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Inflammatory bowel diseases (IBD) affect over 5 million individuals in the industrialized world, with an increasing incidence rate worldwide. IBD also predisposes affected individuals to development of colorectal cancer, which is a leading cause of cancer-related deaths in adults. Mutations in genes encoding molecules in the IL-33 signaling pathway are associated with colitis and colitis-associated cancer (CAC), but how IL-33 modulates gut homeostasis is unclear. Here, we have shown that *Il33*-deficient mice are highly susceptible to colitis and CAC. Mechanistically, we observed that IL-33 promoted IgA production from B cells, which is important for maintaining microbial homeostasis in the intestine. *Il33*-deficient mice developed a dysbiotic microbiota that was characterized by increased levels of mucolytic and colitogenic bacteria. In response to chemically induced colitis, this microbial landscape promoted the release of IL-1α, which acted as a critical driver of colitis and CAC. Consequently, reconstitution of symbiotic microbiota or IL-1α ablation markedly ameliorated colitis susceptibility in *Il33*-deficient animals. Our results demonstrate that IL-33 promotes IgA production to maintain gut microbial homeostasis and restrain IL-1α–dependent colitis and CAC. This study therefore highlights modulation of IL-33, IgA, IL-1α, and the microbiota as a potential therapeutic approach in the treatment of IBD and CAC.

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IL-33 regulates the IgA-microbiota axis to restrain IL-1α-dependent colitis and tumorigenesis

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Inflammatory bowel diseases (IBD) affect over 5 million individuals in the industrialized world, with an increasing incidence rate worldwide. IBD also predisposes affected individuals to development of colorectal cancer, which is a leading cause of cancer-related deaths in adults. Mutations in genes encoding molecules in the IL-33 signaling pathway are associated with colitis and colitis-associated cancer (CAC), but how IL-33 modulates gut homeostasis is unclear. Here, we have shown that Il33-deficient mice are highly susceptible to colitis and CAC. Mechanistically, we observed that IL-33 promoted IgA production from B cells, which is important for maintaining microbial homeostasis in the intestine. Il33-deficient mice developed a dysbiotic microbiota that was characterized by increased levels of mucolytic and colitogenic bacteria. In response to chemically induced colitis, this microbial landscape promoted the release of IL-1α, which acted as a critical driver of colitis and CAC. Consequently, reconstitution of symbiotic microbiota or IL-1α ablation markedly ameliorated colitis susceptibility in Il33-deficient animals. Our results demonstrate that IL-33 promotes IgA production to maintain gut microbial homeostasis and restrain IL-1α-dependent colitis and CAC. This study therefore highlights modulation of IL-33, IgA, IL-1α, and the microbiota as a potential therapeutic approach in the treatment of IBD and CAC.

Introduction

Inflammatory bowel diseases (IBD), such as Crohn’s disease (CD) and ulcerative colitis (UC), affect approximately 1.6 million individuals in the USA and 3.5 million individuals in Europe, constituting a major health problem (1, 2). IBD involves chronic inflammation in the intestine with episodes of acute flare-ups that manifest as abdominal pain, diarrhea, rectal bleeding, and body weight loss (1, 2). The mucus and the epithelial layer form a physical barrier between the microbiota and underlying host immune system, and deterioration of this barrier is prominent in IBD patients (3, 4). In addition, rodent studies have linked disruption of the epithelial barrier to cytokine imbalances in the gut that eventually lead to the development of IBD (5). IBD also predispose patients to the development of colorectal cancer, referred to as colitis-associated cancer (CAC) (6). Colorectal cancer is a leading cause of adult cancer-related deaths, with 160,000 cases being diagnosed annually in the USA, and colitis is associated with approximately 90% of these cases (7).

IL-33 is a member of the IL-1 cytokine family that is expressed constitutively in epithelial cells and reticular fibroblasts and can be induced in immune cells (8). IL-33 is usually localized in the nucleus and released upon cell death (9). However, unlike IL-1β and IL-18, which require cleavage by caspase 1 for bioactivity, IL-33 is bioactive in the full-length form (9, 10). IL-33 serves as an amplifier of the type 2 cytokine response from T_{H}2, ILC2, mast cells, and alternatively activated macrophages during asthma, allergic hypersensitivity, and helminth infection (11–15). In addition, IL-33 has been reported to promote antiviral responses by enhancing cytokine production from cytotoxic T cells (16) and to exacerbate arthritis by hyperactivating mast cells (17).

