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Participation of MyD88 and Interleukin-33 as Innate Drivers of Th2 Immunity to *Trichinella spiralis*

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Trichinella spiralis **is a highly destructive parasitic nematode that invades and destroys intestinal epithelial cells, injures many different tissues during its migratory phase, and occupies and transforms myotubes during the final phase of its life cycle. We set out to investigate the role in immunity of innate receptors for potential pathogen- or danger-associated molecular patterns (PAMPs or DAMPs). Focusing on the MyD88-dependent receptors, which include Toll-like receptors (TLRs) and interleukin-1 (IL-1) family members, we found that MyD88-deficient mice expelled worms normally, while TLR2/4-deficient mice showed accelerated worm expulsion, suggesting that MyD88 was active in signaling pathways for more than one receptor during intestinal immunity. A direct role for PAMPs in TLR activation was not supported in a transactivation assay involving a panel of murine and human TLRs. Mice deficient in the IL-1 family receptor for the DAMP, IL-33 (called ST2), displayed reduced intestinal Th2 responses and impaired mast cell activation. IL-33 was constitutively expressed in intestinal epithelial cells, where it became concentrated in nuclei within 2 days of infection. Nuclear localization was an innate response to infection that occurred in intestinal regions where worms were actively migrating. Th2 responses were also compromised in the lymph nodes draining the skeletal muscles of ST2-deficient mice, and this correlated with increased larval burdens in muscle. Our results support a mechanism in which the immune system recognizes and responds to tissue injury in a way that promotes Th2 responses.**

The parasitic nematode *Trichinella spiralis* induces potent Th2 responses that both clear intestinal infections and regulate immunity in extraintestinal sites (1–5). The innate cellular and molecular drivers of Th2 immunity appear to vary among nematode infections, and the identification of mechanisms that initiate Th2 responses continues to be an area of active research (reviewed in reference 6). Key roles for pattern recognition receptors, including Toll-like receptors (TLRs), Nod-like receptors (NLR), and C-type lectins, have been described for Th2 responses (7–13), although a central paradigm of Th2 activation that parallels that of TLR/interleukin-12 (IL-12) and Th1 immunity has yet to be defined. TLR4 has been implicated in Th2 immunity in several different systems, including the response to parasitic worms, ovalbumin (OVA)-lipopolysaccharide (LPS), and house dust mite allergen (8, 14–17). In an indirect mechanism, eosinophil-derived neurotoxin promotes Th2 immunity by binding to TLR2 (18). MyD88 is a signaling adaptor molecule that propagates the response to most TLRs, as well as responses to IL-1 family members, including IL-18 and IL-33 (19–21). MyD88 binds to the intracellular Toll/IL-1 receptor (TIR) domain of these receptors (directly or via a bridging adaptor such as MyD88-adaptor-like [MAL]), where it initiates a signaling cascade that ultimately leads to the activation of proinflammatory genes by the transcription factor NF-κB (22, 23).

Trichinella spiralis is a highly destructive pathogen that is cleared from the intestine by a Th2-driven mechanism that depends upon mucosal mast cells (24, 25). Infection is initiated when first-stage larvae (L_1) invade the intestinal epithelium. As they penetrate and move through epithelial cells, larvae and adult worms release glycoproteins that bear both novel and conserved glycan modifications (26–29). One of these glycans incorporates the dideoxyhexose tyvelose (26, 30). Another dideoxyhexose, ascarylose, is found in ascaroside lipids in nematodes across the phylum *Nematoda* (31), suggesting potential for these molecules to serve as pathogen-associated molecular patterns (PAMPs). A second glycan modification in *T. spiralis* first-stage larvae incorporates phosphorylcholine. This structure is also found in filarial worms and is documented to bind TLR4 (14).

Epithelial cells occupied by the worm do not survive, and migrating larvae and adult worms leave behind trails of dead cells (32). Adult female worms in the intestine release newborn larvae that may migrate through a variety of tissues prior to finding skeletal muscle where they penetrate, occupy, and transform myotubes. Thus, cellular injury and death are prominent features of all aspects of *T. spiralis* infection, and both are known to influence adaptive immunity in other contexts, for example, binding of host cell actin by DNGR-1 on dendritic cells or macrophage uptake of apoptotic neutrophils (33, 34).

Necrotic cells release so-called alarmins, or danger-associated molecular patterns (DAMPs), such as IL-33 and high-mobility group protein B1 (HMGB1), which can give rise to inflammation (35–37). The receptor for IL-33 is ST2, a member of the IL-1 family of cytokine receptors (20, 38). Unlike other IL-1 family cytokines, IL-33 is released in its active form by necrotic cells (36,

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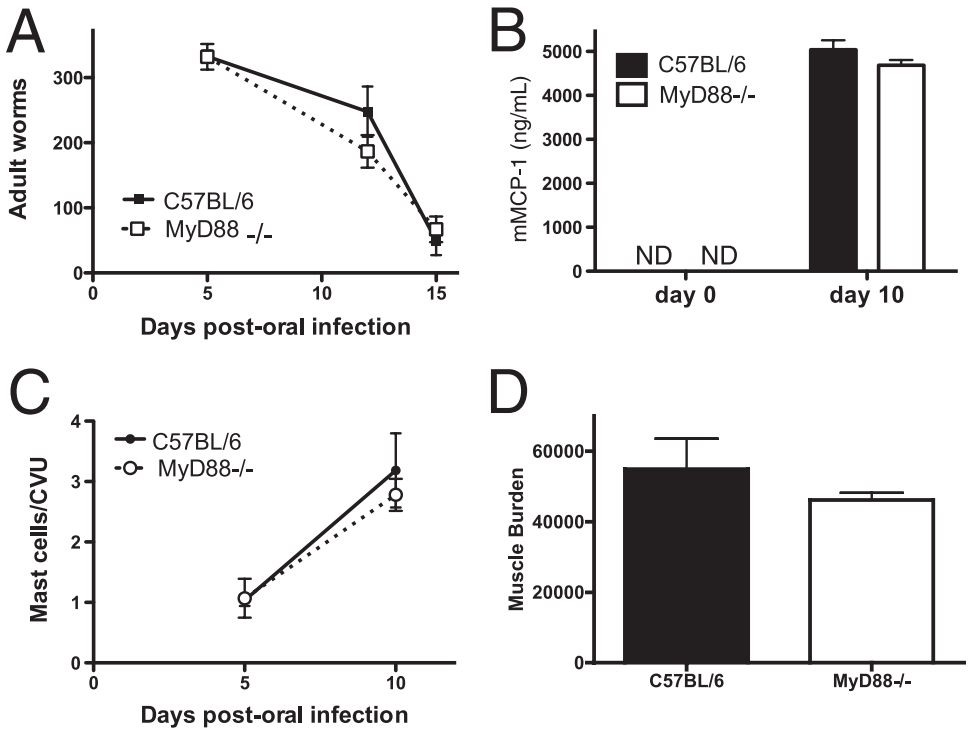

