

Mast cells orchestrate type 2 immunity to helminths through regulation of tissue-derived cytokines

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Edited by Ruslan Medzhitov, Yale University School of Medicine, New Haven, CT, and approved March 16, 2012 (received for review July 27, 2011)

Mast cells (MCs) are potent inflammatory cells that are distributed throughout mucosal barrier tissues and respond rapidly to pathogenic stimuli. During helminth infections, MCs play an important role as late-stage effectors. However, it is currently unknown whether MCs contribute to the early innate events that determine the priming of adaptive immunity. MC-deficient mouse strains and mice treated with the MC stabilizing agent cromolyn sodium had dramatically reduced Th2 priming and type 2 cytokine production and harbored increased parasite burdens following infection with gastrointestinal helminths (*Heligmosomoides polygyrus bakeri* and *Trichuris muris*). In addition, early production of the tissue-derived cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) was significantly diminished in MC-deficient mice and resulted in decreased numbers of infection-elicited IL-25-dependent (Lin[−]CD45[−])CD34⁺Sca-1⁺ progenitors, which produced type 2 cytokines and could be differentiated into mast cells *ex vivo*. Finally, repair of MC deficiency increased production of IL-25, IL-33, and TSLP, restored progenitor cell numbers and Th2 priming, and reduced parasite burden. Our data reveal an innate IgE-independent role for MCs in orchestrating type 2 immune responses via the regulation of IL-25, IL-33, and TSLP.

Mast cells (MCs) are preferentially distributed throughout barrier tissues such as the skin and mucosa, including the intraepithelial space of the intestine. Their ability to respond rapidly via release of prestored inflammatory mediators means they are primed to contribute to the first line of response to pathogens (1). Although MCs are classically considered important late-stage effector cells during Th2-associated immune responses including host responses against parasitic helminths in mucosal tissues (2), the innate role of MCs during the initial stages of the immune response is relatively neglected. Recent reports have begun to identify critical roles for MCs in the early orchestration of an immune response. For example, MCs help to maintain tissue homeostasis (3), express Toll-like receptors important in early pathogen sensing (4), and contribute to the induction of immune responses in the tissues (5). MCs are found in close contact with dendritic cells (DCs) and epithelial cells in the tissues and provide innate adjuvant stimuli via the release of histamine, proteases, and cytokines, which lead to the activation of DCs and recruitment of neutrophils and T cells to the site of infection (5–7).

The importance of early events at mucosal tissues in orchestrating immune responses has also been highlighted by the critical roles for tissue-derived cytokine signals in the development of polarized Th2 immune responses, in particular the cytokines IL-25 (also known as IL-17E) (8), IL-33 (9), and thymic stromal lymphopoietin (TSLP) (10). Mice deficient in IL-25 have been shown to be susceptible to infection with the cecal-dwelling helminth *Trichuris muris* (8), whereas mice with defective production of TSLP are unable to mount Th2-driven worm expulsion (11), and TSLP has a crucial role in conditioning APCs for efficient priming of Th2 cell responses (12–14). Similarly, administration of recombinant IL (rIL)-33 to mice normally susceptible to *T. muris* infection can enhance Th2 immunity and restore worm expulsion (9). All three cytokines have been shown to have important roles

in limiting the expression of proinflammatory cytokines such as IL-23p40 and IFN- γ (12, 15). Recently, IL-25 and IL-33 were reported to induce several novel type 2 cytokine producing innate cell types, including CD34⁺Sca-1⁺ multipotent progenitor cells (MPP^{type2}) (16) and c-kit^{int} effector cell types (17–20), which play important roles in Th2 amplification.

The mechanisms by which IL-25, IL-33, and TSLP are triggered and regulated are relatively poorly defined, although exposure to microbial Toll-like receptor ligands or tissue damage may play an important role (reviewed in ref. 21). Even though they are largely considered to be epithelial and endothelial cell-derived cytokines, recent studies identified alternative cell sources including MCs, which are sources and targets of IL-25, IL-33, and TSLP (22, 23). Thus, we aimed to explore the role of MCs in the early events determining the orchestration and priming of Th2 immune responses following infections with the intestinal helminths *Heligmosomoides polygyrus bakeri* (*Hp*) and *T. muris* (*Tm*). Here we investigated the early innate IgE-independent role of MCs in the regulation of the tissue-derived cytokines IL-25, IL-33, and TSLP and subsequently the development of innate and adaptive type 2 immunity.