Multiple studies have demonstrated that IL-33, ST2 (the binding site for IL-33), and soluble ST2 (sST2, a decoy receptor) are upregulated in the mucosa of patients with IBD, and polymorphisms in IL33 and ST2 have been associated with the development of IBD (18–22). Expression levels of ST2 correlate inversely with the grade of colon tumors, suggesting that IL-33 signaling is protective in CAC (22). However, the role of IL-33 in IBD and colon tumorigenesis is unclear. Genetic ablation of Il33 in mice of a mixed genetic background lowered clinical symptoms in the early phase of experimental colitis, but also delayed the resolution of inflammation (23). Administration of recombinant IL-33 was also shown to ameliorate colitis in Il33-deficient (WT) mice, suggesting a protective role for IL-33 in colitis (24, 25). By utilizing Il33–/– mice generated on a congenic C57BL/6 background and employing cohousing strategies and littermate controls, we show that IL-33 protected from colitis and CAC. IL-33 was critical to maintaining gut homeostasis by regulating IgA production in the colon. Reduced intestinal IgA in Il33–/– mice led to a dysbiotic microbiota characterized by increased levels of mucolytic and colitogenic bacteria. Consequently, administration of dextran sulfate sodium (DSS) in Il33–/– mice led to increased release of proinflammatory IL-1α, which was a critical driver of CAC. Remarkably, cohousing Il33–/– mice with WT controls equilibrated the level of mucolytic bacteria and thereby protected Il33–/– mice from colitis and epithelial hyperplasia. These findings reveal a critical role for IL-33 in regulating the immune responses and microbiota in the gut.

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IL-33 regulates the development of colitis and associated cancer.

To determine the role of IL-33 in pathogenesis of IBD, WT and Ii33\(^{-/-}\) mice were administered with DSS in drinking water for 6 days, followed by regular drinking water. Following administration of 3% DSS, Ii33\(^{-/-}\) animals displayed significantly greater body weight loss and disease activity score when compared with WT mice (Figure 1, A and B). Further, 100% of the Ii33\(^{-/-}\) mice reached humane end point of the experiment (>20% body weight loss) by day 8, while all the WT mice survived (Figure 1C). At a lower dose of DSS (2%), Ii33\(^{-/-}\) mice still displayed significantly greater weight loss and disease activity scores (Figure 1D), although they survived the experiment. These results suggest that IL-33 plays a protective role in dextran sulfatesodium (DSS)-induced colitis.
Shortening of colon length is a feature of acute colitis (26), and Il33−/− mice had significantly shorter colons at day 8 (Figure 1, F and G). Histological analysis revealed progressive increase in inflammation, ulceration, edema, and hyperplastic proliferation of epithelial cells in the colons of Il33−/− mice during DSS administration (Figure 1, H and I). Breakdown of the epithelial barrier in Il33−/− mice was associated with dissemination of bacteria to the draining lymph nodes by day 8 (Figure 1J). Together, these data demonstrate that IL-33 ameliorates susceptibility to DSS-induced colitis.

Lack of IL-33 leads to increased secretion of IL-1α during DSS administration. To determine the immunological basis of increased colitis and CAC in Il33−/− mice, cytokine and chemokine production were analyzed in the colon during DSS administration. Day 4 was considered as a preclinical time point because there was no difference in body weight loss between WT and Il33−/− mice at that time, while day 8 was considered an acute time point because significant differences in weight change and disease activity score were observed (Figure 1D). In the colon explants, significantly increased levels of IL-1α were observed at both the preclinical time point (day 4) and acute time point (day 8) during DSS administration (Figure 3A). In contrast with the early increase in IL-1α, there was no difference in levels of the neutrophil chemokine KC (also known as CXCL1), granulocyte-CSF (G-CSF), IL-6, IL-1β, macrophage inflammatory protein-1α (MIP-1α, also known as CCL3), IL-10, and IFN-γ-induced protein 10 (IP-10, also known as CXCL10) at day 4, while they were significantly enhanced at day 8 (Figure 3A and Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI88625DS1). Levels of other cytokines, such as IL-17, granulocyte-macrophage CSF (GM-CSF), monocyte chemotactic protein-1 (MCP-1), TNF, and CCL5, were unchanged during the course of the experiment (Supplemental Figure 1). These data suggest that the release of IL-1α precedes the enhanced inflammatory response in Il33−/− mice in response to DSS administration. While IL-1α was upregulated at protein and transcript levels by day 8, the levels of IL-1α protein and transcript were similar between WT and Il33−/− mice at day 4 during DSS administration (Figure 3, B and C). Therefore, DSS administration leads to the release of the preformed pool of IL-1α early during disease progression.