FIG 1 The role of MyD88 in immunity to *T. spiralis*. MyD88$^{-/-}$ mice and C57BL/6 littermates were infected orally with 400 L_1, and data were collected on the days indicated. (A) Intestinal worm burdens; (B) serum mMCP-1 concentrations; (C) jejunal mast cell numbers, as defined by mMCP-1 staining; (D) larval burdens in muscle, 28 days postinfection. Values shown are means ± standard deviations (SD). ND, not detected.

39, 40). Normally found in epithelial and endothelial cells, adipocytes, astrocytes, and airway smooth muscle cells, IL-33 may also be detected in mast cells, dendritic cells, and monocytes under inflammatory conditions (41–47).

We hypothesized that *T. spiralis* may promote Th2 immunity in three ways: directly via engagement of host receptors by parasite-derived molecular patterns, indirectly by enabling access of intestinal microbiota to normally sequestered TLRs, or by the triggering release of DAMPs by injured host cells. Our findings support a mechanism involving ST2, the receptor for IL-33, and show that in the intestine, the early innate response of intestinal epithelial cells (IECs) to *T. spiralis* infection includes redistribution of IL-33 within cells. Late in infection, muscle larval burdens in ST2-deficient mice were significantly higher than in wild-type controls, suggesting that IL-33 is a key regulator that limits parasite survival.

MATERIALS AND METHODS

Mice and rats. All mouse strains were on a C57BL/6 background. TLR2/4$^{-/-}$, MyD88$^{-/-}$, and MyD88$^{+/+}$ littermates (provided by S. Akira, Osaka, Japan) (48), C57BL/6, and RAG2$^{-/-}$ mice (Taconic) were bred and/or maintained at Cornell under specific pathogen-free conditions according to AAALAC guidelines. Albino Oxford (AO) rats served as parasite donors. Rodents were infected at 7 to 10 weeks of age and euthanized by CO_2 inhalation, and blood was collected by cardiac puncture. Experiments performed at Cornell University were approved by the IACUC.

ST2$^{-/-}$ mice (provided by A. McKenzie, Cambridge, United Kingdom) (49) were bred and maintained at Trinity College Dublin in compliance with Irish Department of Health and Children regulations and with the approval of Trinity College Dublin's BioResources ethical review board.

Parasite and infections. Maintenance of *Trichinella spiralis* (pig strain), L_1 recovery, and intestinal worm or muscle larval burdens were estimated as described previously (3, 50, 51). Mice were infected by oral gavage with 400 L_1. Crude L_1 extract, excretory-secretory (ES) antigens, and purified phosphorylcholine-bearing proteins were prepared as described previously (27, 52).

Culture of leukocytes and cytokine ELISA. Cells from cervical lymph nodes (CLN) or mesenteric lymph nodes (MLN) were cultured, and supernatants were assayed for IL-4, IL-5, and IL-10 by enzyme-linked immunosorbent assay (ELISA), as described previously (53). Similar assays were used for IL-13 (eBioscience, 2 μg/ml capture clone ebio13A, 0.2 μg/ml detection antibody clone eBio1316H) and IFN-γ (BD Biosciences, 1 μg/ml capture antibody clone AN-18; eBioscience, 0.125 μg/ml detection antibody clone XMG1.2), using recombinant cytokine standards (eBioscience). Mouse mast cell protease-1 (mMCP-1) was measured in serum by ELISA (eBioscience).

Histology. For evaluation of skeletal muscle, tongues were fixed in formalin. Samples of small intestine were cut longitudinally, prepared as Swiss rolls (54), and fixed in formalin. Proximal and distal small intestine (SI) were collected as separate rolls. Immunohistochemistry (IHC) was performed as previously described to detect inducible nitric oxide synthase (iNOS) (53) or IL-33 (3 μg/ml goat anti-IL-33 antibody [R&D Systems] with 0.6 μg/ml biotinylated rabbit anti-goat antibody [Vector Laboratories]) and mMCP-1 (5 μg/ml rat anti-mMCP-1 antibody [R&D Systems] with 1 μg/ml biotinylated rabbit anti-rat antibody [Vector Laboratories]). Cells positive for mMCP-1 were counted per crypt-villus unit (CVU) for a minimum of 50 CVU per section. Microscopy was performed using a BX51 microscope, and images were captured with Microsuite Basic Edition software (Olympus).