Results

MC Deficiency Results in Impaired Th2 Responses and Reduces Host Protection Following Intestinal Helminth Infection. It is currently unknown whether MCs contribute to the early events that lead to the priming and orchestration of Th2 immunity during gastrointestinal helminth infection. To investigate the role of MCs, we infected WT (*Kit*^{+/+}) and MC-deficient mice (*Kit*^W/*Kit*^{W-v} and *Kit*^{W-sh}) with the small intestinal-dwelling helminth *Hp*. MC degranulation was already detected within the first few days of infection with *Hp* as indicated by increased levels of mouse mast cell protease-1 (mMCP-1) in the sera of WT mice (Fig. 1A), and was undetectable in MC-deficient *Kit*^W/*Kit*^{W-v} mice (Fig. 1A, white bars; below detection). MC-deficient mice harbored significantly increased numbers of viable adult worms compared with WT controls at day 21 post infection (p.i.; *Kit*^W/*Kit*^{W-v}, Fig. 1B; *Kit*^{W-sh}, Fig. S1A), and, similarly, shedding of parasite eggs into the feces during *Hp* infection was significantly increased in both strains of MC-deficient mice (Fig. 1C and Fig. S1B). Analysis of *Hp* antigen (Ag)-specific immune responses of draining lymph node cells at days 0, 6, and 21 p.i. demonstrated the induction of a strong Th2-polarized response in WT mice, characterized by high levels of the type 2 cytokines IL-4, IL-5, IL-9, IL-10, and IL-13 (Fig. 1D). In contrast, the Th2 response was significantly impaired in MC-

Author contributions: M.R.H., M. Metz, M. Maurer, and S.H. designed research; M.R.H., E.D.-L., S.R., and C.K. performed research; M. Metz and M. Maurer contributed new reagents/analytic tools; M.R.H., E.D.-L., S.R., C.K., and S.H. analyzed data; and M.R.H. and S.H. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1112268109/-DCSupplemental.

