IL-33-induced alterations in murine intestinal function and cytokine responses are MyD88, STAT6, and IL-13 dependent

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IL-33 is a recently identified cytokine member of the IL-1 family that also includes IL-1α, IL-1β, IL-18, and IL-37 (16, 36). Multiple cell types are able to produce IL-33, including macrophages and dendritic cells, Th2 cells, B1 cells, mast cells, and nonhematopoietic structural cells including epithelial and endothelial cells (16, 36). The biological activity of IL-33 has been associated with the promotion of Th2 immunity. Exogenous administration of IL-33 in mice caused pathological changes in most mucosal tissues such as the lung and the gastrointestinal (GI) tract associated with increased production of type 2 cytokines and chemokines (36). For example, early studies showed that IL-33 can activate Th2 cells, mast cells, or basophils to produce type 2 cytokines such as IL-4, IL-5, and IL-13. Recently, several novel cell populations, collectively referred as “type 2 innate lymphoid cells,” were identified as being IL-33 responsive and are thought to be important for initiating type 2 immunity (25, 27, 32, 34). Nevertheless, the underlying mechanisms by which IL-33 promotes type 2 immunity in the GI tract remains to be fully understood.

The receptor for IL-33 is a heterodimer consisting of IL-1R accessory protein (IL-1RAP) and ST2, a member of the Toll-like receptor/IL1R superfamily. IL-33 binding to ST2 induces formation of a complex with IL-1RAP, which then leads to recruitment of adapter protein MyD88 and activation of NF-κB and MAPK pathways (16, 36). IL-33/ST2 axis plays a critical role in various autoimmunity and inflammatory diseases because of its pro- or anti-inflammatory activities. High levels of IL-33 were found in humans and mice with asthma (11, 31). Administration of exogenous IL-33 exacerbated allergen-induced airway inflammation (12, 35), whereas neutralizing IL-33 with antibody or genetic deletion of IL-33 signaling in mice attenuated pulmonary eosinophilia and airway hyperresponsiveness (11, 17). Increased production of IL-33 was also observed in patients with rheumatological diseases, inflammatory skin disorders, and inflammatory bowel disease, especially ulcerative colitis (23). On the other hand, IL-33 can inhibit certain Th1- or Th17-associated pathologies. Treatment of mice with IL-33 reduced the development of atherosclerosis associated with increased type 2 but decreased type 1 cytokines (22) and attenuated experimental autoimmune encephalomyelitis by suppressing IL-17 and IFN-γ production and inducing alternatively activated macrophages (M2) (9).

In the GI tract, a hallmark of type 2 immunity is the characteristic changes in intestinal function including smooth muscle hypercontractility, epithelial hyposecretion, and increased mucosal permeability (39). One of the most well-characterized models of type 2 immunity is the response to enteric nematode infection in which various immune cells recruited to the intestine lead to increased production of various type 2-associated cytokines/mediators including IL-4, IL-5, IL-9, and IL-13 (3). IL-4 and IL-13, prototype Th2 cytokines, bind to the type 1 and/or type 2 IL-4 receptors and activate the STAT6 signaling pathway to upregulate various
downstream effector molecules important for intestinal function (37, 44, 45). During enteric nematode infection, epithelial cells are a major source of the IL-33 that is thought to initiate type 2 protective immunity. The effects of IL-33 on gut function, however, are unknown. The present study was designed to 1) investigate the involvement of MyD88, STAT6, and IL-13 in IL-33-induced type 2 immune response in the intestine; 2) examine the effects of IL-33 on the functions of intestinal smooth muscle and epithelium; 3) determine the role of STAT6 pathways in IL-33 regulation of intestinal function; and 4) elucidate the contribution of IL-13 vs. IL-4 effects of IL-33-dependent intestinal function.