Evaluation of nuclear IL-33. Histologic sections of intestine were blind scored for nuclear localization of IL-33 in epithelial cells. Briefly, the percentage of a given villus epithelium that demonstrated nuclear IL-33 staining was estimated to be 0 to 25%, 25 to 50%, 50 to 75%, or 75 to 100%. At least 100 villi were counted per section (proximal or distal SI of a single mouse). The data are reported as the percentage of villi in each

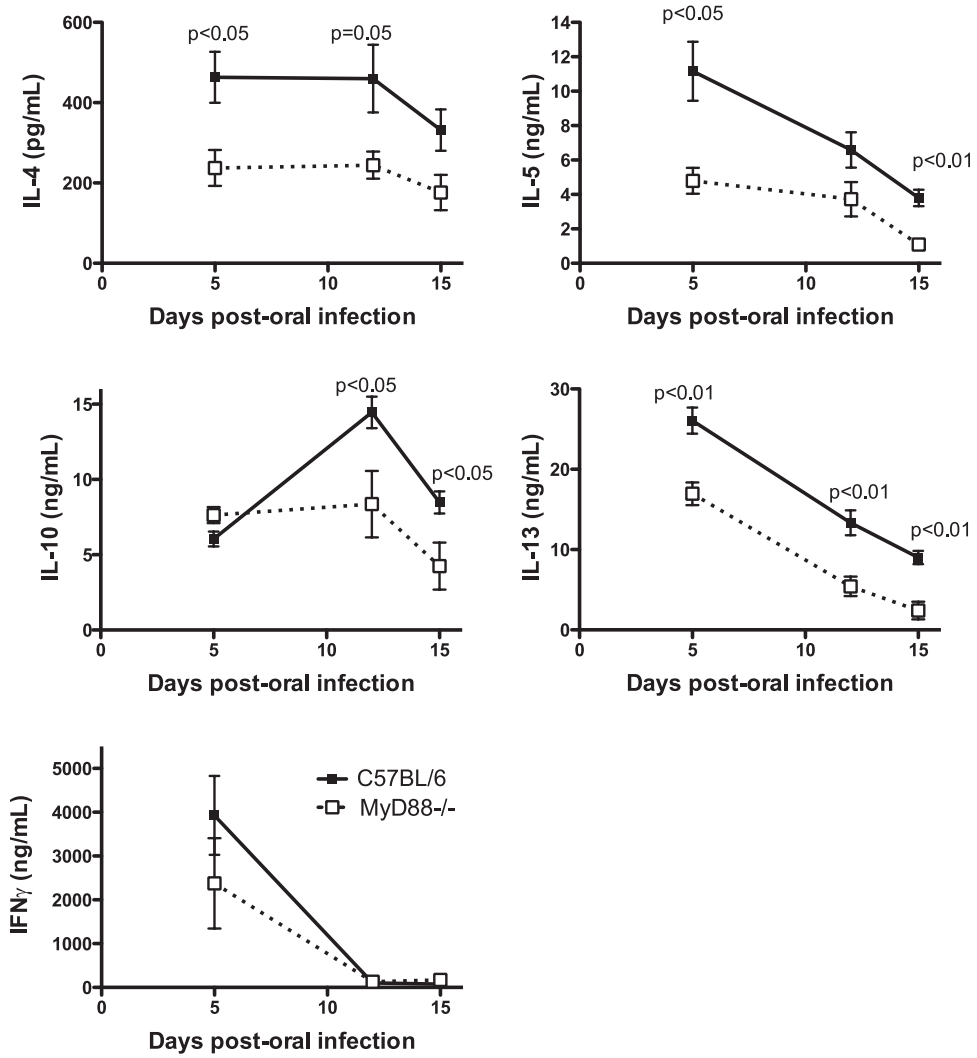

FIG 2 Cytokine responses in the MLNs of MyD88$^{-/-}$ mice. MLN cells were collected from infected mice on the days indicated. Cytokines were assayed in cell cultures following stimulation with *T. spiralis* cAg. Values shown are means ± SD.

range for a given tissue section. Those values were assigned to the mouse, and means were calculated for each group of mice.

TLR transactivation assay. HEK293 cells were transfected with an NF-κB luciferase reporter construct, a constitutive β-galactosidase construct (to control for transfection efficiency), and a plasmid encoding the indicated TLR, as previously described (55). Cells were cultured overnight with crude parasite extract (10 μg/ml), ES antigens (1 μg/ml), or purified phosphorylcholine-bearing proteins (2 μg/ml), each at the highest concentration determined by titration to be nontoxic for the cells. Data were analyzed by determining the fold induction of NF-κB luminescence relative to that of the β-galactosidase control (relative light units [RLU]). Peptidoglycan, poly(I:C), LPS, flagellin, loxoribine, CpG DNA, and profilin were used as positive controls.

Antibiotic treatment. C57BL/6 mice were provided with drinking water containing 0.5 mg/ml bacitracin and streptomycin (Sigma-Aldrich), *ad libitum*, starting 1 week prior to *T. spiralis* infection. This protocol has been previously characterized as clearing all culturable fecal bacteria (56). To confirm antibiotic efficacy, fecal pellets were cultured on LB agar plates under aerobic conditions, and results are reported as CFU per gram of feces.

Quantitative RT-PCR. Longitudinal, 3-cm sections of jejunum and ileum were collected from infected and uninfected C57BL/6 mice. Peyer's patches were removed, total RNA was isolated from the remaining tissue following disruption in TRIzol (Invitrogen) with a handheld homogenizer (Fisher Scientific), and cDNA was prepared (SuperScript III first-strand cDNA synthesis system; Invitrogen). A sample lacking reverse transcriptase served as a negative control. IL-33 expression was measured with TaqMan quantitative reverse transcription-PCR (qRT-PCR) ($\Delta\Delta C_T$ method) using an ABI Prism 7500 sequence detection system. HPRT expression (not shown) was not affected by infection and served as the endogenous control. Primers and probes were purchased (Applied Biosystems).