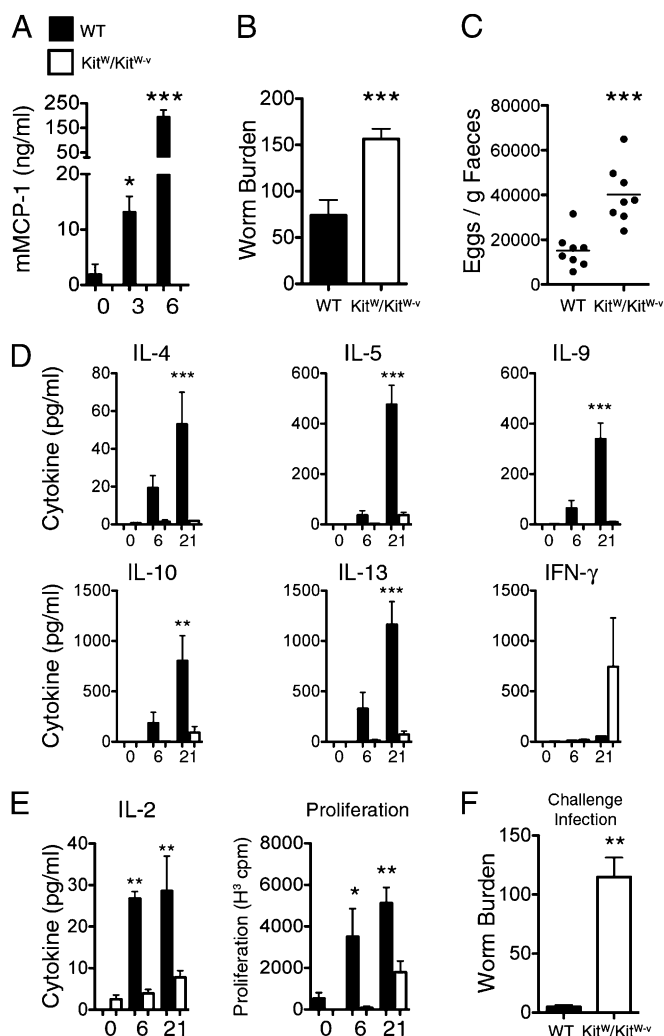


Fig. 1. MC-deficient *Kit^W/Kit^{W-v}* mice exhibit impaired Th2 responses and increased parasite burden following *Hp* infection. (A) Serum levels of mMCP-1 at days 0, 3, and 6 after *Hp* infection in WT (black bars) and *Kit^W/Kit^{W-v}* mice (white bars). (B) Adult worm numbers assessed at day 21 p.i. in WT and *Kit^W/Kit^{W-v}* mice and (C) median parasite egg output per gram feces of three measurements per mouse taken on days 14, 16, and 18 p.i. (D) Th1/Th2 cytokine production and (E) IL-2 production and proliferation of MLN cells restimulated with *Hp* Ag at days 0, 6, and 21 p.i. (F) Adult worm burdens of WT and *Kit^W/Kit^{W-v}* mice at day 14 p.i. following secondary challenge infection. Data are representative of six independent experiments ($n = 6-8$ mice per group $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

deficient mice, and MC-deficient mice exhibited variably increased IFN- γ in the later stages of infection (Fig. 1D and Fig. S1C). MC-deficient mice also showed reduced draining lymph node hypertrophy and significantly lower levels of IL-2 in the mesenteric lymph node (MLN), and had impaired proliferation to *Hp*-Ag (Fig. 1E). MC-deficient mice were unable to clear a challenge infection, which is indicative of an impaired Th2 memory response (Fig. 1F). Diminished Th2 responses were also found in MC-deficient mice infected with the cecal-dwelling helminth *Tm*, as *Kit^{W-s/h}* mice harbored significantly higher parasite burdens than WT mice at day 21 p.i. (Fig. S2A) and exhibited substantially reduced proliferation and type 2 cytokine production in response to *Tm* Ag restimulation (Fig. S2B and C).

MC-Deficient Mice Have Decreased T-Cell Imprinting and Th2 Polarization. Optimal Th2 priming requires the migration of DCs from the tissues to the lymph node, where Ag can be presented to naive

T cells. Conditioning of DCs by the mucosal tissue-derived cytokines IL-25 and TSLP has previously been shown to stimulate migration of DCs to the draining lymph node and enhance Th2 priming capacity via up-regulation of the costimulatory molecule OX40-L (12–14). We detected an influx of intestinal-derived CD103⁺CD11c⁺ DCs in the MLN of WT mice following *Hp* infection, including a subset expressing OX40-L (Fig. 2A). In contrast, no significant increase vs. baseline in CD11c⁺CD103⁺OX40-L⁺ DC or total DC numbers was detected in *Kit^W/Kit^{W-v}* mice at any time following *Hp* infection (Fig. 2A). DC migration correlated with CD4⁺ T-cell $\alpha 4\beta 7$ integrin imprinting (Fig. 2B), necessary for migration to the small intestine, and T-cell activation (CD44⁺CD62-L^{lo}; Fig. 2C), which was enhanced in WT mice but not MC-deficient mice. WT mice exhibited induction of the Th2 transcription factor GATA-3 in CD4⁺ T cells in the MLN by day 4 p.i., whereas T cells from MC-deficient mice had fourfold lower frequency of GATA-3⁺ cells (WT, 3.0% $\pm$ 0.4% vs. 0.7% $\pm$ 0.1% for *Kit^W/Kit^{W-v}*; day 4 p.i.). Confounded by the reduced proliferation of lymphocytes to parasite Ag, this translated to a highly significant reduction in total GATA-3⁺ Th2 cell numbers in *Kit^W/Kit^{W-v}* mice (Figs. 1E and 2D). Similar findings were also found during *Tm* infection (Fig. S2D).

