunofluorescence

For immunofluorescent staining of IL-33 with F4/80 on colon sections, sections were deparaffinized, rehydrated, and antigen was unmasked in a high pH buffer (Vector Labs, Burlingame, CA). Sections were stained with goat anti-mouse IL-33 (AF3626, R&D Systems, Minneapolis, MN) followed by anti-goat-Alexa Fluor-594 (Life Technologies, Carlsbad, CA) with anti-F4/80-FITC (11-4801-11, eBioscience, San Diego, CA) and sections were counterstained with Vectashield with DAPI (Vector Laboratories, Burlingame, CA).

T84 cells grown on coverslips were subsequently fixed with 4% paraformaldehyde for 30 minutes. Cells were blocked with goat serum and then stained with rabbit anti-ST2 (ab25877, AbCam, Cambridge, MA) followed by goat anti-rabbit-Alexa Fluor488 (Jackson Immunoresearch, West Grove, PA), and Alexa Fluor635-Phalloidin (Life Technologies, Carlsbad, CA) and counterstained with Vectashield with DAPI (Vector Laboratories, Burlingame, CA).

## Mice

IL-33$^{-/-}$, ST2$^{-/-}$, and wild type (WT) C57BL/6 mice and were bred at CCHMC and VUMC under specific pathogen free conditions. The generation of the IL-33$^{-/-}$ and ST2$^{-/-}$mice were previously described.[28,29] This study was carried out following recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Vanderbilt University and CCHMC Institutional Animal Care and Use Committees approved the protocol.

## Histopathology

Mouse colon sections were stained with hematoxylin and eosin and analyzed by light microscopy. A pathologist (M.K.W.) blinded to genotype and treatment scored each mouse using a previously described 15-point scale, with a maximum of 3 points each for enterocyte loss, crypt hyperplasia, crypt inflammation, neutrophil infiltrate, and mononuclear cell infiltrate.[30] Mouse colon sections were stained with periodic acid Schiff (PAS) and staining was quantified using a modified nuclear algorithm with Aperio Imagescope software (Buffalo Grove, IL). In brief, positive (yellow) and negative (blue) regions are segmented based upon color. The algorithm returns the number of positive and negative nuclei, as well as the average size and intensity of each type. The percent positive is calculated based upon both nuclear counts and area ratio.

## Oxazolone Colitis

Anesthetized 5–7 week-old male mice were sensitized by topically applying 3% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; Sigma-Aldrich, Saint Louis, MO) in 100% ethanol (150 μL) on the shaved abdomen. Seven days later, 1.5% oxazolone in 50% ethanol (150 μL) was administered intrarectally with a 5 French plastic infant feeding tube (C. R. Bard, Covington, GA). Control mice were treated with ethanol vehicle alone. Mice were sacrificed 3 days after rectal oxazolone administration.

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### Tissue Cytokines

Mouse colon tissues were suspended in 400 uL CelLytic MT lysis buffer (Sigma-Aldrich, St. Louis, MO) with protease inhibitors (EMD Millipore, Billerica, MA) and homogenized at 50Hz for 2 minutes with a stainless steel bead using a TissueLyser LT (Qiagen, Germantown, MD). Homogenates were incubated on ice for 10 minutes prior to a 7 minute, 14,000 × *g* centrifugation at 4°C. Total protein concentration of the resulting supernatants was determined by colorimetric protein assay and aliquots were stored at -80°C. Cytokine concentrations (G-CSF, GM-CSF, IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, IP-10, KC, MCP-1, MIP-1α, MIP-1β, MIP-2, RANTES, TNFα) were determined by multiplex analysis using a Milliplex MAP Mouse Magnetic Bead Panel (EMD Millipore, Billerica, MA). IL-13 was not detected by Luminex assay and an ELISA was used (eBioscience, San Diego, CA). Tissue lysate concentrations were normalized to total protein concentrations.

### RNA Expression

RNA was isolated from tissue using the RNeasy Mini Kit (Qiagen, Valencia, CA) per the manufacturer's instructions. RNA (1 μg) was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Real-time PCR was performed with TaqMan Gene Expression Assays (Life Technologies, Carlsbad, CA) for *IL33* (Hs01125942_m1),*GAPDH* (Hs02758991_g1), *Muc2* (Mm01276696_m1) and *Gapdh* (Mm99999915_g1). All reactions were performed on a StepOnePlus real-time PCR system (Life Technologies, Carlsbad, CA). Relative mRNA levels were determined using the $2^{-\ \ CT}$ method with *Gapdh* as the reference.

### Cell Culture

T84 cells were grown in 1:1 DMEM/Ham's F-12 medium containing 5% fetal calf serum. All media contained 100 U/mL penicillin, and 100 μg/mL streptomycin. Cultures were incubated at 37°C in a 95% air/5% $CO_2$ atmosphere. Cells were treated with 10 ng/mL human IL-33 (R&D systems, Minneapolis, MN), 10 ng/mL IL-13 (Peprotech, Rocky Hill, NJ), 6.25-25 μM PD 98,059 (Sigma Aldrich, St. Louis, MO).

For measurements of transepithelial resistance (TER), T84 cell monolayers were grown on standing mixed cellulose esters membrane inserts (Millipore, Billerica, MA). TER across each monolayer was assessed by using a voltmeter (WPI, Sarasota, FL). Measurements were calculated in $\Omega \cdot cm^2$ and expressed as a percentage of baselines. For all experiments, baseline TER was > 1000 $\Omega \cdot cm^2$.

### *Ex Vivo* Intestinal Permeability

Colon was mounted in Ussing chambers and after 15 min stabilization, baseline transepithelial resistance (TER) was measured. FITC-dextran (2.2 mg/mL, 4.4 kDa; Sigma-Aldrich, St. Louis, MO) was added to the luminal bath, and medium (0.25 ml out of 10 ml) was removed from the serosal reservoir and replaced with fresh medium every 20 min over a period of 180 min for measurement of FITC-dextran translocation.[31]

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### Western Blot Analysis

Membranes were blocked with 5% nonfat dry milk in TBS-Tween (0.05%) for 1 hour and incubated overnight at 4°C with primary antibodies against tubulin (Santa Cruz Biotechnology, Santa Cruz, CA), Phospho-p44/42 MAPK (ERK1/2), Phospho-SAPK/JNK, Phospho-p38, and Phospho-NFκB (all from Cell Signaling, Danvers, MA). Membranes were incubated with HRP-linked secondary antibodies, anti-rabbit- or anti-mouse and visualized using ECL Prime (RPN2232, GE Healthcare, Piscataway, NJ) and FujiFilm LAS-4000 Gel Documentation system (GE Healthcare, Piscataway, NJ).

### Statistical Analysis

For each set of experiments yielding continuous data in three or more groups, one-way ANOVA was applied as a global test for differences in the primary outcome variable. Histopathologic scoring, as an ordinal variable, was compared globally among groups using the nonparametric Kruskal–Wallis test. Predetermined pair-wise comparisons of interest were made only when an overall effect was detected using the Student t test or Mann–Whitney U test followed by Hochberg procedure to adjust for multiple comparisons. Repeated-measures two-way ANOVA with Bonferroni correction was used to compare measurements of mouse weight and epithelial membrane TER over time among experimental groups.