To determine the cellular source of IL-33 and IL-1α in the colon, we assessed for expression of Il33 and Il1a in epithelial (Epcam+CD45−) and immune cells (CD45+Epcam−) from the mice were injected with DNA damaging agent azoxymethane (AOM), followed by 3 rounds of low-dose (2%) DSS treatment (27). Il33−/− mice lost significantly more body weight during each round of DSS treatment (Figure 2A). At the end of the experiment, WT mice exhibited few small tumors restricted to the distal colon (Figure 2, B–D). In contrast, Il33−/− mice exhibited a significantly increased number of tumors that were of a larger size and distributed throughout the distal and middle colon (Figure 2, B–D). Histological analysis revealed few low-grade adenomas in the colons of WT mice, whereas both low- and high-grade adenomas were observed more frequently in the colons of Il33−/− mice (Figure 2, E and F). These data demonstrate that IL-33 protects mice from colitis and CAC.
Figure 3. Lack of IL-33 leads to early release of pathogenic IL-1α. See also Supplemental Figure 2. (A) Quantification of cytokines in supernatants of colon explants and (B) quantification of IL-1α in clarified homogenates of colons of WT and Il33−/− mice at indicated days after DSS administration. (C) qRT-PCR analysis of Il1α expression in whole colon tissue at indicated days after DSS administration. n = 8–10 mice per time point per group. (D) Body weight loss and (E) disease activity index of WT and Il33−/− mice during DSS and control IgG or anti–IL-1α antibody administration. (F) Colon length measurement and (G) representative colon images at day 8 after DSS. (H) Representative images of H&E-stained colon sections and (I) colon histology analysis at day 8 after DSS. Original magnification, ×10. n = 5 mice for WT+CIgG and 9 to 10 mice for other groups. Data represent 2 independent experiments and were analyzed by Kruskal-Wallis test (A, B, C, F, and I) or 2-way ANOVA (D and E) followed by Holm-Šídák post test. Error bars represent mean ± SEM, and each symbol represents an individual mouse. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
antigen-presenting cells, but was not detectable in the CD90+ lymphocytes (Supplemental Figure 2C). Expression of Il1a was upregulated by epithelial cells, antigen-presenting cells, and the CD90+ lymphocytes after DSS administration (Supplemental Figure 2C). Therefore, both epithelial and immune cells of the colon contributed to increased production of IL-33 and IL-1α during DSS treatment.

To determine the mechanism of early IL-1α release during DSS treatment, we evaluated inflammasome activation by analyzing epithelia and antigen-presenting cells (CD45+CD90 MHCII+) and CD90+ lymphocytes (CD45+CD90 MHCII) from the lamina propria. IL-33 was detectable in the colon tissue of WT mice under homeostatic conditions and was increased at both protein and RNA levels at day 8 (Supplemental Figure 2, A and B and Supplemental Methods), consistent with previous reports (24, 25). Increased Il33 expression was observed in both epithelial cells and immune cells of the epithelial fraction. Within the lamina propria fraction, expression of Il33 was increased in the antigen-presenting cells, but was not detectable in the CD90+ lymphocytes (Supplemental Figure 2C). Expression of Il1a was upregulated by epithelial cells, antigen-presenting cells, and the CD90+ lymphocytes after DSS administration (Supplemental Figure 2C). Therefore, both epithelial and immune cells of the colon contributed to increased production of IL-33 and IL-1α during DSS treatment.
ing caspase 1 maturation in the colon homogenates. There was no difference in activation of caspase 1 (observed by the presence of the p10 band) in the colons of WT and Il33–/– mice at day 4, demonstrating that IL-33 does not regulate inflammasome activation to mediate early IL-1α release (Supplemental Figure 2D). However, increased ulceration and necrotic cellular morphology were observed at day 4 and day 8 in the colon sections of Il33–/– mice after DSS administration (Figure 1, H and I, and Supplemental Figure 2E), suggesting that enhanced cell death leads to early IL-1α release from the Il33–/– colonocytes.