MATERIALS AND METHODS

Mice. C57BL/6 wild-type (WT) mice were purchased from the Small Animal Division of the National Cancer Institute or Jackson Laboratory (Bar Harbor, ME). Mice deficient in STAT6 (STAT6−/−) on C57BL/6 background were purchased from Jackson Laboratory and bred in the USDA/Beltsville animal facility. Mice deficient in MyD88 (MyD88−/−) were obtained from the National Institutes of Allergy and Infectious Diseases Taconic contract. Mice deficient in MyD88 (MyD88−/−) were the kind gift of Dr. Shizuo Akira and were bred at the University of Maryland, Baltimore. These studies were conducted with institutional approval from both the University of Maryland, Baltimore and the USDA Beltsville Area Animal Care and Use Committees, in accordance with principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, Health and Human Services Publication (National Institutes of Health 85-23, revised 1996).

Administration of IL-33. Mice were injected intraperitoneally with 1 g of mouse recombinant IL-33 (R&D Systems, Minneapolis, MN) in 100 g of saline daily for 3 days or as otherwise indicated. Control mice were administered BSA. The dose of cytokine was determined in preliminary experiments showing that this dose of IL-33 induced a prominent upregulation of type 2 cytokines in the intestine. Five mice per group were used for each independent experiment with three sets of experiment for WT mice, two sets of experiment for STAT6−/−, as well as IL-13−/− mice, and one set of experiment for MyD88−/− mice.

In vitro smooth muscle contractility in organ baths. In vitro smooth muscle contractility was measured as described previously (43). Smooth muscle responses to acetylcholine, a cholinergic neurotransmitter, and to electric field stimulation (EFS), which mimics nerve stimulation, as well as amplitude of spontaneous contractions were determined. Tension was expressed as force per cross-sectional area (42).

In vitro epithelial cell ion transport in Ussing chambers. Musclease segments of small intestine were mounted in Ussing chambers as described previously (38). Concentration-dependent changes in short-circuit current (Isc) were determined in response to the addition of acetylcholine to the serosal side or glucose to the mucosal side. Responses from all tissue segments exposed to acetylcholine or glucose from an individual animal were averaged to yield a mean response per animal.

Micro-Snapwell assay for mucosal TEER and paracellular permeability. The modified micro-Snapwell system is a miniaturized version of the standard Ussing chamber that has been engineered to measure the transepithelial electrical resistance (TEER) (5). Segments of mouse intestine stripped of both muscle and serosal layers were placed in the micro-Snapwell system. DMEM (250 µl) containing 4.5 g/l glucose, 4 mM L-glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, and MEM with 1 mM nonessential amino acids was added to the mucosal-side compartment, and 3 ml of the same medium was added to the serosal-side compartment. The system was incubated at 37°C with 5% CO2 for 30 min to stabilize the pH, and the baseline TEER measurement was taken. To measure paracellular permeability, Cascade blue-labeled dextran (3K MW; source) was added to the mucosal-side compartment. Then 100 µl of medium from the serosal-side compartment was collected 4 h later for measuring the influx of labeled dextran across the mucosal layer.

RNA extraction, cDNA synthesis, and real-time qPCR. Total RNA was extracted from midjejunum whole tissue as described previously (24). Total RNA samples (2 µg) were reverse-transcribed to cDNA by using the First Strand cDNA Synthase Kit (MBI Fermentas, Hanover, MD) with random hexamer primers. Real-time quantitative PCR (qPCR) was performed on an iCycler detection system (Bio-Rad). PCR was performed in a 25-µl volume with SYBR Green Supermix. The fold changes in mRNA expressions for targeted genes were relative to the respective vehicle group of mice after normalization to 18s rRNA. Primer sequences were designed by using Beacon Designer 7.0 (Premier Biosoft International, Palo Alto, CA), and synthesized by the Biopolymer and Genetics Laboratory (University of Maryland) or by Sigma.

Solutions and drugs. Krebs buffer contained (in mM) 4.74 KCl, 2.54 CaCl2, 118.5 NaCl, 1.19 NaH2PO4, 1.19 MgSO4, 25.0 NaHCO3, and 11.0 glucose. All drugs were obtained from Sigma (St. Louis, MO) unless otherwise indicated. Appropriate dilutions of acetylcholine were made from a stock solution on the day of the experiment.