For the multiplex luminex analysis, one-way ANOVA was applied after log transformation. For analytes with a false discovery rate (FDR)-adjusted significance by ANOVA, individuals comparisons were between groups made using the Student T test, with *P*-values again adjusted for FDR. Correlation between analytes were assessed using the Pearson correlation coefficient. After removal of highly correlated analytes ($r > 0.85$), a subset of analytes with differences detected by ANOVA were entered into a principal component analysis (PCA). Statistical analyses were performed using Prism 5.0b (GraphPad Software, La Jolla, CA) and R 3.0.0 (R Working Group, http://www.r-project.org/).

### Ethical Considerations

Pediatric and adult patients were enrolled through protocols approved by the Institutional Review Boards at CCHMC and VUMC, respectively. Adult biopsies specimens were obtained as a part of the study “Effects of L-Arginine in Colitis and Colon Cancer” (clinicaltrials.gov NCT01091558). Animal studies were carried out following recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by both the Vanderbilt University Medical Center and Cincinnati Children’s Hospital Medical Center Institutional Animal Care and Use Committees.

## Results

### IL-33 expression in UC

Several studies have now demonstrated that mucosal IL-33 expression is increased in adult patients with UC compared to healthy patients, but expression of IL-33 in pediatric IBD has not been reported.[17–20] We performed immunohistochemistry for IL-33 on rectal endoscopic

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biopsy tissue from children with UC and non-inflamed control patients to examine cellular expression and localization of IL-33 in pediatric UC. In non-inflamed rectal tissue from children without IBD, we observed nuclear IL-33 expression in endothelial cells, with limited expression in lamina propria immune cells (Fig. 1A). In UC patients, we observed pronounced increased nuclear and cytoplasmic expression in epithelial and lamina propria immune cells compared to non-inflamed controls (Fig. 1A). We quantified IL-33 mRNA expression by real-time PCR in pediatric rectal biopsies (Fig. 1B), and found increased *IL33* in pediatric UC patients compared to non-IBD controls (Fig 1B). In addition, we similarly observed increased mucosal IL-33 expression in rectal biopsies of adult patients with UC compared to healthy controls (Fig. 1C).

### IL-33 expression in OXA colitis

Previously, we determined that colon mucosal IL-33 mRNA expression is increased in OXA colitis and correlates with histopathologic severity.[27] In the current study, we examined tissue localization of IL-33 protein in WT ETOH and OXA mice. IHC analyses demonstrated that IL-33 is increased in OXA colitis compared to ETOH-treated mice (Fig 2A). IL-33 localized to both epithelial cells and cells in the lamina propria, with both nuclear and cytoplasmic staining (Fig. 2B). To further elucidate the source of IL-33 in the lamina propria, we performed IF analyses for F4/80 and IL-33. We demonstrate that IL-33 colocalizes with F4/80+ myeloid-derived cells in the lamina propria of OXA- treated mice (Fig. 2C).

### Increased severity of OXA colitis in IL-33$^{-/-}$ and ST2$^{-/-}$ mice

To determine the role of IL-33 *in vivo*, we induced colitis with OXA in WT, IL-33$^{-/-}$ and ST2$^{-/-}$ mice. IL-33$^{-/-}$ and ST2$^{-/-}$ mice receiving ETOH enemas did not lose weight or develop significant disease pathology compared to WT mice (Fig. 3). While WT, IL-33$^{-/-}$ and ST2$^{-/-}$ mice treated with OXA lost similar weight by Day 1, weight recovery was significantly impaired in IL-33$^{-/-}$ and ST2$^{-/-}$ mice compared to WT mice (Fig. 3A). Colon histopathologic examination of WT OXA mice at Day 3 revealed mild expansion of the lamina propria with neutrophils and mononuclear cells, cryptitis, and regenerative epithelial changes compared to WT ETOH mice. In contrast to WT OXA mice, both IL-33$^{-/-}$ and ST2$^{-/-}$ mice exhibited more marked expansion of the lamina propria, epithelial regenerative changes, and crypt distortion (Fig. 3B). Reflective of these findings, median histologic scores were increased in IL-33$^{-/-}$ and ST2$^{-/-}$ mice (6 and 6 respectively) compared to WT mice treated with OXA (3.5,, $P < 0.05$ vs. IL-33$^{-/-}$ and ST2$^{-/-}$ mice, respectively, Fig. 3C).

### Deletion of IL-33 or ST2 does not alter the mucosal cytokine response in OXA colitis

Since IL-33 can induce cytokine production by lymphoid cells, we examined tissue cytokine levels by multiplex assay. Global differences were detected by ANOVA for 15 of 25 cytokines assayed (Fig. S1). Several of these 15 analytes were highly correlated with each other including IL-6 and G-CSF (r = 0.98), MIP-1$\alpha$ and MIP-1$\beta$ (r = 0.91), and IL-1$\beta$ and TNF-$\alpha$ (r = 0.88). Therefore, we performed PCA with the subset of 12 significant cytokines (after eliminating 3 highly correlated analytes) to determine if experimental groups could be distinguished by tissue cytokine response. While PCA clearly distinguished mice treated

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with ETOH versus those treated with OXA, it did not distinguish the WT OXA, IL-33$^{-/-}$ OXA and ST2$^{-/-}$ OXA mice (Fig. 4A). Both type 1 and type 2 cytokines, and multiple pro-inflammatory chemokines were increased in OXA colitis compared to ETOH (Fig. 4B, Fig. S1). Although IL-33 has a known role in promoting type 2 immune responses, we observed no differences in type 2 cytokines between WT OXA and IL-33$^{-/-}$ or ST2$^{-/-}$ OXA (Fig. 4, Fig. S1). Although we did not observe histological differences between ST2$^{-/-}$ and IL-33$^{-/-}$ OXA mice, IL-17A and IL-15 were increased in ST2$^{-/-}$ OXA mice compared to IL-33$^{-/-}$ OXA mice (Fig. S1). Notably, these cytokines were not increased in WT OXA mice compared to WT ETOH mice. Collectively, these data suggest that the observed protective effect of IL-33 is not mediated by alterations in the mucosal cytokine milieu.

### Deficiency of IL-33 or ST2 induces goblet cell depletion in the setting of OXA colitis

Since we observed no consistent influence of IL-33 and ST2 deficiency on inflammatory cytokines in OXA colitis, and exogenous IL-33 has effects on intestinal epithelium *in vivo*,[3] we examined the effect of IL-33 and ST2 deficiency on the colon epithelium in the setting of colitis. Injection of IL-33 induces goblet cell hypertrophy and hyperplasia in WT mice.[3] Thus, we performed PAS staining (Fig. 5A) with software quantification (Fig. 5B) to assess goblet cells in IL-33$^{-/-}$ and ST2$^{-/-}$ in the setting of colitis. While WT, IL-33$^{-/-}$ and ST2$^{-/-}$ mice treated with ETOH control exhibited similar percentages of PAS+ goblet cells in the colon, there were 60 and 55% decreases in PAS+ goblet cells in IL-33$^{-/-}$ and ST2$^{-/-}$ mice, respectively, compared to WT mice in the setting of OXA colitis (Fig 5A, B). Consistent with the decrease in mucin-secreting goblet cells, we observed significantly decreased mucosal Muc2 mRNA expression in ST2$^{-/-}$ and IL-33$^{-/-}$ OXA mice compared to WT OXA (Fig. 5C).