Statistical analysis. Each experiment was performed at least twice with 3 to 8 mice or samples per group. Data were analyzed using Student's *t* test or analysis of variance (ANOVA) with Tukey's *post hoc* test for multiple means. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

MyD88 is required for amplification of the Th2 cytokine response to infection. To investigate a role for innate receptors in the response to *T. spiralis* infection, we infected mice deficient in MyD88. Adult worm expulsion, serum mMCP-1, and intestinal

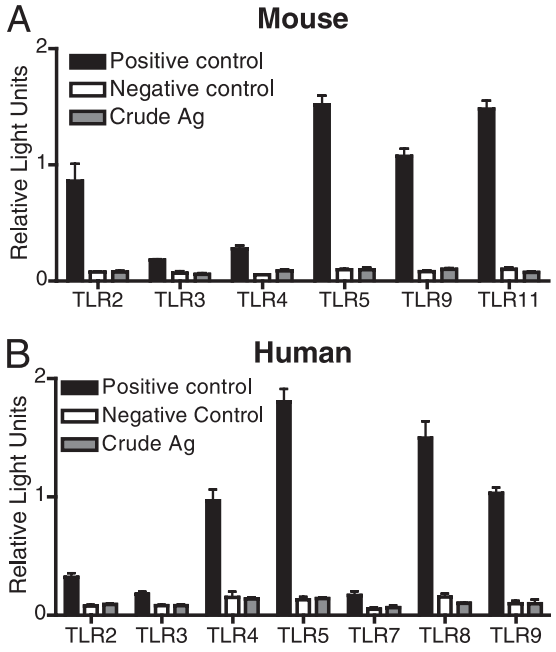

FIG 3 TLR responses to *T. spiralis* extracts *in vitro*. Activation of mouse (A) and human (B) TLRs by *T. spiralis* crude extract or the appropriate positive control (see Materials and Methods) for each TLR in an HEK293 cell luciferase reporter assay. Values are means ± SD. Each positive control was significantly different from the negative control for that receptor ($P < 0.05$).

mast cells were similar in MyD88$^{-/-}$ mice and littermate controls (Fig. 1A, B, and C). Muscle larval burdens were also similar between the two strains, confirming that MyD88 deficiency did not alter the outcome of infection (Fig. 1D). Nevertheless, in cell cultures from MyD88$^{-/-}$ MLN, the canonical Th2 cytokines IL-4, IL-5, and IL-13 were significantly diminished, as was IL-10, while there was no difference in gamma interferon (IFN-γ) production (Fig. 2). There were no differences in cytokine production between the groups in the absence of antigen stimulation; cytokines in most unstimulated cultures were at or below the detection limit of the ELISA (data not shown).

***T. spiralis* does not directly activate TLRs *in vitro*.** In order to determine whether MyD88-dependent enhancement of cytokine responses was promoted by PAMPS interacting directly with TLRs, we assayed extracts and purified glycoprotein preparations from first-stage *T. spiralis* larvae in a TLR transactivation assay. Crude extracts, containing both large and small molecules, did not stimulate any of the human or mouse receptors included in the panel (Fig. 3). We also tested the glycoprotein-rich excretory-secretory products and phosphorylcholine-bearing glycoproteins purified from first-stage larvae. All results were negative (not shown), suggesting that the MyD88-dependent effect was not driven by a direct interaction of larval molecular patterns with the TLRs assayed.