**IL-1α is a critical driver of colitis and CAC.** We posited that the enhanced IL-1α release is a critical instigating event in the induction of colitis in Il33–/– mice. To test this hypothesis, IL-1α was depleted in the Il33–/– mice during early time points of DSS administration by injecting IL-1α–neutralizing antibody. Anti–IL-1α treatment significantly ameliorated body weight loss and other clinical signs of the disease in Il33–/– mice (Figure 3, D and E). Anti–IL-1α treatment also significantly protected Il33–/– mice from colon shortening and histological changes associated with DSS administration (Figure 3, F–I).

**Figure 5. IL-33 promotes intestinal IgA production.** (A and B) Immunoglobulin ELISA from colon explants and sera from naive WT and Il33–/– mice. (C) Immunoglobulin ELISA from colon explants at days 0, 4, and 8 after AOM administration. (D) Quantification of CD138+IgA+ cells (intracellular stain for IgA) in the colon lamina propria of naive WT and Il33–/– mice by flow cytometry. (E) Representative images for immunohistochemistry for IgA in colon sections from naive WT and Il33–/– mice. Original magnification, ×40. (F) Immunoglobulin ELISA from media supernatants or (H) percentage and (I) number of IgA+B cells from WT splenic B cells (CD19+) treated with LPS, TGF-β, and/or IL-33 for 4 days. (J) qRT-PCR analysis of Pigr expression in whole colon tissue at indicated days after DSS administration. Data represent 3 independent experiments (A–D, F, and J) or 3 technical replicates that are representative of 3 independent experiments. Data in G–I were analyzed by Kruskal-Wallis test followed by Holm-Šídák post test. Error bars represent mean ± SEM, and each symbol represents an individual mouse. **P < 0.01; ***P < 0.001; ****P < 0.0001.
Similar to results with antibody-mediated neutralization of IL-1α, mice deficient in both IL33 and Il1a (Il33−/−Il1a−/−) were protected from AOM and DSS-induced body weight loss, clinical signs of disease, colon shortening, and histological changes when compared with Il33−/− mice (Figure 4, A–E). Epithelial hyperplasia is an early event in tumorigenesis, which normally correlates with the extent of inflammation after AOM and DSS treatment. We therefore evaluated the colonic epithelial hyperplasia in WT, Il33−/−, and Il33−/−Il1a−/− mice at day 14 of AOM and DSS treatment. Consistent with decreased inflammation in the colons of Il33−/−Il1a−/− mice, a lower proportion of Il33−/−Il1a−/− mice showed epithelial hyperplasia (30%) when compared with Il33−/− mice (100%) (Figure 4, E–G). Therefore, genetic ablation of Il1a also protects Il33−/− mice from epithelial hyperplasia. To specifically determine whether tumor development in Il33−/− mice is decreased by Il1a ablation, we treated WT, Il33−/−, and Il33−/−Il1a−/− mice with AOM followed by 3 rounds of DSS. Il33−/−Il1a−/− mice lost significantly lower body weight when compared with Il33−/− mice during each round of DSS administration (Figure 4H). Consistent with decreased hyperplasia at day 14, Il33−/−Il1a−/− mice also harbored a significantly reduced number of tumors in their colons at day 49 when compared with Il33−/− mice (Figure 4, I and J). The number of tumors in Il33−/−Il1a−/− mice was in fact similar to that in WT mice. Therefore, epithelial hyperplasia data at day 14 and tumor burden at day 49 clearly demonstrate that genetic ablation of Il1a protects Il33−/− mice from CAC.