To further examine the effect of IL-33 and ST2 deficiency on the colon epithelium in the setting of colitis, we assessed epithelial proliferation and apoptosis by PH3 and TUNEL staining, respectively. We observed no differences between WT OXA and IL-33$^{-/-}$ and ST2$^{-/-}$ OXA mice (Fig S2).

### IL-33 signaling augments epithelial barrier function *in vitro* and *in vivo*

Since impaired barrier function has been implicated in the pathogenesis of UC,[32] we examined whether IL-33 exerts a direct effect on epithelial barrier function using T84 colon epithelial cell monolayers. Immunofluorescence staining demonstrated the presence of ST2 at the cell membrane of T84 cells, suggesting capacity for IL-33-induced signaling in these cells (Fig. 6A). T84 cells were plated on semi-permeable transwells and treated with IL-33 (10 ng/mL) for 48 hours. Mean TER as a percentage of baseline was significantly greater in IL-33-treated cells at 12, 24 and 48 hours compared to PBS-treated cells. As a control we also treated cells with IL-13 and observed the expected decrease in TER at 24 and 48 hours (Fig 6B). To determine the intracellular mechanisms mediating IL-33 augmentation of TER in T84 cells, we examined signaling pathway activation downstream of IL-33 by Western Blot analysis. Others have previously reported activation of MAPK and NF-κB signaling in HEK293 cells.[3] We found that IL-33 induced phosphorylation of ERK1/2, but did not significantly induce the phosphorylation of JNK, p38 or NF-κB p65 in T84 colon epithelial

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cells (Fig. 6C). Densitometry revealed a 2.6-fold increase in phospho-ERK1/2 after 60 minutes of activation with IL-33 (Fig. 6D).

We next determined the role of ERK1/2 signaling on IL-33 induced alterations in barrier function *in vitro*. The ERK1/2 inhibitor, PD98,059, significantly inhibited ERK1/2 activation at concentrations ranging from 6.25 – 25μM (Fig 6E). When added to T84 monolayers exposed to IL-33, PD98,059 effectively inhibited IL-33-induced augmentation of TER (Fig 6F), indicating that ERK1/2 signaling downstream of IL-33 is important for IL-33-induced augmentation of TER.

To link our *in vitro* findings that IL-33 augments barrier function in colon epithelial cells back to the murine model, we used Ussing chambers to compare baseline colon barrier function between WT and ST2$^{-/-}$ mice *ex vivo*. We observed significantly decreased colon TER in ST2$^{-/-}$ mice compared to WT mice (Fig 6G). Furthermore, FITC-dextran permeability was increased in ST2$^{-/-}$ mice compared to WT, indicating a role for IL-33 signaling in maintaining colon barrier function *in vivo* (Fig 6H).

## Discussion

In the present study, we demonstrate that deficiency of either IL-33 or it's receptor, ST2, exacerbates OXA colitis in mice, indicating a protective role for endogenous IL-33 in colitis. Furthermore, we provide the first description to our knowledge of upregulated mucosal IL-33 protein and gene expression in pediatric UC patients. Despite a known role for IL-33 in inducing pro-inflammatory cytokines, we observed few effects of IL-33 or ST2 deficiency on the mucosal cytokine milieu in OXA colitis. Rather, we demonstrate protective effects of IL-33 on the epithelium including preservation of goblet cells and *Muc2* expression in the setting of colitis and augmentation of epithelial barrier function *in vitro*.

Our findings support those of others demonstrating a protective role for IL-33 during experimental colitis.[23,24,33] In the only other report of experimental colitis in IL-33$^{-/-}$ mice, while IL-33 deficiency conferred modest protection from acute dextran sulfate sodium(DSS)-induced colitis, IL-33$^{-/-}$ mice exhibited delayed weight recovery compared WT mice.[29] Similarly, administration of recombinant IL-33 during the recovery phase of acute DSS-induced colitis enhances mucosal healing, and IL-33 attenuates chronic DSS-induced colitis, possibly through inhibition of the Th1 response.[23] Other groups have reported that IL-33 protects from experimental colitis by acting on ST2$^{+}$ regulatory T cells to induce their proliferation and suppressive function.[24,33]

OXA colitis was originally described as driven by type 2 inflammation;[26,34] however, we and others have observed a mixed cytokine response.[27,35] IL-33 is known to support type 2 immune responses[3,36] and we previously reported increased mucosal IL-33 expression in OXA colitis correlating with histopathologic severity.[27] Furthermore, *ex vivo* stimulation of MLN T cells from mice with OXA and IL-10$^{-/-}$ colitis with IL-33 has been shown to increase type 2 cytokine production.[27,37] Interestingly, however, we did not observe any changes in the type 2 mucosal cytokine response associated with exacerbation of OXA colitis in IL-33$^{-/-}$ and ST2$^{-/-}$ mice. While several groups have demonstrated that systemic

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administration of recombinant IL-33 potently induces type 2 cytokines, others have shown that endogenous IL-33 signaling is not required to generate type 2 immune responses.[3,36,38–40] This apparent discrepancy may be explained by redundant roles of other cytokines. For example, both IL-33 and IL-25 induce type 2 ILCs in the gut, but, in the setting of helminth infection, only absence of both receptors prevents ILC induction and worm expulsion.[8]

Given the absence of consistent changes in the mucosal cytokine response associated with exacerbation of OXA colitis in both IL-33$^{-/-}$ and ST2$^{-/-}$, we directed our investigations toward the effect of IL-33 on the intestinal epithelium. The initial report of the identification of IL-33 demonstrated that administration of exogenous recombinant IL-33 to mice results in intestinal goblet cell hypertrophy and hyperplasia.[3] Accordingly, we observed that in the setting of colitis, IL-33$^{-/-}$ and ST2$^{-/-}$ mice exhibited marked goblet cell depletion associated with decreased mucosal *Muc2* expression compared to WT OXA mice. Similarly, others observed that exogenous IL-33 given during DSS-induced colitis prevented the loss of goblet cells through suppression of Notch signaling, although it actually exacerbated colitis.[22] Previous studies demonstrated that IL-33-induced goblet cell hyperplasia is dependent on IL-13 *in vivo*;[3,38] however we did not observe that deficiency of either IL-33 or ST2 influenced IL-13 levels in oxazolone colitis. Further studies are necessary to determine whether IL-33 can directly affect goblet cell differentiation or mucus production. Our findings support the concept that elevations of endogenous mucosal IL-33 in response to colitis may serve a protective role by preserving goblet cells.

To further elucidate the mechanisms by which IL-33 is protective, we assessed the effect of IL-33 signaling on colon epithelial barrier function both *in vitro* and *ex vivo*. We demonstrated that T84 cells express ST2, and IL-33 acts directly on colon epithelial cells to augment TER in an ERK-dependent fashion. ERK1/2 signaling is known to promote increased tight junction expression in various cell types, including T84 cells.[41] For example, TGF-β increases T84 barrier function *in vitro*, and a probiotic mixture increases intestinal barrier function *in vivo* through an ERK1/2-dependent mechanism.[42,43] In line with our *in vitro* results, we observed that ST2$^{-/-}$ mice exhibit impaired colon barrier function as evidenced by decreased TER and increased macromolecule permeability compared to WT mice. These data support a homeostatic role for IL-33 in maintaining epithelial barrier integrity.