Influence of the microbiota on Th2 immunity. To determine

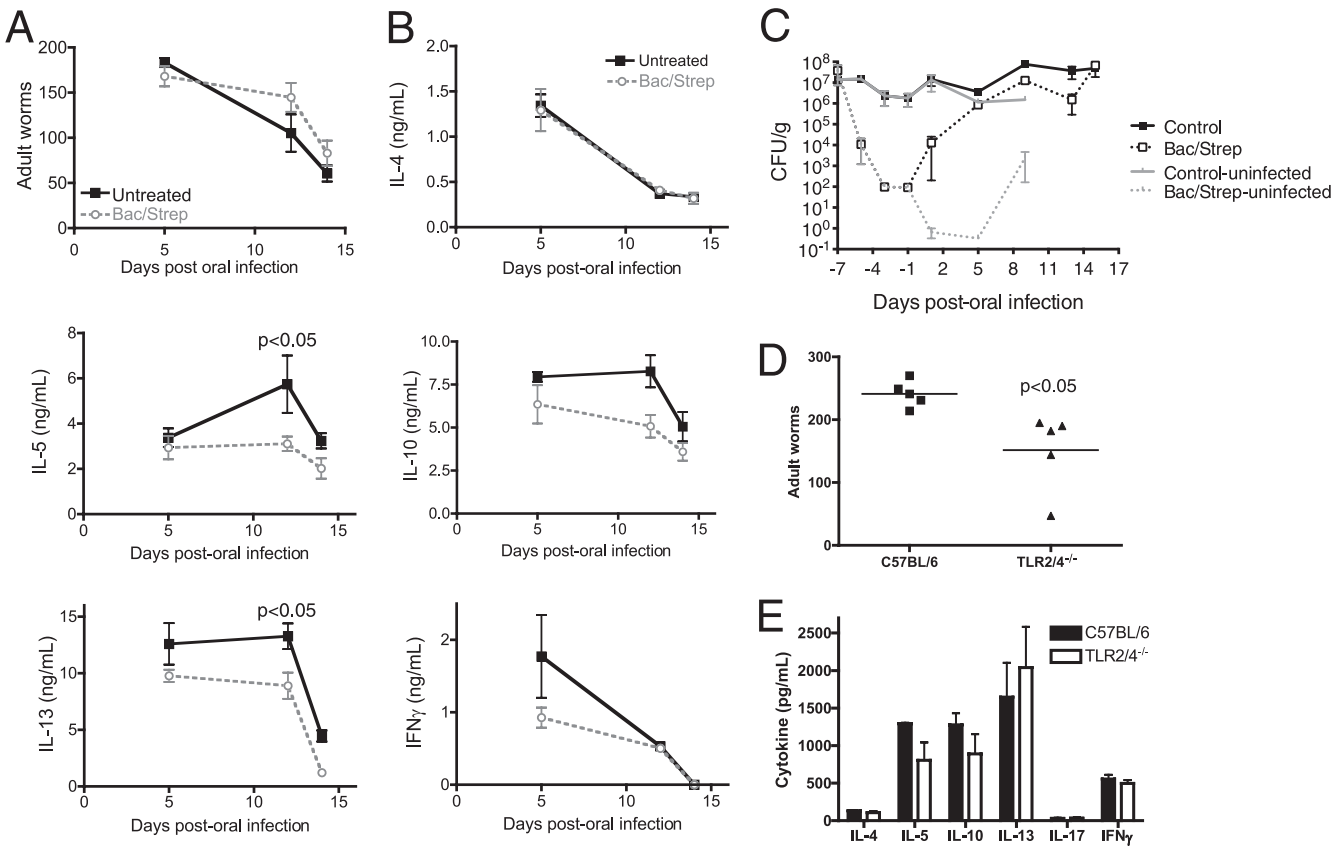

FIG 4 Effect of the intestinal microbiota and TLR2/4 on intestinal immunity to *T. spiralis*. (A to C) C57BL/6 mice were treated with bacitracin and streptomycin for 7 days prior to oral infection with 400 L_1 (on day 0), and data were collected on the days indicated. (A) Intestinal worm burdens; (B) cytokines in cAg-restimulated MLN cell cultures; (C) fecal bacteria (CFU/g); (D and E) TLR2/4$^{-/-}$ and C57BL/6 mice were infected with 400 L_1; (D) intestinal worm burdens at 12 days postinfection; (E) cytokines in cAg-restimulated MLN cells collected 12 days postinfection. Values shown are means ± SD.

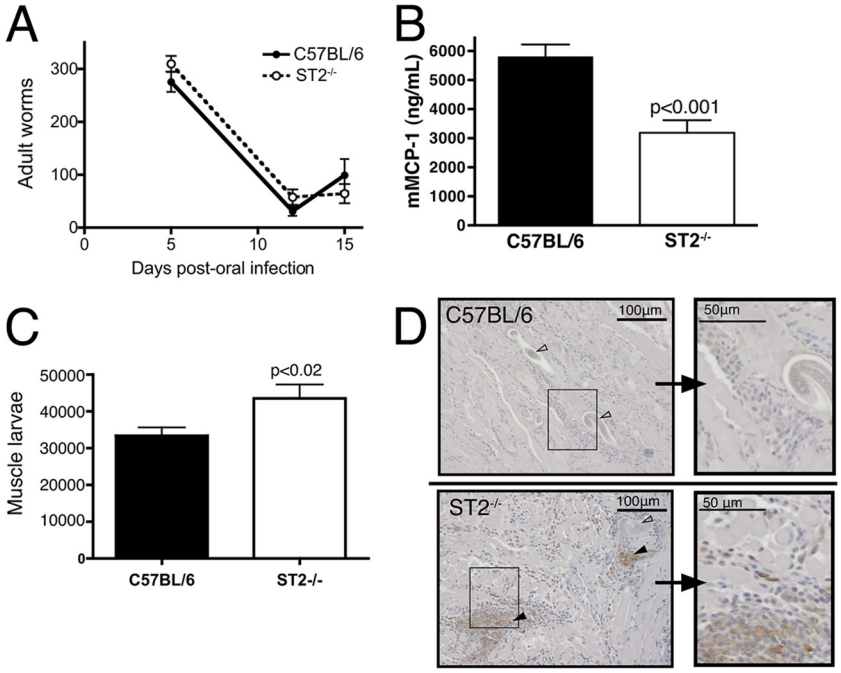

FIG 5 The role of ST2 in protective immunity to *T. spiralis*. ST2$^{-/-}$ and C57BL/6 mice were infected orally with 400 L_1. (A) Intestinal worm burdens; (B) serum mMCP-1 concentrations, 15 days postinfection; (C) muscle burdens at 28 days postinfection; (D) immunohistochemical detection of iNOS in the tongue (muscle), 17 days postinfection. Filled arrows indicate areas of brown iNOS staining; hollow arrows indicate cross sections of larvae. Thin boxes show areas included in zoom image (on right). Values shown are means ± SD.

whether the results obtained in MyD88-deficient mice were attributable to infection-induced disturbance of the intestinal microbiota such that microbes gained access to TLRs, we employed a treatment regimen that has been reported to eliminate all culturable bacteria from the intestine (56). Antibiotic treatment had no effect on parasite expulsion (Fig. 4A) or IL-4 production in MLN cultures (Fig. 4B). IFN-γ and IL-10 were not reduced significantly, and IL-5 and IL-13 were reduced at a single time point, consistent with a modest contribution of the intestinal microbiota toward inducing the intestinal Th2 response to *T. spiralis*. We observed that culturable fecal bacteria were effectively eliminated after 1 week of antibiotic treatment; however, *T. spiralis* infection induced growth of antibiotic-resistant bacteria (Fig. 4C). A similar pattern of bacterial clearance followed by infection-induced bacterial growth was observed in animals treated with enrofloxacin (not shown). Compared with results of the bacitracin-streptomycin treatment, enrofloxacin had an equally modest effect on Th2 cytokine production (not shown).

Because TLR2 and TLR4 bind bacterial products and have been implicated as drivers of other Th2 responses, we infected mice deficient in both receptors. In contrast to what was observed in MyD88$^{-/-}$ and antibiotic-treated mice, TLR2/4$^{-/-}$ mice showed accelerated worm expulsion (Fig. 4D), together with MLN cytokine responses similar to wild-type mice (Fig. 4E). Taken together, the results indicate that although TLRs are not directly activated in a MyD88-dependent fashion by larval products, and intestinal bacteria have only a modest influence on immunity, TLR2/4 are influential (perhaps via MyD88-independent signaling) by limiting rather than promoting intestinal immunity.