Data analysis. Data presented are pooled from or representative of the independent experiments with 5–10 mice per treatment. Agonist responses were fitted to sigmoid curves (GraphPad, San Diego, CA). Statistical analysis was performed by one-way ANOVA followed by Student-Newman-Keuls test to compare the responses and gene expression among the different treatment groups or by Student’s t-test between the means of two different treatment groups.

RESULTS

IL-33 upregulated the expression of type 2 cytokines in the intestine by an MyD88-dependent, but IL-13/STAT6-independent, pathway. It is known that IL-33 promotes type 2 immunity, yet the underlying mechanisms are not well defined. To address this question, we injected IL-33 to C57BL/6 WT mice or mice genetically deficient in MyD88, STAT6, or IL-13. qPCR revealed that IL-33 induced upregulation of IL-4, -5, and -13 mRNA expression (Fig. 1, A–C) in the intestines of WT mice, consistent with previous reports (36). Increased expression was observed also in the intestines of STAT6−/− or IL-13−/− mice but was absent in MyD88−/− mice (Fig. 1, A–C).

IL-25 is a member of Th17 cytokine family. Like IL-33, IL-25 can be produced by epithelial cells and has been associated with induction of type 2 immunity. A modest but significant upregulation of IL-25 expression was observed in the intestine of IL-33-treated WT, not MyD88−/− mice (Fig. 1D). Interestingly, there was a downregulation of IL-25 in the intestines of IL-33-treated STAT6−/− or IL-13−/− mice (Fig. 1D), suggesting that the IL-13/STAT6 signaling pathway is required for induction of IL-25 by IL-33. A similar downregulation of thymic stromal lymphopoietin (TSLP) expression was also observed in IL-33-treated STAT6−/− and IL-13−/−, but not WT mice (Fig. 1E). In contrast, IL-33 downregulated IL-17A expression in WT but not STAT6−/− or IL-13−/− mice (Fig. 1F).

Exogenous IL-33 induced MyD88-dependent changes in intestinal smooth muscle and epithelial function. Type 2 immunity in the GI tract features characteristic alterations in intestinal smooth muscle and epithelial function (reviewed in...
Smooth muscle strips from IL-33-treated WT mice exhibited a hypercontractility response to stimulation with acetylcholine or EFS, as well as an increase in the amplitude of spontaneous contraction (Fig. 2, A–C). In addition, the intestinal mucosa from IL-33-treated mice exhibited significantly decreased secretion in response to acetylcholine, as well as inhibited glucose absorption compared with mice treated with BSA (Fig. 2, D and E). Moreover, a disrupted mucosal barrier was observed in mice after IL-33 administration, as evidenced by a significantly decreased TEER (Fig. 2 F) and increased influx of Cascade blue-labeled dextran-3K across the intestinal mucosa (0.38 ± 0.11% in vehicle vs. 1.72 ± 0.49% in IL-33-treated, P < 0.019, Student’s t-test, n = 5). Notably, these IL-33-induced changes in intestinal function were entirely absent in MyD88−/− mice (Fig. 2 and data not shown).

STAT6 plays divergent roles in IL-33-induced effects on intestinal smooth muscle vs. epithelial function. Since IL-4 and IL-13 are the two major downstream effector molecules for IL-33 (36), we sought to investigate whether STAT6 signaling plays a role in the IL-33 effects on intestinal function. The intestinal smooth muscle from STAT6−/− mice that received IL-33 did not differ in response to acetylcholine compared with that from mice receiving BSA (Fig. 3A) but retained the hypercontractile response to EFS (Fig. 3B) and the increased amplitude of spontaneous contraction (Fig. 3C). In contrast, no significant difference in the mucosal response to acetylcholine or glucose was detected between STAT6−/− mice treated with IL-33 or BSA (Fig. 3, D and E). In addition, the IL-33-induced decrease in TEER of intestinal mucosa seen in WT mice was absent in STAT6−/− mice (Fig. 3F). These data indicated that IL-33-induced changes in intestinal mucosal function depended entirely on STAT6, whereas the effects on smooth muscle function were only partially dependent on STAT6, suggesting a role for non-IL-4/IL-13 mediators.