In contrast to our observation, others have reported that IL-33 acts to impair epithelial barrier function.[25,44] Yang and colleagues reported decreased small intestine mucosal TER in mice treated with recombinant IL-33 that is IL-13-dependent.[44] IL-13 is well known to impair epithelial barrier function.[45] It may be that while endogenous IL-33 can act directly on epithelial cells to support barrier function, administration of high doses of recombinant IL-33 results in such a systemic induction of IL-13 that the net effect is a reduction in epithelial resistance. Also in contrast to our findings, Sedhom and colleagues observed that IL-33 acts directly on Caco2 cells to decrease TER.[25] We chose to study T84 colon epithelial cells because they are characteristic of the chloride secretory cells of the colonic crypts.[46] Further studies in primary epithelial cell models are warranted to determine whether IL-33 has differing effects on enterocytes and colonocytes.

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While many studies have demonstrated that IL-33 can affect the immune response, its role as a nuclear protein versus a protein released from cells remains controversial. Although IL-33 has a nuclear localization domain,[47] our immunohistochemical analyses demonstrate IL-33 localization to both the nucleus and cytoplasm of epithelial and lamina propria cells (Fig. 1 and 2).[19] Furthermore, parallel experiments in IL-33$^{-/-}$ and ST2$^{-/-}$ mice demonstrated that signaling through its receptor, ST2, drives the protective role of IL-33 in oxazolone colitis. Epithelial cells expressing ST2 were also able to respond directly to IL-33 stimulation *in vitro*. Additional evidence for a major extracellular role of IL-33 comes from a recently reported mouse with a deletion of the nuclear localization domain in IL-33.[48] These mice exhibit increased serum levels of IL-33, ST2-dependent multi-organ inflammation, increased mortality.[48] These studies provide further evidence that IL-33 exerts its predominant effects through extracellular signaling.

The role of IL-33 in IBD has been controversial, and contrary to our findings, others have reported a pathogenic role for IL-33 in murine models of colitis. Several groups have administered recombinant IL-33 to determine its role in colitis. Administration of IL-33 exacerbates acute DSS-induced colitis[22,23] and IL-10$^{-/-}$ colitis.[49] Similarly, deletion of the nuclear localization domain in IL-33 causes markedly increased serum IL-33 resulting in multi-organ inflammation, including colitis.[48] Therefore, it is plausible that while supra-physiologic levels of IL-33 may induce sufficient inflammation to be detrimental during colitis, physiologic levels of endogenous IL-33 are important for protecting the intestine from damage. A study by Sedhom and colleagues calls this model into question, as they reported a role for endogenous ST2 signaling impairing mucosal healing in DSS- and trinitrobenzene sulphonic acid- (TNBS)-induced colitis.[25] These models differ from the OXA colitis model in method of induction and predominant cytokine response, suggesting the predominant role of IL-33 may be specific to the type of injury/inflammation. Our study is strengthened by two features of our approach. First, to our knowledge, the effect of genetic deletion of IL-33, specifically, on colitis has only been reported once previously, and ours is the first report of genetic deficiency of IL-33 in a colitis model other than the DSS injury-repair model.[29] Second, our results are strengthened by our demonstration of the same overall effect in both mice deficient for IL-33 and its receptor ST2.

In conclusion, mucosal IL-33 is upregulated in pediatric as well as adult UC. Our data in a relevant murine model of UC suggests that induction of endogenous IL-33 signaling through ST2 is an important protective mechanism in the setting of colitis. Furthermore, our study supports that the protective effect of IL-33 is mediated through preserving epithelial homeostasis – namely barrier function and mucin production. Inhibition of IL-33 function may not be a viable therapeutic strategy for UC, as it could exacerbate disease. Further study of the mechanisms underlying the protective effect of IL-33 would inform the development of novel therapeutic approaches to maintain barrier integrity and control inflammation in UC.

## Supplementary Material

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## References

1. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2005; 353:2462–2476. [PubMed: 16339095]
2. Allison J, Herrinton LJ, Liu L, et al. Natural history of severe ulcerative colitis in a community-based health plan. Clin Gastroenterol Hepatol. 2008; 6:999–1003. [PubMed: 18774533]
3. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005; 23:479–490. [PubMed: 16286016]
4. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov. 2008; 7:827–840. [PubMed: 18827826]
5. Pei C, Barbour M, Fairlie-Clarke KJ, et al. Emerging role of interleukin-33 in autoimmune diseases. Immunology. 2014; 141:9–17. [PubMed: 24116703]
6. Pichery M, Mirey E, Mercier P, et al. Endogenous IL-33 Is Highly Expressed in Mouse Epithelial Barrier Tissues, Lymphoid Organs, Brain, Embryos, and Inflamed Tissues: In Situ Analysis Using a Novel Il-33-LacZ Gene Trap Reporter Strain. J Immunol. 2012:3488–3495. [PubMed: 22371395]
7. Hardman CS, Panova V, McKenzie ANJ. IL-33 citrine reporter mice reveal the temporal and spatial expression of IL-33 during allergic lung inflammation. Eur J Immunol. 2012:488–498. [PubMed: 23169007]
8. Neill DR, Wong SH, Bellosi A, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature. 2010; 464:1367–1370. [PubMed: 20200518]
9. Kurowska-Stolarska M, Stolarski B, Kewin P, et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. J Immunol. 2009; 183:6469–6477. [PubMed: 19841166]
10. Louten J, Rankin AL, Li Y, et al. Endogenous IL-33 enhances Th2 cytokine production and T-cell responses during allergic airway inflammation. Int Immunol. 2011; 23:307–315. [PubMed: 21422152]
11. Barlow JL, Bellosi A, Hardman CS, et al. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. J Allergy Clin Immunol. 2012; 129:191–198. e191–194. [PubMed: 22079492]
12. Humphreys NE, Xu D, Hepworth MR, et al. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. J Immunol. 2008; 180:2443–2449. [PubMed: 18250453]
13. Hung LY, Lewkowich IP, Dawson LA, et al. IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. Proc Natl Acad Sci U S A. 2013; 110:282–287. [PubMed: 23248269]
14. Bonilla WV, Frohlich A, Senn K, et al. The alarmin interleukin-33 drives protective antiviral CD8(+) T cell responses. Science. 2012; 335:984–989. [PubMed: 22323740]