ST2$^{-/-}$ mice have impaired Th2 immunity. In the absence of compelling evidence that TLRs promote Th2 immunity to *T. spiralis*, we turned our attention to another MyD88-dependent receptor, the IL-33 receptor ST2 (19, 38). Similar to MyD88$^{-/-}$ mice, ST2$^{-/-}$ mice displayed normal parasite expulsion (Fig. 5A). (Although the number of intestinal worms recovered from both strains did not decline significantly between days 12 and 15 of infection in the experiment shown, a second experiment confirmed that there was no difference between the strains and that worms were largely eliminated by day 15.) Unlike MyD88$^{-/-}$ mice, serum mMCP-1 was reduced (Fig. 5B) consistent with an influence of ST2 on mastocytosis or mast cell activation. Reduction of Th2 cytokine production by MLN cells was evident in ST2$^{-/-}$ mice, particularly for IL-5 and IL-13 (Fig. 6A).

In contrast to MyD88$^{-/-}$ mice, larval burdens in the muscle tissue of ST2$^{-/-}$ mice were significantly higher than in those of wild-type controls (Fig. 5C). This increase was associated with a strongly diminished Th2 cytokine response by CLN cells (Fig. 6B). Although IFN-γ production in the draining lymph node was not increased in ST2$^{-/-}$ mice relative to controls, the robust infiltration of infected tongue muscle by iNOS^{+} leukocytes in ST2$^{-/-}$ mice (Fig. 5D) may indicate Th1 skewing in the local milieu. Overall, the impact on infection and immunity was greater for ST2 deficiency than MyD88 deficiency.

Cellular distribution of IL-33 is altered by infection. Transcription of IL-33 in intestinal tissue was not upregulated by infection (Fig. 7A); however, the distribution of IL-33 protein within epithelial cells changed dramatically in regions where parasites were actively migrating (Fig. 7B). Prior to infection, IL-33 was diffusely distributed in the cytoplasm of IECs, as well as some leukocytes in the lamina propria. Two days after infection, IL-33 was concentrated in the nuclei of IECs. The change in the numbers of cells that demonstrated nuclear localization was statistically significant in

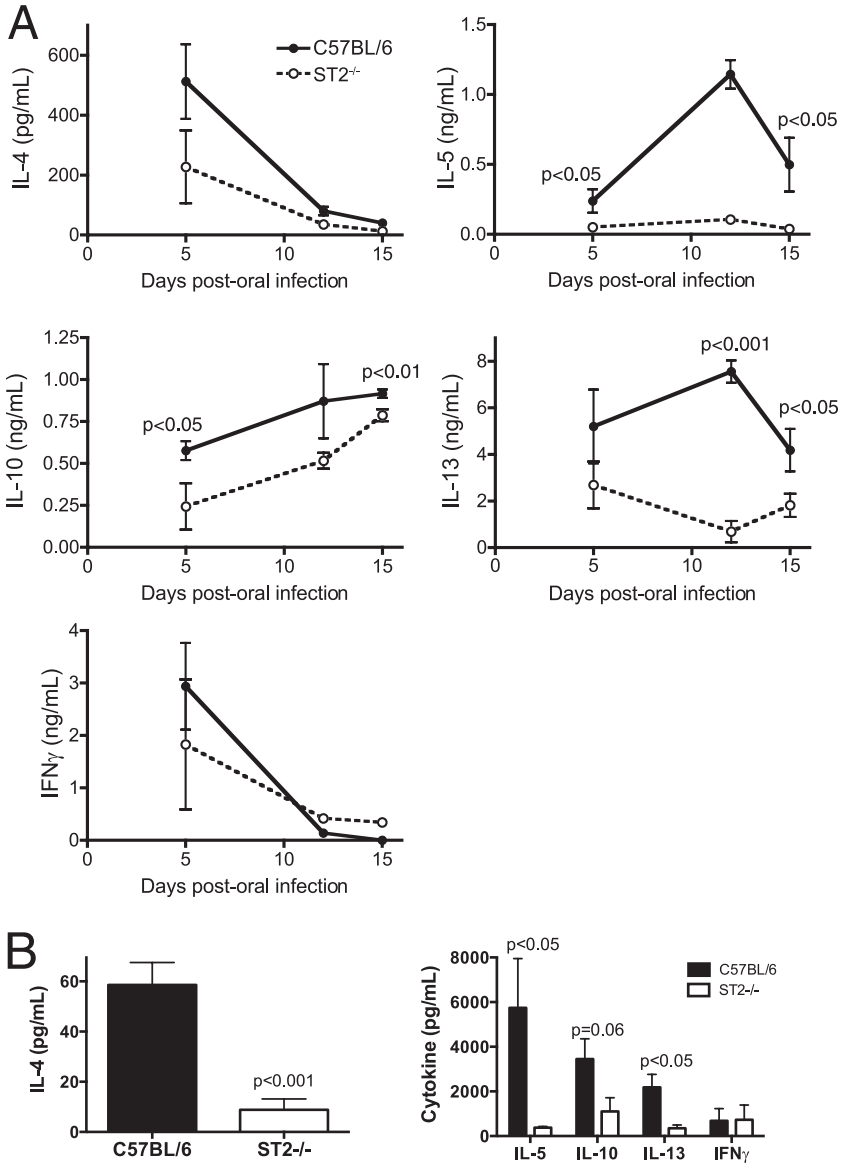

FIG 6 Cytokine response of lymph node cells during infection of ST2$^{-/-}$ mice. (A) MLN cells were collected from infected mice on the days indicated. Cytokines were assayed in cell cultures following stimulation with *T. spiralis* cAg. (B) Cytokines in cAg-restimulated cultures of CLN cells collected during muscle infection, 17 days postinfection. Values shown are means ± SD.

the proximal SI by day 2 and in the distal SI by day 4 (Fig. 7C), consistent with the distal migration of worms during the course of infection (57). Epithelial IL-33 redistribution also occurred at day 4 postinfection in the proximal SI of RAG2$^{-/-}$ (Fig. 7D), MyD88$^{-/-}$, and ST2$^{-/-}$ (not shown) mice. Thus, nuclear concentration of IL-33 in IECs is an innate response that is not driven in an autocrine fashion, is not triggered by MyD88-dependent signaling, and occurs independently of the adaptive immune response.