Contribution of IL-13 to the effects of IL-33 on intestinal smooth muscle and epithelial function. To further differentiate the contribution of IL-13 vs. IL-4 to mediate the effects of IL-33 on intestinal function, we injected IL-33 into mice deficient in IL-13. IL-13−/− mice had a constitutively lower intestinal smooth muscle response to EFS (5,428 ± 952 in WT vs. 2,437 ± 269 in IL-13−/−, 20 Hz) and smaller amplitude of spontaneous contraction compared with WT mice (3,515 ± 543 in WT vs. 1,679 ± 253 in IL-13−/−). Administration of IL-33 to IL-13−/− mice increased the smooth muscle contractile responses to acetylcholine and EFS, as well as the amplitude of spontaneous contraction (Fig. 4, A–C). The IL-33-induced hypercontractility in IL-13−/− mice was less prominent than that of WT mice. Similarly, IL-33-treated IL-13−/− mice had decreased secretory response to acetylcholine and reduced glucose absorption (Fig. 4, D–F). These data indicated that both IL-13 and IL-4 contribute to the effects of IL-33 on intestinal function.

The dramatically decreased TEER in the intestinal mucosa of IL-33-treated WT mice was not observed in IL-33-treated IL-13−/− mice. Coupled with the data from STAT6−/− mice, it can be concluded that IL-13 is the major downstream effector
molecule of IL-33 that acts on disruption of the intestinal mucosal barrier.

The IL-33-induced smooth muscle hyperplasia of the intestine is dependent on IL-13-mediated activation of STAT6. Smooth muscle hypercontractility of the intestine is often accompanied by smooth muscle hypertrophy/hyperplasia. Thickness of the intestinal smooth muscle layer was therefore examined to determine whether this were the case for IL-33-treated mice. As expected, thickened smooth muscle layers were observed in IL-33-treated WT mice compared with BSA-treated mice (Fig. 5, A and B). However, this smooth muscle hypertrophy/hyperplasia was not found in IL-33-treated STAT6−/− or IL-13−/− mice (Fig. 5, A and B). Goblet cell hypertrophy and hyperplasia were also observed in the jejunal epithelia from mice treated with IL-33, consistent with a previous study (36). There appeared no other evident histological abnormalities in the jejunal epithelia from mice treated with IL-33.

Insulin-like growth factor-1 (IGF-1) and transforming growth factor (TGF)-β were shown previously to contribute to muscle hypertrophy/hyperplasia. Thickness of the intestinal smooth muscle layer was therefore examined to determine whether this were the case for IL-33-treated mice. As expected, thickened smooth muscle layers were observed in IL-33-treated WT mice compared with BSA-treated mice (Fig. 5, A and B). However, this smooth muscle hypertrophy/hyperplasia was not found in IL-33-treated STAT6−/− or IL-13−/− mice (Fig. 5, A and B). Goblet cell hypertrophy and hyperplasia were also observed in the jejunal epithelia from mice treated with IL-33, consistent with a previous study (36). There appeared no other evident histological abnormalities in the jejunal epithelia from mice treated with IL-33.

Insulin-like growth factor-1 (IGF-1) and transforming growth factor (TGF)-β1 were shown previously to contribute to muscle hypertrophy of the intestine (7, 46). IL-33 upregulated expression of IGF-1 in WT, but not in STAT6−/− or IL-13−/− mice, indicating a dependence on the IL-13/STAT6 axis (Fig. 5C). In contrast, IL-33 administration did not affect expression of TGF-β1 in any of the mouse strains examined including WT, MyD88−/−, STAT6−/−, and IL-13−/− mice (data not shown).