Il1a−/− mice themselves were protected from acute colitis after 4% DSS administration (Supplemental Figure 3, A and B), consistent with a previous report (28). Moreover, upon AOM and DSS treatment with a 3.5% DSS dose, Il1a−/− mice lost significantly less body weight than WT controls at chronic time points of days 66 to 90 (Supplemental Figure 3C). Histological analysis revealed reduced inflammation and epithelial dysplasia in the Il1a−/− colon (Supplemental Figure 3, D–G). Therefore, IL-1α is an important driver of colitis and CAC.

**IL-33 promotes intestinal IgA production.** Next, we examined the mechanism behind increased IL-1α release from Il33−/− colonoocytes after DSS administration. In order to reach the colonoocytes, DSS must first penetrate the mucus barrier. IL-33 has been shown to upregulate the production of mucus after helminth challenge and oxazolone treatment (14, 29). We therefore examined possible defects in the mucus production in Il33−/− mice. There were no significant differences in the expression of mucins Muc1, Muc2, Muc3, Muc4, or Muc5ac at basal or preclinical time points during DSS administration between WT and Il33−/− colons (Supplemental Figure 4A). Further, the number of goblet cell was similar between the colons of WT and Il33−/− mice under homeostatic conditions (day 0). Also, consistent with increased colitis in Il33−/− mice, frequency of goblet cells was decreased in the colons of Il33−/− mice during DSS treatment (Supplemental Figure 4, B and C). IL-33 has also been shown to be an amplifier of type 2 cytokine response from Tγ2 and ILC2 cells after helminth and allergen challenge (14, 29). Monticelli et al. showed that exogenous IL-33 administration expands ILC2s in the GALT and upregulates amphiregulin production (24). However, the impact of IL-33 deficiency on ILC2 and Tγ2 response in the gut under homeostatic conditions or during DSS administration has not been directly tested. The levels of cytokines associated with ILC2 and Tγ2 response, IL-5, IL-13, and amphiregulin, were similar in the colons of WT and Il33−/− mice at day 0 and day 4 and increased in the colons of Il33−/− mice at day 8 (Supplemental Figure 5A). We also evaluated the number of ILC2s (CD19 CD45+Lin CD90.2+CD127−GATA-3−) and Tγ2 cells (CD19 CD45+Lin CD90.2+CD127−GATA-3−) in the colons of WT and Il33−/− mice during DSS administration. We found that the number of ILC2 and Tγ2 cells in the colons of Il33−/− mice were similar to WT levels at day 0. At day 8, the number of ILC2s remained similar, but the number of Tγ2 cells was increased in the colons of Il33−/− mice (Supplemental Figure 5, B–D). Increase in the number of Tγ2 cells correlated with increased levels of Il5 and Il13 and the extent of inflammation in the Il33−/− mice. These data are consistent with findings from Waddell et al., who showed that the colonic type 2 cytokine response is preserved in Il33−/− mice after oxazolone treatment (29).

Expression of epithelium-healing genes, such as Il22 and its associated antimicrobial peptides (AMPs) Reg3g and Reg3b, was also unperturbed at day 4 of DSS administration (Supplemental Figure 6A). Furthermore, expression of other AMPs such as lipocalin, pentraxin, and SI100a9 (Supplemental Figure 6B) and epithelial tight junction proteins occludin and ZO1 (Supplemental Figure 6C) were also similar between the colons of WT and Il33−/− mice until day 8 (Supplemental Figure 6, A–C). Schiering et al. have also shown that IL-33 promotes differentiation of Tregs and thereby protects lymphopenic mice from colitis resulting from infusion of naive T cells (30). However, there was no defect in the number of Tregs in the colons of Il33−/− mice at day 0, and they were increased during DSS administration (Supplemental Figure 6D). Collectively, these data demonstrate that perturbation in production of mucins, type 2 cytokines, AMPs, tight junction proteins, and epithelial restitution factors is not observed in the colons of Il33−/− mice until overt inflammation is established. Therefore, while perturbation in these pathways may promote disease pathogenesis, they are unlikely to be responsible for the early release of pathogenic IL-1α.