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15. Turnquist HR, Zhao Z, Rosborough BR, et al. IL-33 expands suppressive CD11b+ Gr-1(int) and regulatory T cells, including ST2L+ Foxp3+ cells, and mediates regulatory T cell-dependent promotion of cardiac allograft survival. J Immunol. 2011; 187:4598–4610. [PubMed: 21949025]
16. Xu D, Jiang HR, Kewin P, et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. Proc Natl Acad Sci U S A. 2008; 105:10913–10918. [PubMed: 18667700]
17. Beltran CJ, Nunez LE, Diaz-Jimenez D, et al. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. Inflamm Bowel Dis. 2010; 16:1097–1107. [PubMed: 20014018]
18. Kobori A, Yagi Y, Imaeda H, et al. Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. J Gastroenterol. 2010; 45:999–1007. [PubMed: 20405148]
19. Pastorelli L, Garg RR, Hoang SB, et al. Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. Proc Natl Acad Sci U S A. 2010; 107:8017–8022. [PubMed: 20385815]
20. Seidelin JB, Bjerrum JT, Coskun M, et al. IL-33 is upregulated in colonocytes of ulcerative colitis. Immunol Lett. 2010; 128:80–85. [PubMed: 19913053]
21. Latiano A, Palmieri O, Pastorelli L, et al. Associations between genetic polymorphisms in IL-33, IL1R1 and risk for inflammatory bowel disease. PLoS One. 2013; 8:e62144. [PubMed: 23634226]
22. Imaeda H, Andoh A, Aomatsu T, et al. Interleukin-33 suppresses Notch ligand expression and prevents goblet cell depletion in dextran sulfate sodium-induced colitis. Int J Mol Med. 2011; 28:573–578. [PubMed: 21667014]
23. Grobeta P, Doser K, Falk W, et al. IL-33 attenuates development and perpetuation of chronic intestinal inflammation. Inflamm Bowel Dis. 2012; 18:1900–1909. [PubMed: 22508383]
24. Duan L, Chen J, Zhang H, et al. Interleukin-33 ameliorates experimental colitis through promoting Th2/Foxp3(+) regulatory T-cell responses in mice. Mol Med. 2012; 18:753–761. [PubMed: 22426954]
25. Sedhom MA, Pichery M, Murdoch JR, et al. Neutralisation of the interleukin-33/ST2 pathway ameliorates experimental colitis through enhancement of mucosal healing in mice. Gut. 2013; 62:1714–1723. [PubMed: 23172891]
26. Boirivant M, Fuss IJ, Chu A, et al. Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. J Exp Med. 1998; 188:1929–1939. [PubMed: 9815270]
27. Rosen MJ, Chaturvedi R, Washington MK, et al. STAT6 deficiency ameliorates severity of oxazolone colitis by decreasing expression of claudin-2 and Th2-inducing cytokines. J Immunol. 2013; 190:1849–1858. [PubMed: 23303670]
28. Townsend MJ, Fallon PG, Matthews DJ, et al. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. J Exp Med. 2000; 191:1069–1076. [PubMed: 10727469]
29. Oboki K, Ohno T, Kajiwara N, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. Proc Natl Acad Sci U S A. 2010; 107:18581–18586. [PubMed: 20937871]
30. Kennedy RJ, Hoper M, Deodhar K, et al. Interleukin 10-deficient colitis: new similarities to human inflammatory bowel disease. Br J Surg. 2000; 87:1346–1351. [PubMed: 11044159]
31. Forbes EE, Groschwitz K, Abonia JP, et al. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. J Exp Med. 2008; 205:897–913. [PubMed: 18378796]
32. Schmitz H, Barmeyer C, Fromm M, et al. Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. Gastroenterology. 1999; 116:301–309. [PubMed: 9922310]
33. Schiering C, Krausgruber T, Chomka A, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. Nature. 2014; 513:564–568. [PubMed: 25043027]
34. Heller F, Fuss IJ, Nieuwenhuis EE, et al. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. Immunity. 2002; 17:629–638. [PubMed: 12433369]
35. Iijima H, Neurath MF, Nagaishi T, et al. Specific regulation of T helper cell 1-mediated murine colitis by CEACAM1. J Exp Med. 2004; 199:471–482. [PubMed: 14970176]

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36. Li J, Razumilava N, Gores GJ, et al. Biliary repair and carcinogenesis are mediated by IL-33-dependent cholangiocyte proliferation. J Clin Invest. 2014; 124:3241–3251. [PubMed: 24892809]
37. Seidelin JB, Coskun M, Kvist PH, et al. IL-33 promotes GATA-3 polarization of gut-derived T cells in experimental and ulcerative colitis. J Gastroenterol. 2015; 50:180–190. [PubMed: 25112700]
38. Kondo Y, Yoshimoto T, Yasuda K, et al. Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. Int Immunol. 2008; 20:791–800. [PubMed: 18448455]
39. McSorley HJ, Blair NF, Smith KA, et al. Blockade of IL-33 release and suppression of type 2 innate lymphoid cell responses by helminth secreted products in airway allergy. Mucosal Immunol. 2014; 7:1068–1078. [PubMed: 24496315]
40. Hoshino K, Kashiwamura S, Kuribayashi K, et al. The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. J Exp Med. 1999; 190:1541–1548. [PubMed: 10562328]
41. Gonzalez-Mariscal L, Tapia R, Chamorro D. Crosstalk of tight junction components with signaling pathways. Biochim Biophys Acta. 2008; 1778:729–756. [PubMed: 17950242]
42. Howe HS, Cheung PL, Kong KO, et al. Transforming growth factor beta-1 and gene polymorphisms in oriental ankylosing spondylitis. Rheumatology (Oxford). 2005; 44:51–54. [PubMed: 15479754]
43. Dai C, Zhao DH, Jiang M. VSL#3 probiotics regulate the intestinal epithelial barrier in vivo and in vitro via the p38 and ERK signaling pathways. Int J Mol Med. 2012; 29:202–208. [PubMed: 22089663]
44. Yang Z, Sun R, Grinchuk V, et al. IL-33-induced alterations in murine intestinal function and cytokine responses are MyD88, STAT6, and IL-13 dependent. Am J Physiol Gastrointest Liver Physiol. 2013; 304:G381–389. [PubMed: 23257921]
45. Heller F, Florian P, Bojarski C, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology. 2005; 129:550–564. [PubMed: 16083712]
46. Dharmsathaphorn K, McRoberts JA, Mandel KG, et al. A human colonic tumor cell line that maintains vectorial electrolyte transport. Am J Physiol. 1984; 246:G204–208. [PubMed: 6141741]
47. Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'? PLoS One. 2008; 3:e3331. [PubMed: 18836528]
48. Bessa J, Meyer CA, de Vera Mudry MC, et al. Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. J Autoimmun. 2014; 55:33–41. [PubMed: 24786898]
49. Sattler S, Ling GS, Xu D, et al. IL-10-producing regulatory B cells induced by IL-33 (Breg(IL-33)) effectively attenuate mucosal inflammatory responses in the gut. J Autoimmun. 2014; 50:107–122. [PubMed: 24491821]

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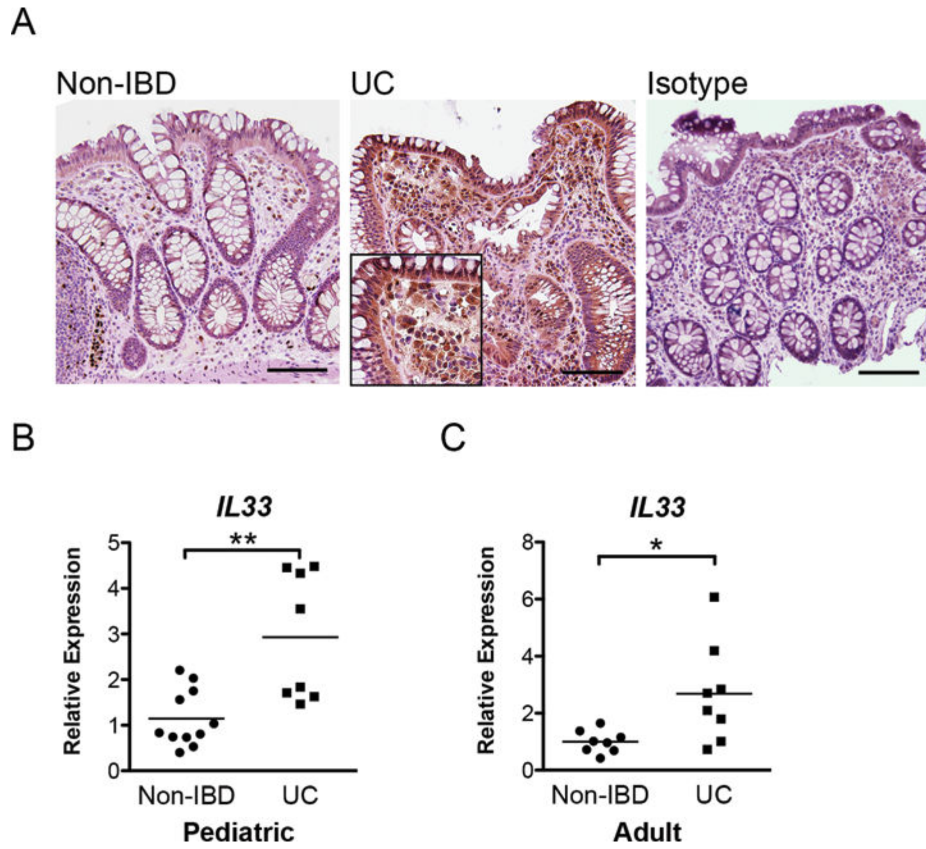