MCs Are Critical for Helminth Infection-Induced Increases in Intestinal MC Progenitors. Recent studies revealed a critical role for innate IL-25/IL-33-elicited c-kit-expressing cells in the priming and enhancement of Th2 adaptive responses during helminth infection (16–20). This included a unique IL-25-elicited type 2 cytokine-producing myeloid progenitor population in the gut-associated lymphoid tissue, termed MPP^{type2}, which were characterized by their Lin[−]CD34^{+/−}Sca-1⁺ phenotype and ability to differentiate into other myeloid cell types, including MCs (16). We aimed to assess whether MC-deficient mice may also have defects in the infection induced expansion of innate type 2 cytokine producing progenitors. On day 6 after *Hp* infection, WT and *Kit^W/Kit^{W-v}* mice showed an increase in the frequency of cells lacking classical lymphoid and lineage markers (Lin[−]CD45[−]) in the lamina propria and Peyer patches of the small intestine (Fig. 3A and F and Fig. S3A and B), whereas these cells were not

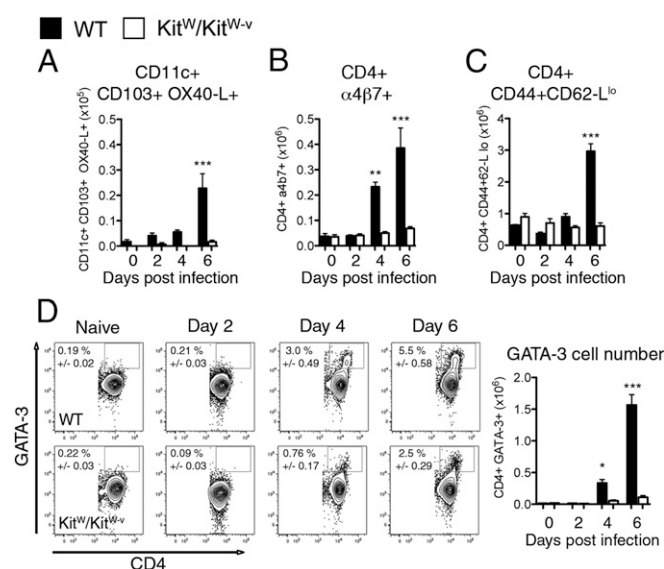


Fig. 2. *Kit^W/Kit^{W-v}* mice have defective T-cell imprinting and Th2 priming. Total MLN cell numbers of (A) CD11c⁺CD103⁺OX40-L⁺, (B) CD4⁺ $\alpha 4\beta 7$ ⁺, and (C) CD4⁺CD44⁺CD62-L^{lo}, and (D) frequency and total MLN cell numbers of GATA-3⁺ CD4⁺ (Th2) cells at days 0, 2, 4, and 6 after *Hp* infection of WT (black bars) and *Kit^W/Kit^{W-v}* mice (white bars). Data are representative of three independent experiments ($n = 5$ mice per group $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