DISCUSSION

IL-4 is essential for efficient elimination of intestinal adult *T. spiralis* (2). Although the threshold of Th2 response necessary for expulsion of *T. spiralis* has not been defined, our findings suggest that relatively weak responses are sufficient for normal worm expulsion. MyD88 deficiency weakened the Th2 response without compromising parasite clearance. Interpretation of this result is complicated by the fact that MyD88 serves as a signaling adaptor for a variety of TLRs and IL-1 family receptors, and it is possible that there are contradictory influences mediated by different receptors. For example, we found that TLR2/4$^{-/-}$ mice expelled worms more rapidly, while MyD88$^{-/-}$ did not. In another example, IL-18 is an IL-1 receptor family member that is known to suppress mastocytosis and the Th2 cytokine response to *T. spiralis* (21, 58); however, we found that another IL-1 receptor family member, ST2, is necessary for the Th2 response to be fully manifest. Thus, the more modest effects observed in MyD88$^{-/-}$ mice may represent the conflicting influences of molecules that suppress (e.g., IL-18, TLR2/4) and promote (e.g., IL-33) Th2 cytokine and effector responses.

First-stage *T. spiralis* larvae initiate intestinal infection, making

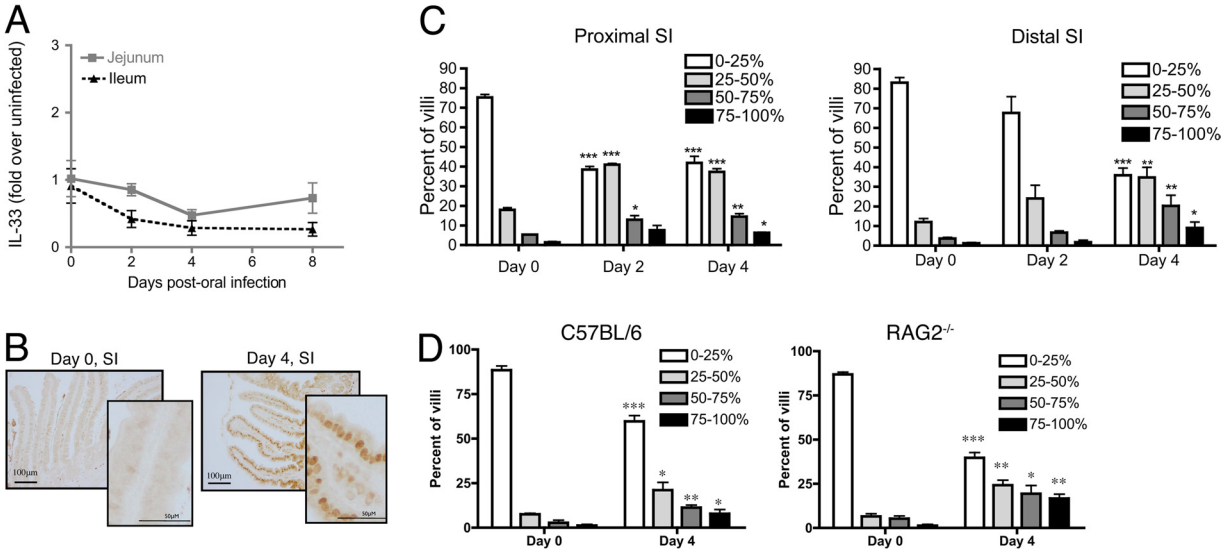

FIG 7 IL-33 gene expression and protein distribution in the small intestine during *T. spiralis* infection. (A) IL-33 mRNA isolated from the jejunum and ileum of infected C57BL/6 mice was measured using TaqMan qRT-PCR. Values shown are means ± SD. (B) Immunohistochemical detection of intestinal IL-33 showing nuclear concentration of IL-33 during infection of the SI. (C) Quantification of epithelial cells per villus displaying nuclear IL-33, expressed as the percentage of total villi counted in the proximal and distal small intestines of C57BL/6 mice. (D) Quantification of epithelial cells per villus displaying nuclear IL-33 in the proximal SI of RAG2$^{-/-}$ and control mice. Values shown are means ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared to day 0.

first contact with the innate immune system. We did not find evidence in support of direct activation of TLRs by larval extracts (containing both large and small molecules), phosphorylcholine-bearing glycoproteins, or ES products that are dominated by dideoxyhexose-bearing glycoproteins. Nevertheless, deficiency of TLR2 and TLR4 did impact worm expulsion, demonstrating that these receptors are engaged during *T. spiralis* infection. One hypothesis regarding the mechanism of this TLR2/4-dependent effect is that impaired gut homeostasis in the absence of these TLRs contributes to a "leaky gut," thus promoting parasite expulsion. *T. spiralis* infection occurs within the intestinal epithelial layer, which is exposed to vast numbers of bacteria (100 trillion bacteria representing 1,000 different species). Under homeostatic conditions, intestinal bacteria do not promote intestinal inflammation (59, 60), and most remain physically separated from the epithelium by a 50-μm zone maintained by the secreted antibacterial lectin RegIIIγ (61, 62). The destruction of the epithelial monolayer by *T. spiralis* would likely disturb this protective zone or perhaps alter the composition of the microbiota, providing access to intestinal cells for bacterial TLR ligands; however, our findings document that dysbiosis (as we observed during antibiotic treatment) had only a modest impact on local Th2 responses and no effect on worm clearance from the intestine. The contradiction of results from antibiotic treatment and TLR2/4$^{-/-}$ mice may be rationalized if TLR2 and TLR4 are activated by ligands other than bacterial cell wall constituents during *T. spiralis* infection or if their activation by bacteria is normally masked by more potent innate responses, as described above. The dysbiosis we observed during infection is not surprising given the many factors (e.g., diet, immune milieu) that are known to affect the composition of the gut microbiota. It seems unlikely that bacteria were carried into the mouse by the parasite, as there is no evidence that *T. spiralis* carries bacterial symbionts; genomic studies performed on larvae, prepared by the same protocol used here, failed to identify bacterial sequences (63). It seems more probable that an existing commensal strain was given the opportunity for robust growth in the infected gut when competing, antibiotic-sensitive bacteria had been eliminated.