IL-33 induced alternative activation of macrophages in the intestine via STAT6-dependent and -independent pathways. Increased infiltration and alternative activation of macrophages (M2) are also characteristics of type 2 immunity (3). Administration of IL-33 to mice increased the expression of F4/80 mRNA, a macrophage marker, in the intestines of WT, but not MyD88−/−, STAT6−/−, or IL-13−/− mice (Fig. 6A). In addition, IL-33 significantly upregulated the expression of M2 markers arginase I, YM-1, and FIZZ1 in WT intestine (Fig. 6, B and C and not shown). This M2 development was absent in MyD88−/− mice. Notably, the expression of M2 markers (arginase I, YM-1) in the intestines of IL-33-treated STAT6−/− mice was significantly higher than that of BSA-treated mice, but also significantly lower than that of IL-33-treated WT mice (Fig. 6, B and C). That IL-13 deficiency in mice did not completely abolish the upregulation of M2 markers induced by IL-33 suggests a role for IL-4 (Fig. 6, B and C). In addition, IL-33 administration induced a significant downregulation of nitric oxide synthase-2, a marker for classically activated macrophages (M1), in the intestines of WT mice that was not seen in IL-33-treated MyD88−/−, STAT6−/−, or IL-13−/− mice.

**DISCUSSION**

IL-33 is a key immune modulator in the gut mucosa and has been implicated in host protective immunity against enteric nematode infection and gut inflammatory pathologies such as ulcerative colitis (16, 29, 30). The present study demonstrated that IL-33 promotes potently type 2 immunity in the intestine. The cytokine response to IL-33 was dependent on MyD88, but not IL-13 or STAT6, whereas the effects on intestinal function...
and macrophage activation were mediated by both STAT6-dependent and -independent mechanisms.

IL-33 is found throughout the GI tract and expressed by many cell types ranging from major innate immune cells to nonhematopoietic cells (16). Epithelial cells in the intestine produce IL-33 that is thought to be important in initiating type 2 immunity (36). IL-33 binds to a heterodimeric receptor consisting of IL-1 receptor accessory protein and ST2, which may lead to activation of MyD88-dependent and -independent signaling pathways. Studies have further identified that NF-κB and all three MAPKs, JNK1/2, ERK1/2, and P38, are among the key signaling components linking IL-33 to various downstream molecules including type 2-related cytokines (IL-4, IL-5, IL-13) and chemokines (CCL2, CCL17, and CCL24) (36). The present study shows that exogenous IL-33 induces upregulation of type 2 cytokines in the intestine including IL-4 and IL-13 that contribute to alterations in intestinal function as well as IL-5 that is linked to eosinophilia (33). The IL-33-promoted type 2 immunity was completely absent in mice deficient in MyD88, consistent with an essential role for the MyD88 pathway. This is also consistent with previous studies showing that IL-33 induced a MyD88-dependent production of IL-5 and IL-13 from mast cells, basophils, and T cells (8, 13, 14). On the other hand, STAT6 is not required for IL-33-induced upregulation of type 2 cytokines despite its essential role in the development of classical Th2 immunity, supporting the concept that innate immune cells, most likely type 2 innate lymphoid cells rather than Th2 cells, are a major source of the type 2 cytokines (40).

IL-25 and TSLP are the other two epithelial-derived cytokines implicated in type 2 immunity. Previous studies showed that mice deficient in IL-25 or TSLP had impaired host protective immunity against nematode infection associated with diminished intestinal smooth muscle and epithelial cell response to the infection (28, 41, 47). IL-25 and IL-33 belong to different cytokine families but share many similarities in promoting type 2 immunity including the responsive cells and downstream effector molecules. Our present study indicated that exogenous IL-33 modestly upregulated IL-25 expression in the intestine, suggesting that the two cytokines may work together. Notably, a downregulation of IL-25 expression was observed when STAT6 or IL-13 was genetically deleted in mice. The underlying mechanisms have not been identified but may involve reciprocal regulation between IL-17 and type 2 cytokines as suggested by a previous study (4). In agreement with that, IL-33-induced inhibition of IL-17A was absent in mice deficient in STAT6 or IL-13. These data also indicate that IL-13 activation of STAT6 plays a critical role in the inhibition of Th17 immunity.