**FIGURE 1.**

IL-33 upregulation in pediatric and adult UC patients. (A) Colon endoscopic biopsy tissues from pediatric UC and non-IBD patients (sections representative of 5 per group) were evaluated by IHC for IL-33 expression and localization. (Scale bars, 100 μm). RT PCR analysis of *IL33* expression in (B) pediatric and (C) adult patients. *$P < 0.05$, **$P < 0.01$.

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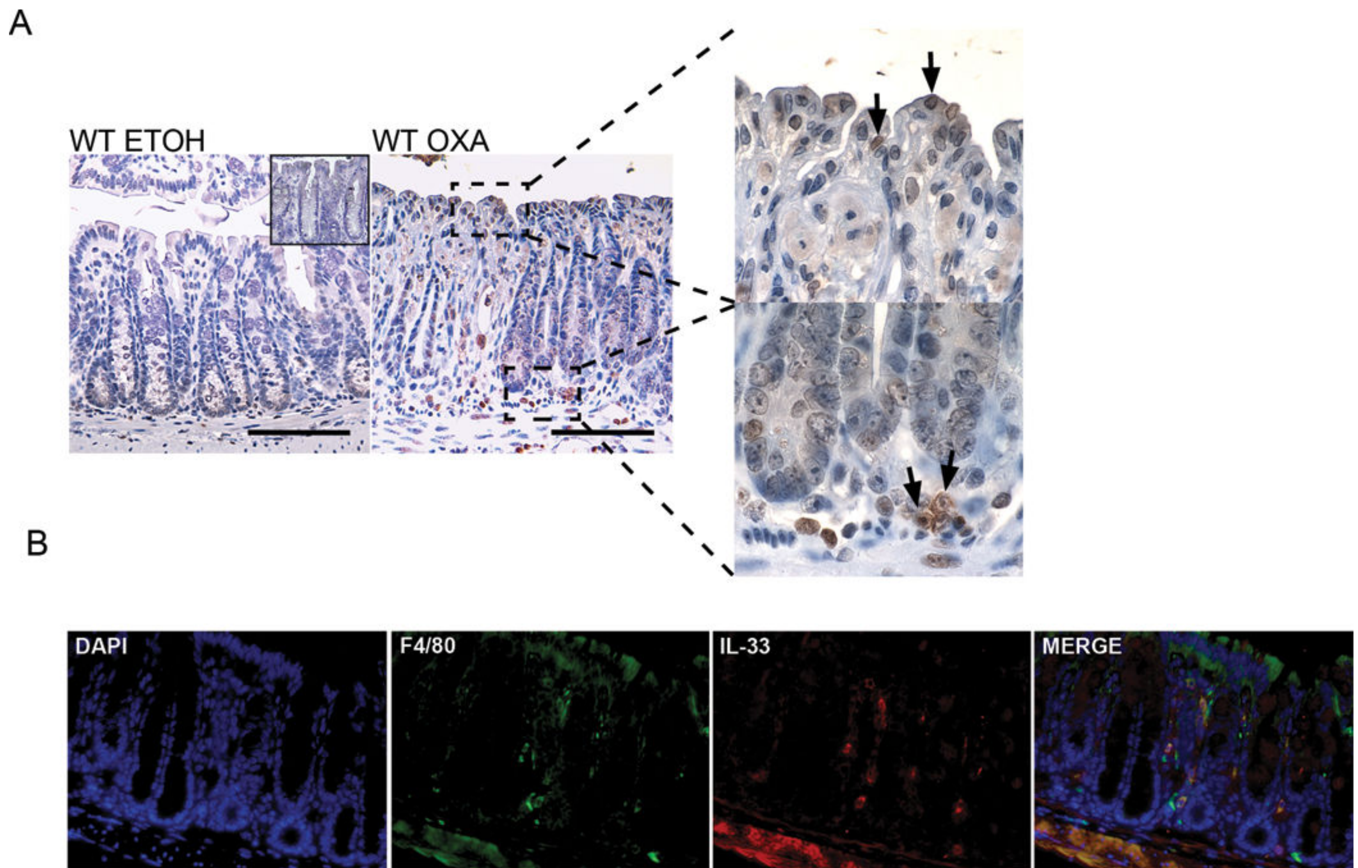


**FIGURE 2.**

Upregulation and localization of IL-33 in OXA colitis. (A) Representative immunohistochemical staining for IL-33 in WT ETOH and WT OXA mice with increased positive staining in epithelial cells (arrows, top panel) and lamina propria cells with morphologic characteristics of macrophages (arrows, bottom panel) in OXA colitis. Inset, IL-33$^{-/-}$ OXA negative control. (Scale bars, 100 μm) (B) Immunofluorescence labeling for F4/80 and IL-33 with DAPI nuclear stain in the colon of an OXA-treated mouse.