Intestinal IgA is a critical factor in promoting gut homeostasis. Intestinal IgA has been shown to neutralize toxins and preferentially target IBD-promoting gut bacteria for clearance by M cells and lamina propria macrophages (31). Further, IgA deficiency predisposes humans to gut infections and IBD and increases severity of DSS-induced colitis in mice (32–34). Therefore, we evaluated the level of intestinal IgA in Il33−/− mice. Il33−/− mice had significantly decreased levels of IgA in colon explants, while the levels of other immunoglobulins—IgM, IgG2b, IgG2c, IgG3, and IgG1—were similar to WT levels (Figure 5A). Levels of IgA and other immunoglobulins were similar in sera, suggesting a gut-specific role of IL-33 in regulating IgA production (Figure 5B). Furthermore, levels of intestinal IgA stayed low in the Il33−/− mice during DSS administration (Figure 5C). Decrease in intestinal IgA was also confirmed by a decrease in the number of IgA-producing plasma cells (gated as CD138+IgA+ with intracellular staining for IgA) in the colons of Il33−/− mice (Figure 5, D and E). The intestinal IgA level in Il1a−/− mice was similar to the WT level, while the level in Il33−/−Il1a−/− mice was similar to the level in Il33−/− (Figure 5F). Therefore, protection from colitis and CAC that was conferred by IL-1α blockade or deficiency was independent of modulation of intestinal IgA production.
To assess whether IL-33 promotes IgA production from B cells, we utilized an in vitro class-switching assay. WT splenic B cells were stimulated with LPS in the presence of IL-33, TGF-β, or both. TGF-β significantly increased the production of IgA and decreased production of IgM and IgG3 (Figure 5G), consistent with previous reports (35). Addition of IL-33 alone did not affect the isotype or quantity of immunoglobulin production. However, addition of both TGF-β and IL-33 further enhanced IgA production (Figure 5G). Increase in IgA secretion was consistent with increase in both the proportion and total number of IgA+ cells in B cells treated with both TGF-β and IL-33 (Figure 5, H and I). IL-33, however, did not affect the expression of polymeric immunoglobulin receptor (Pigr), a receptor required for secretion of IgA into the lumen (Figure 5). Therefore, IL-33 synergizes with TGF-β to promote IgA production, and absence of IL-33 leads to decreased IgA levels in the intestine.

Colitis susceptibility in Il33-deficient mice is dependent on dysbiotic microbiota. We and others have shown that gut microbial dysbiosis contributes to susceptibility toward colitis and CAC (36–38). IgA is known to regulate the gut microbial landscape, and mice deficient in IgA and pIgR are known to harbor a dysbiotic microbiota that contributes to their increased susceptibility to colitis (33, 39–41). We therefore posited that decreased colonic IgA in Il33–/– mice would lead them to developing a dysbiotic microbiota. To assess whether IL-33 has an effect on gut microbial homeostasis, we performed real-time quantitative PCR (qPCR) analysis of the gut microbiota derived from colon contents of separately housed WT and Il33–/– mice. Relative to WT mice, Il33–/– mice harbored increased levels of Akkermansia muciniphila and segmented filamentous bacteria (SFB), while the levels of bacteria belonging to other class and phyla were similar (Figure 6, A and B). A. muciniphila is an anaerobic bacterium that specializes in degrading the mucus (42) and is highly targeted (Figure 6, A and B). The Journal of Clinical Investigation

To determine whether IL-33 actively regulates microbial homeostasis, we cohoused WT and Il33–/– mice for 2 weeks and then separated them for another 4 weeks. While cohousing equilibrated the level of Akkermansia and intestinal IgA in the stool samples, Il33–/– mice regained the separately housed gut ecological landscape of increased Akkermansia (Figure 7A) and decreased intestinal IgA levels (Figure 7B) after 4 weeks of separation. In
Discussion