It is well established that type 2 immunity in the GI tract is characterized by changes in intestinal function that are primarily dependent on IL-4/IL-13-activation of STAT6 (18, 19, 38, 43). The present study extended previous findings demonstrating that IL-33-induced upregulation of type 2 cytokines, it was understandable that IL-33-induced changes in intestinal function...
were completely absent in mice lacking MyD88. Interestingly, the STAT6 pathway appeared to contribute distinctively to the IL-33 effects on smooth muscle vs. epithelial function, despite the fact that IL-4 and IL-13 are the major downstream molecules of IL-33 (36). In particular, STAT6 was essential for IL-33-induced alterations in epithelial function, including the decreased response to acetylcholine and glucose as well as increased mucosal permeability. The IL-33-induced effects on epithelial function were only attenuated in IL-13<sup>−/−</sup> mice, suggesting a role for IL-4 that is induced by exogenous IL-33 in IL-13<sup>−/−</sup> mice. These data are consistent with our previous findings in mice with enteric nematode infection or administration of IL-4/IL-13 (18, 19, 38, 43). Unlike a complete STAT6 dependence of epithelial responses, IL-33 affected the smooth muscle function through both STAT6-dependent and -independent mechanisms. Specifically, the hypercontractility

![Graph](image1)

**Fig. 4.** Contribution of IL-13 to IL-33 induced changes in intestinal smooth muscle and epithelial function. Mice deficient in IL-13 were injected with IL-33 or BSA daily for 3 days. Intestinal strips were suspended longitudinally in organ baths for in vitro contractility in response to acetylcholine (10 nM–0.1 mM; A) or EFS (1–20 Hz, 80 V; B) or for spontaneous contraction (C). Muscle-free mucosa were mounted in Ussing chambers for changes in Lsc of epithelial response to (D) acetylcholine (10 nM–1 mM; D) or glucose (E) or in micro-Snapwell for the measurement of TEER (F). Data presented are pooled from the independent experiments with 10 mice per treatment. *P < 0.05 vs. the respective BSA.

were completely absent in mice lacking MyD88. Interestingly, the STAT6 pathway appeared to contribute distinctively to the IL-33 effects on smooth muscle vs. epithelial function, despite the fact that IL-4 and IL-13 are the major downstream molecules of IL-33 (36). In particular, STAT6 was essential for IL-33-induced alterations in epithelial function, including the decreased response to acetylcholine and glucose as well as increased mucosal permeability. The IL-33-induced effects on epithelial function were only attenuated in IL-13<sup>−/−</sup> mice, suggesting a role for IL-4 that is induced by exogenous IL-33 in IL-13<sup>−/−</sup> mice. These data are consistent with our previous findings in mice with enteric nematode infection or administration of IL-4/IL-13 (18, 19, 38, 43). Unlike a complete STAT6 dependence of epithelial responses, IL-33 affected the smooth muscle function through both STAT6-dependent and -independent mechanisms. Specifically, the hypercontractility

![Graph](image2)

**Fig. 5.** IL-33-induced intestinal smooth muscle hypertrophy is accompanied by changes in IGF-1 expression. C57BL/6 or mice deficient in STAT6 or IL-13 were injected with IL-33 or BSA daily for 3 days. A: smooth muscle thickness was assessed under the microscope in sections prepared from frozen blocks of intestines. B: representative pictures taken from hematoxylin and eosin-stained tissue slides of jejunum with 5 mice in each group. C: qPCR was performed to measure mRNA expression of IGF-1. The fold changes were relative to WT-BSA after normalization to 18s rRNA. Data in bar graphs are pooled from the independent experiments with 10 mice per treatment. *P < 0.05 vs. the respective BSA.
response to acetylcholine depended entirely on STAT6, whereas the response to EFS and increased spontaneous contractions relied only partially on STAT6. Acetylcholine induced muscle contraction via binding directly to the muscarinic receptor M3 on muscle cells coupled to Gq protein, leading to activation of phospholipase C (26). It is known that IL-4 and IL-13 can increase the expression and affinity of muscarinic receptors as well as induce muscle hypertrophy via STAT6 (1, 2). Therefore, it can be inferred that IL-33 induced hypercontractility to acetylcholine via IL-4/IL-13-activation of STAT6. The significantly attenuated, but not abolished, hypercontractile response to acetylcholine in IL-33-treated IL-13/−/− mice emphasized the contributions of both IL-4 and IL-13. GI smooth muscle activities are coordinated by enteric nerves that innervate the muscle. Emerging evidence indicated that inflammation or infection can alter GI functions through immune mediators that interact with and influence the neurophysiological, neurochemical, and morphological properties of enteric nerves (20). IL-33 induced a hypercontractile response to EFS and increased spontaneous contractions that were retained partially in both IL-4 and IL-13. GI smooth muscle activities are coordinated by enteric nerves that innervate the muscle. Emerging evidence indicated that inflammation or infection can alter GI functions through immune mediators that interact with and influence the neurophysiological, neurochemical, and morphological properties of enteric nerves (20). IL-33 induced a hypercontractile response to EFS and increased spontaneous contractions that were retained partially in both IL-4 and IL-13. One possible explanation is that IL-33 increased the production of IL-4 that, in turn, acted on enteric nerves in a STAT6-independent manner as demonstrated by our previous study (43). Alternatively, IL-33 may interact with enteric nerves directly or indirectly via mediators other than IL-4/IL-13. This speculation will be investigated in future studies.