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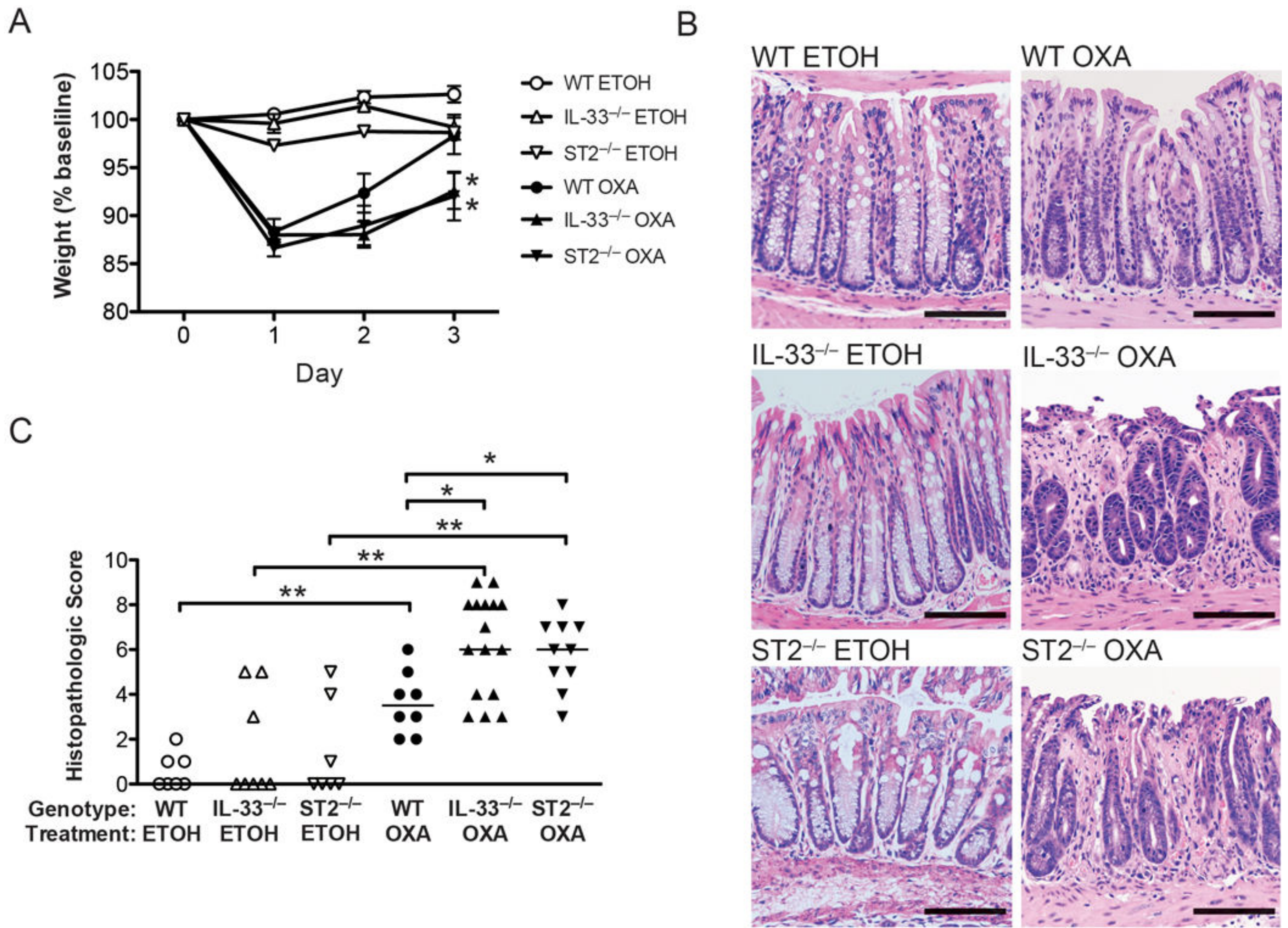


**FIGURE 3.**

Increased severity of OXA colitis in IL-33$^{-/-}$ and ST2$^{-/-}$ mice. WT, IL-33$^{-/-}$, and ST2$^{-/-}$ mice were administered OXA or ETOH vehicle rectally after skin sensitization and sacrificed on Day 3. (A) Colitis severity was assessed by weight loss. (B) Representative H&E-stained histologic sections (Scale bars, 100 μm). (C) Results of histopathologic scoring (maximum score = 15). *$P < 0.05$, **$P < 0.01$.

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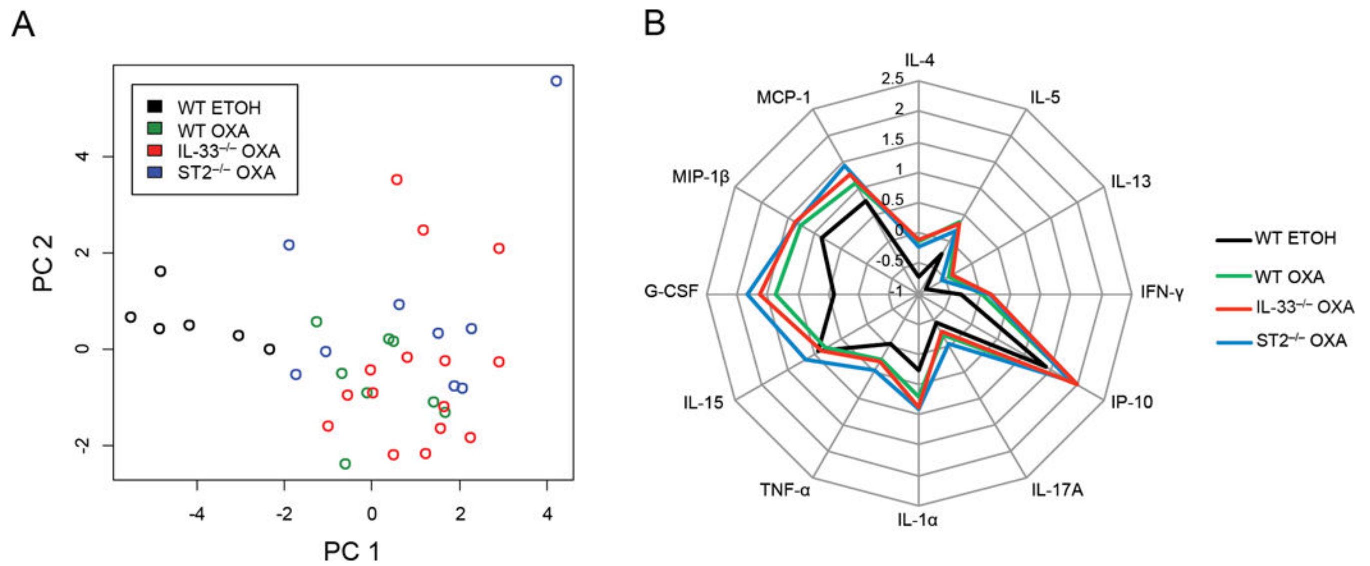


**FIGURE 4.**

Tissue cytokines distinguish between mice treated with OXA and ETOH, but not between IL-33$^{-/-}$, ST2$^{-/-}$, and WT treated with OXA. (A) PCA and (B) spider plot of results of multiplex assay of 12 tissue cytokines statistically different amongst groups by ANOVA. The spider plot depicts mean log cytokine levels for each group.

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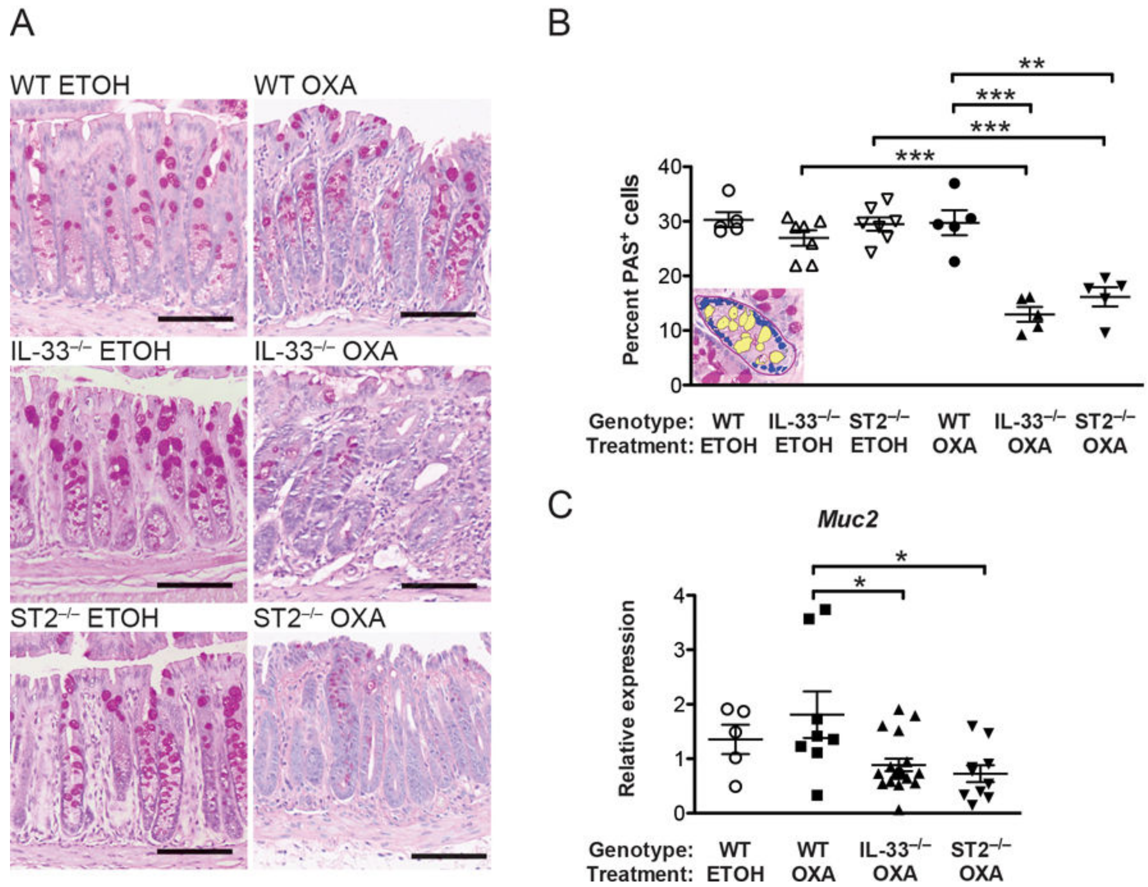