Only 25% of patients achieve sustained disease remission with the currently available anti-TNF biologics, while another 30% require total colectomy (50). Therefore, further investigations into the cytokine networks that underlie IBD are required to develop novel therapeutic strategies. Expression of IL-1α, IL-33, ST2, and sST2 are increased in the mucosa of IBD patients, and SNPs in IL33 and ST2 are associated with the development of IBD (18–22). Other studies that utilized distinct models of colitis or exogenous IL-33 infusion have attributed the functions of upregulating the production of mucus, type 2 cytokines and cells, and Tregs to the IL-33/ST2 signaling pathway (24, 29, 30). Consistent with these studies demonstrating a protective role of IL-33 in the gut, we show here that Il33−/− mice were more susceptible to DSS-induced colitis. However, Il33−/− colon explants released high levels of IL-1α early during disease onset. Increased IL-1α was pathogenic, as its neutralization or genetic deletion markedly protected Il33−/− mice from colitis.
IBD is a strong predisposing factor for CAC, and expression of IL-33 and ST2 is increased in low-grade tumors, but decreased in high-grade tumors, suggesting a protective role of IL-33 in CAC (22). In line with this hypothesis, we showed that IL33-deficient mice were highly susceptible to AOM/DSS-induced CAC. Furthermore, we showed that IL-1α was a major driver of colitis and CAC in both IL33-deficient and IL33-sufficient settings. Maywald et al. showed that IL-33 signaling promotes small intestinal tumorigenesis in a model that is dependent on aberrant Wnt signaling instead of inflammation (51). Therefore, the role of IL-33 in restraining colitis-associated colon cancer is due to its dominant role in preventing IL-1α-mediated overt inflammation. IL-1α-blocking monoclonal antibody was well tolerated in phase I clinical trials for psoriasis and solid tumors (52, 53). Therefore, further trials in testing its efficacy in ameliorating IBD and CAC are warranted.

Immune system components can regulate the host microbiota that modulate the susceptibility to colitis and CAC (38, 48, 54, 55). Intestinal IgA is one such immune component that preferentially targets colitogenic members of the microbiota, including SFB and A. muciniphila (34, 39, 41, 56, 57). Reciprocally, certain pathobionts can produce proteolytic enzymes that degrade intestinal IgA and thereby enhance colitis susceptibility (40). We showed here that IL-33 was essential for efficient production of IgA in the intestine, even under homoecostatic conditions. Similar to the role of IL-33 in Treg differentiation (30), IL-33 promoted IgA production in a TGF-β-dependent manner. Consistent with decreased levels of intestinal IgA in IL33−/− mice, they also harbored a dysbiotic microbiota associated with increased levels of SFB and, in particular, A. muciniphila. Verrucomicrobia family member Akkermansia expresses mucinases that allow it to degrade the inner mucus layer and utilize it as a carbon and nitrogen source (42). As a consequence, experimental Akkermansia colonization weakens the mucus barrier and promotes epithelial injury after administration of the cytotoxic agent heme (43). Repeated dosing with Akkermansia also leads to increased severity of DSS-induced colitis in WT mice (44) and promotes colon tumorigenesis in a mouse model of aberrant Wnt signaling in the epithelium (58). In line with these studies, we found that deletion of Akkermansia by metronidazole treatment protected IL33-deficient mice from DSS-induced colitis and associated epithelial hyperplasia.

It is of particular clinical relevance that Akkermansia has also been shown to improve obesity and metabolic disorders (59). We have also previously shown that the antibiotic cocktail that increases the levels of Akkermansia also ameliorates IL-1β-mediated autoinflammation (49). We showed here that the same antibiotic cocktail also strikingly increased colitis severity. Further, it was recently shown that oral delivery of IgA reactive to pathobionts can decrease colitis susceptibility (39). Therefore, along with modulation of IL-1α and IL-33 levels, discovering tools that lead to specific manipulation of the microbiota, such as a tailored diet, targeted antibiotic regimen, or oral IgA supplementation, holds great promise of ameliorating inflammatory diseases and cancer.

Methods

Mice. C57BL/6 WT and Il1α−/− (60) mice were described previously. Il33−/− (komp) (Il33-deficient) embryos were obtained from the Knock-out Mouse Project (KOMP; https://www.komp.org/) and rederived in C57BL/6 mice in the colony. Mice were maintained in a specific pathogen-free facility.