Increased production of growth factors, such as IGF-1 and TGF-β1, and M2 macrophage producing arginase I contribute to smooth muscle hypertrophy (7, 46). IL-33-induced intestinal smooth muscle hypertrophy was associated with upregulation of arginase I and IGF-1, but not TGF-β1. There was a disassociation between arginase I upregulation and muscle hypertrophy in IL-33-treated mice that lack STAT6 or IL-13 and a temporally correlated expression level of IGF-1 with muscle hypertrophy in all strains of mice. Thus the IL-13-STAT6-IGF-1 axis, but not arginase I pathway, was the major pathway for IL-33-induced smooth muscle hypertrophy. It is likely that the smooth muscle hypertrophy, together with the hyperactivity of the smooth muscle/nerves shown by our previous studies (43–45), contributes to the hypercontractility of intestinal smooth muscle.

In the intestine, IL-33 increased macrophage infiltration through IL-13 activation of STAT6 and MyD88 pathways. IL-33-induced M2 development was consistent with previous observations in the lung, spleen, and draining lymph nodes (9, 43–45).
15). Both human and mouse macrophages were previously shown to express functional receptor for IL-33 (6, 10, 21). Direct stimulation of naive macrophages with IL-33 induced the expression of chemokines, reduced foam cell formation, and rendered macrophages more susceptible to M1 or M2 stimuli (6, 10, 21). IL-4 and IL-13 activate macrophages through STAT6, but our data showed that a STAT6-independent pathway was also involved in IL-33-induced M2 development in the intestine. Whether this was due to a direct effect of IL-33 or indirectly through mediators other than IL-4/IL-13 remains to be determined.

It should be noted that present study did not address the role of constitutively expressed IL-33 in the control of intestinal immunity and function. However, mice deficient in MyD88, a pivotal signaling molecule for IL-33 effects, had no defects in the expression of major cytokines and macrophage markers as well as functions of intestinal epithelial and smooth muscle, suggesting that constitutively expressed IL-33 may not be critical for physiological control of intestinal immunity and function. On the other hand, exogenous IL-33 induced characteristic changes in intestinal function similar to that induced by enteric nematode infection that features IL-33 upregulation (18, 19, 38, 43). Therefore, we speculate that IL-33 plays an important role in the pathophysiological regulation of intestinal immunity and function during nematode infection. In summary, the present study established that exogenous IL-33 had potent effects on intestinal immunity and function. IL-33 binds to the receptor ST2 and activates MyD88 signaling pathway leading to increased production of type 2-related cytokines and chemokines. The downstream effector molecules further induce characteristic alterations in intestinal function including smooth muscle hypercontractility/hypertrophy, epithelial hyposecretion, and increased mucosal permeability via STAT6-dependent and -independent mechanisms (Fig. 7).

The ability of IL-33 to promote M2 while suppressing M1 development, and downregulate the expression of IL-17A, further supports a key role for this cytokine in mucosal homeostasis. Thus manipulation of IL-33 or its related signaling pathways represents a potential therapeutic strategy for treating inflammatory diseases associated with dysregulated intestinal function.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