**FIGURE 5.**

Decreased goblet cells and *Muc2* expression in OXA-treated IL-33$^{-/-}$ and ST2$^{-/-}$ mice. (A) Representative photomicrographs of PAS-stained colon tissues. (Scale bars, 100 μm). (B) Results of software image analysis quantifying percent PAS$^{+}$ cells. Inset is image analysis software identification of PAS$^{+}$ cells (yellow) and PAS$^{-}$ cells (blue) highlighted for quantification. (C) Real-time PCR analysis of *Muc2* expression. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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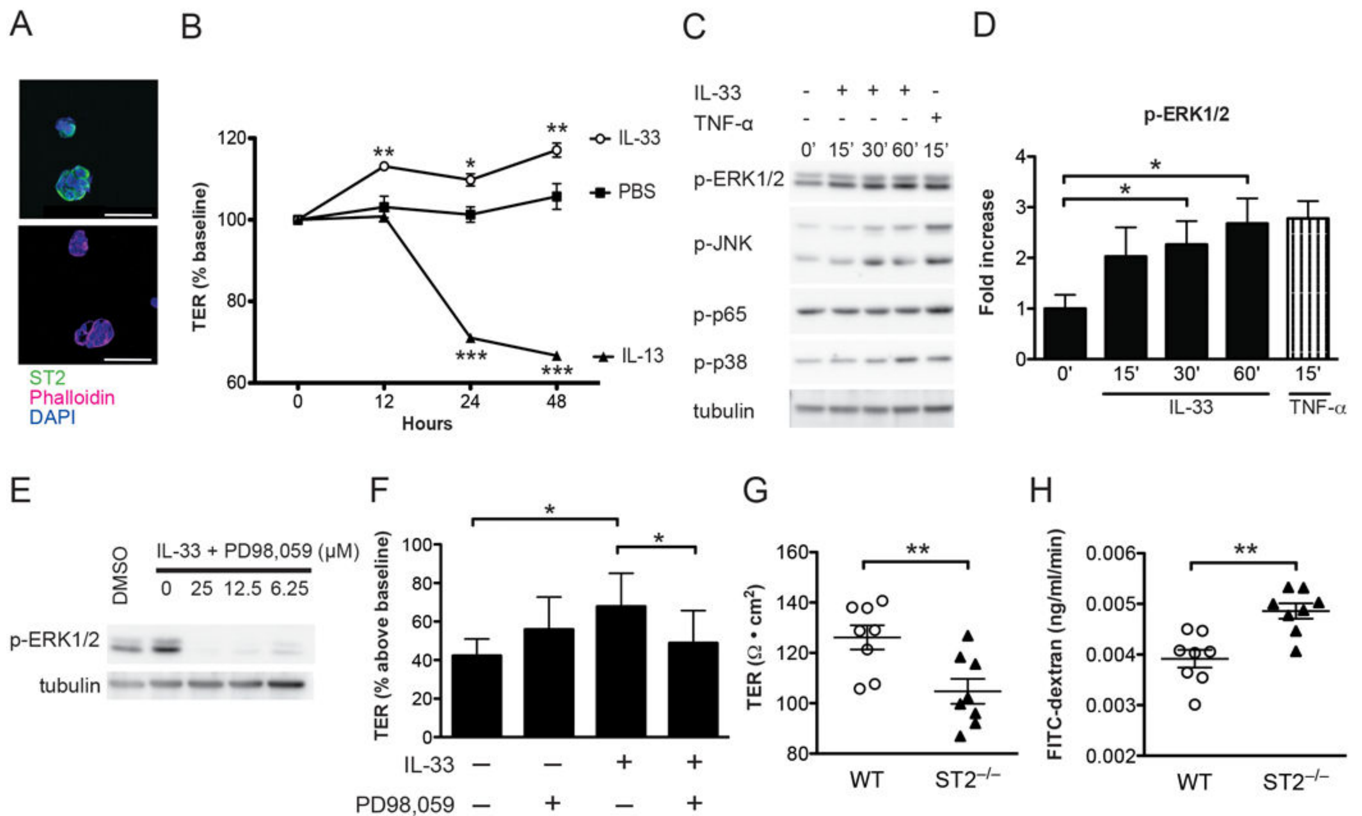


**FIGURE 6.**

IL-33 induced augmentation of epithelial barrier function *in vitro* is dependent on ERK1/2 signaling. (A) Membrane expression of ST2 on T84 cells is demonstrated by representative immunofluorescence staining for ST2 (green) with phalloidin (red) and DAPI (blue) on T84 cells, top panel. Secondary antibody only control for ST2, bottom panel. (B) Percent change in TER of T84 monolayers treated with 10 ng/mL recombinant IL-33 or 10 ng/mL recombinant IL-13. Pooled data of three independent experiments are shown, bars represent means ± SEM. (C) Representative Western blot analysis of phospho-ERK1/2, phospho-JNK, phospho-p65, phospho-p38 and tubulin from T84 cells stimulated with IL-33 (10 ng/mL) or TNF- α (10 ng/mL) for indicated times (minutes). (D) Denistometry quantification of relative p-ERK1/2 levels (normalized to tubulin) graphed as mean ± SEM (data from 3 independent experiments). (E) Representative Western blot of inhibition of p-ERK1/2 following 60 minutes of IL-33 stimulation (10 ng/mL) with the indicated concentrations of PD98,059. (F) Percent increase in TER over baseline of T84 monolayers treated for 48 hours with IL-33 (10 ng/mL) with or without PD98,059 (12.5μM) plotted as mean ± SEM pooled from 6 independent experiments. (F) Baseline TER and (G) luminal-to-serosal flux of FITC-dextran across segments of colon from WT and ST2$^{-/-}$ mice mounted in Ussing chambers. Each data point represents an individual mouse and lines and error bars represent mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.