Induction of colitis and tumors. For DSS models of colitis, mice were administered the indicated dose of DSS (molecular weight 36-40 kDa; Affymetrix) for 6 days, followed by regular water. For neutralization of IL-1α, mice were injected with 100 μg of anti–IL-1α antibody (clone ALF-161, Bio X Cell) in 100 μl sterile PBS on days 1, 3, and 5 via retro-orbital route during DSS administration. The AOM/DSS model for colorectal tumorigenesis has been described previously (61). For metronidazole treatment, mice were administered with 2.5 mg/ml metronidazole (Teva Pharmaceuticals) with 1% sucrose in drinking water. For colitis experiments, equal numbers of female wildtype and female IL33−/− mice were housed in the same cage for 2 weeks before DSS administration and remained cohoused for the duration of the experiment. For separation after cohousing experiments, mice were cohoused for 2 weeks, followed by separation for 4 weeks before DSS administration.

Histology and microscopy analysis. Colon tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with H&E. Histological analysis for inflammation, epithelial hyperplasia, and tumorigenesis was performed by a board-certified pathologist (P. Vogel) as described previously (62). FISH for Akkermansia was performed by utilizing Alexa Fluor 647-conjugated probe as described previously (63).

Cytokines and immunoglobulin measurement. Cytokines in colon explants, homogenates, and sera were measured by ELISA. Culture of colon explants (64) and protein extraction from frozen colon tissues (62) was described previously. Briefly, for explant culture, 0.5 cm of the distal colon was incubated in IMDM media supplemented with 10%FBS and penicillin, streptomycin, and gentamycin for 48 hours. Cytokine levels are presented as pg or ng/ml of the clarified supernatant media. Explants were also weighed before incubation, and equivalent data are obtained if the cytokine levels are normalized to their wet weight. The IL-18 ELISA was from eBioscience; multiplex ELISAs were used for all other cytokines (Millipore) according to the manufacturers’ instructions. Immunoglobulin ELISA was performed by the C57BL/6-HPR clonotyping system (SouthernBiotech) per the manufacturer’s instructions.

Real-time qPCR. RNA was isolated using the RNeasy Kit (Sigma-Aldrich) per the manufacturer’s instructions and converted into cDNA as described earlier (66). Gene expression was assessed using 2x SYBR Green Master Mix according to the manufacturer’s instructions (Applied Biosystems). Sequences for qRT-PCR primers are listed in Supplemental Table 1. qPCR data were analyzed by the 2−ΔΔCT method, with β-actin as the housekeeping gene. For gut microbiota characterization, genomic DNA from feces was extracted using the QiAMP DNA Stool Mini Kit (QIAGEN) with bead beating in tissue lyser (Qia-gen) and used for real-time PCR amplification of indicated microbiota components by SYBR Green Master Mix (Applied Biosystems) as described earlier (65). Data were analyzed with the 2−ΔΔCT method using universal eubacteria primers as control.

B cell class switching assay. The rbc-depleted splenocytes or sort-purified CD19− B cells from naive C57BL/6 WT mice were cultured in media supplemented with anti-CD40 (1 ng/ml), LPS (1 μg/ml, Invivo- gen), TGF-β (1 ng/ml, R&D Systems), and/or IL-33 (30 ng/ml, Pepro-tech) in the indicated combinations. After 4 days in culture, supernatant media were analyzed for immunoglobulins by the C57BL/6-HPR Clonotyping System (SouthernBiotech) per the manufacturer’s instruc-
tions. Supernatant concentrations were 1:10,000 for IgM and 1:100 for IgA and IgGs. Only absorbance values more than 3 SD away from the mean of negative control were considered positive. Cells were used for intracellular staining of IgA (clone mA-E61, eBioscience) and analyzed by flow cytometry.

Statistics. Body weight change data were analyzed by 2-way ANOVA followed by the Holm-Šidák post test. Survival data were analyzed by log-rank (Mantel-Cox) test. Statistical significance for other data sets was determined by parametric or nonparametric tests, where appropriate, and are indicated in the figure legends; *P < 0.05 was considered significant.

Study approval. Animal study protocols were approved by the St. Jude Children’s Research Hospital Committee on the Use and Care of Animals.

Author contributions
AM and TDK conceptualized the project. Methodology was designed by AM, QZ, DS, and RK. Investigation was by AM, QZ, DS, RK, and PV. AM, DS, QZ, PV, and TDK performed formal analysis. AM wrote the original draft. AM, DS, and TDK reviewed and edited the manuscript. Funding acquisition was by TDK. TDK provided resources. TDK supervised the project.

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