Innate receptors may also recognize ligands released by host cells when they are injured during cellular invasion and parasite migration. Indeed, observations in ST2-deficient mice support a role for DAMPs in promoting the Th2 response and parasite clearance. *T. spiralis* worms are much larger than the epithelial cells they invade, and they kill large numbers of cells as they migrate (32, 64). IL-33 in the cytoplasm of epithelial cells would be released when worms penetrate, damage, and then vacate the cells.

Roles for ST2 and IL-33 have been tested in other parasitic worm infections with variable results. Administration of recombinant IL-33 amplifies Th2 immunity to *Trichuris muris* (65). In contrast, ST2$^{-/-}$ mice infected with *Nippostrongylus brasiliensis* manifest normal Th2 responses (66). Thus, it appears that the key mediators of innate immunity vary among worm infections in a manner similar to the variation in effector mechanisms that have been documented for different parasites. These distinctive responses may reflect behaviors or properties intrinsic to each parasite or related to the distinct habitats they occupy.

Our results show that the robust Th2 cytokine response generated after *T. spiralis* infection of mice is dependent on ST2. Although intestinal worms were expelled normally in ST2$^{-/-}$ mice, the concentration of a key mediator in worm expulsion, mMCP-1, was reduced in sera of infected mice, consistent with a diminished mast cell response. Furthermore, the number of larvae that colonized and survived in the muscle was increased in ST2$^{-/-}$ mice, providing evidence that IL-33 binding to ST2 is important in limiting extraintestinal parasite infection. The cytokine data support a role for IL-33 in promoting Th2 responses during the muscle phase of infection and correlate with an increase in production of iNOS (a Th1-associated tissue mediator) at sites of infection. In

other mouse strains (e.g., the Phil, ΔdblGATA, and IL-10$^{-/-}$ strains), local iNOS production is associated with reduced parasite survival in the muscle rather than increased parasite burdens (4, 5). Thus, the ST2-dependent pathways that promote parasite control in the muscle may be more robust in the absence of excess iNOS. In ST2$^{-/-}$ mice, the increase in muscle larvae did not result from prolonged survival of adult worms in the intestine, as worm expulsion was normal. It is possible that there are ST2-dependent effects on worm fecundity. Immune responses that limit fecundity have been documented (67, 68) but are poorly understood. Alternatively, ST2 may affect the migration of newborn *T. spiralis* larvae to skeletal muscle. Newborn larvae are the most destructive of the life stages, causing injury in many tissues, and release of IL-33 during their migration is likely. The influence of IL-33 on fecundity and migration of newborn larvae merits testing.

In contrast to mice infected with *Trichuris muris*, in which IL-33 mRNA expression increases in the cecum (65), we observed constitutive expression of IL-33 in the small intestine. Gene expression was unaffected by *T. spiralis* infection, and IL-33 protein was detected mainly in IECs and lamina propria leukocytes. Following epithelial cell invasion by the parasite, IL-33 became concentrated in the nuclei of IECs that were in the vicinity of migrating worms but were not invaded by them. A similar cellular distribution of IL-33 has been observed in the human intestine, where IL-33 is present in the cytoplasm of IECs in healthy individuals but is concentrated in nuclei during ulcerative colitis (69). IL-33 has also been detected in nuclei of epithelial cells in the lungs of both uninfected and parasite-infected mice (70). The significance of nuclear concentration of IL-33 has not yet been documented, although the phenomenon is consistent with an innate response that sequesters the cytokine or with a mechanism of gene regulation in affected cells.

We observed that the transition from cytoplasmic to nuclear localization *in vivo* did not require ST2 or MyD88, indicating that this response is not a feedback loop by which IL-33 stimulation of ST2 directly leads to suppression of the pathway. Nuclear localization occurred in RAG$^{-/-}$ mice, confirming it to be an innate mechanism. Further studies are necessary to define the nuclear functions of IL-33 in intestinal epithelia and to determine whether nuclear IL-33 constitutes a defensive response in the mucosa that operates independently of ST2. One possibility is that IL-33 accumulates in the nuclei of dying cells; we have investigated the association between IEC cell death and nuclear concentration of IL-33 *in vitro* by using a parasite invasion assay that we have previously described (64). We found that cells with nuclear propidium iodide staining did not demonstrate nuclear IL-33 (data not shown).

We did not observe nuclear IL-33 in IEC in the large intestine (data not shown) nor did redistribution occur *in vitro* when IL-33-expressing DLD-1 cells were infected with *T. spiralis* larvae (experiments performed as described in references 28 and 64) (L. K. Scalfone and J. A. Appleton, unpublished data). Factors intrinsic to the SI may be required to transmit the signal that induces IL-33 to concentrate in IEC nuclei. Nuclear IL-33 was observed along the length of the villus, although the parasite migrates primarily at the crypt-villus junction, suggesting that IECs damaged by migrating larvae or worms may release other molecules that stimulate healthy IECs to concentrate IL-33 in their nuclei.

It is widely accepted that Th2 responses support wound healing and are induced by tissue injury resulting from a variety of causes. Tissue injury is a prominent feature of trichinellosis. Although an influence of parasite-derived molecular patterns on Th2 immunity to *T. spiralis* was not supported by our data, our findings are consistent with a role for tissue and cell damage in promoting Th2 responses during intestinal and extraintestinal phases of infection and with an important role for ST2 in responding to this injury. Infection with *T. spiralis* provides a robust, natural model for investigating the influence of cellular injury on immunity and the role that IL-33 plays in the innate and adaptive responses to parasitic worms.

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