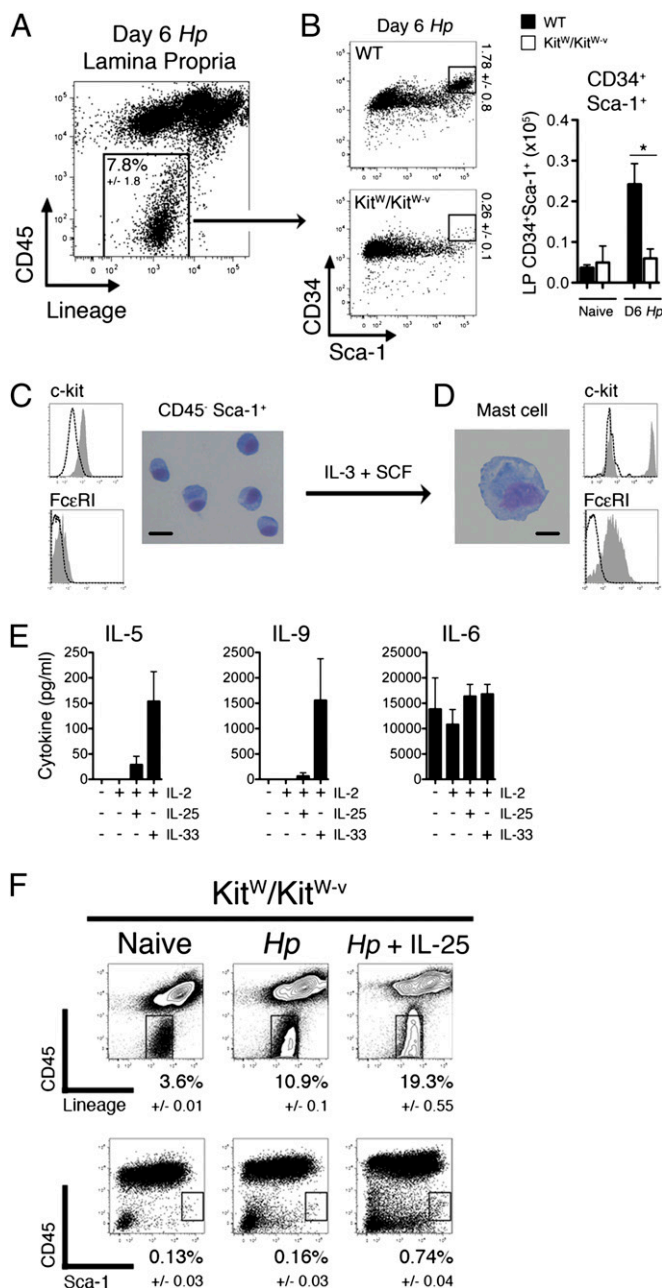


Fig. 3. *Hp* induced increases in an intestinal MC progenitor are MC-dependent. (A) Total frequency of Lin⁺CD45⁺ cell and (B) total frequency and cell numbers of (Lin⁺CD45⁺) CD34⁺Sca-1⁺ cells in the lamina propria of WT (black bars) and Kit^W/Kit^{W-v} mice (white bars). Morphology and surface expression of c-kit and FcεRI of CD45⁺CD34⁺Sca-1⁺ cells (C) ex vivo and (D) following culture for 6 d in the presence of IL-3 and stem cell factor (dotted line, isotype; fill, CD45⁺Sca-1⁺ cells). (Scale bar: 10 μm.) (E) Levels of IL-5, IL-9, and IL-6 following stimulation of isolated CD45⁺Sca-1⁺ cells with combinations of 10 ng/mL rIL-2, rIL-25, and rIL-33 for 5 d. (F) Assessment of Lin⁺CD45⁺ and CD45⁺Sca-1⁺ populations in the Peyer patches of *Hp*-infected Kit^W/Kit^{W-v} mice following treatment with rIL-25. Experiments were repeated three times (A–E) or two times (F; *n* = 6 mice per group ± SEM), and data combined for graphs (**P* < 0.05, ***P* < 0.01, and ****P* < 0.001).

detected in MLN (>98% Lin⁺CD45⁺; Fig. S3B). Further characterization of the Lin⁺CD45⁺ cells revealed a heterogeneous population in WT mice, including a subset expressing high levels of Sca-1 and CD34, phenotypal markers associated with myeloid progenitors (Fig. 3B). WT mice showed a significant in-

crease in the frequency and total cell number of small intestine lamina propria (Lin⁺CD45⁺)CD34⁺Sca-1⁺ cells following *Hp* infection, whereas no significant increase was detected in infected Kit^W/Kit^{W-v} mice (Fig. 3B). This population was present only at low numbers in naive mice, suggesting an infection-induced signal is required for their recruitment to the intestinal tissue (Fig. 3B). The lamina propria (Lin⁺CD45⁺)CD34⁺Sca-1⁺ population was further characterized as c-kit^{int} and FcεRI⁺ (Fig. 3C). To determine the progenitor capacity of the *Hp* infection-induced cells, CD45⁺Sca-1⁺ cells were sorted from the lamina propria of *Hp*-infected WT mice 5 d p.i. and cultured for 6 d with IL-3 and stem cell factor (Fig. 3C). Cultured cells were significantly altered in size and morphology and were c-kit^{hi}FcεRI⁺ and CD11b⁺CD49b⁺, indicative of an MC—but not macrophage or basophil—phenotype, and suggesting monopotent progenitor capacity (Fig. 3D and Fig. S3C). Isolated *Hp*-elicited CD45⁺Sca-1⁺ progenitors were also able to produce the Th2 associated cytokines IL-5 and IL-9 following stimulation with IL-2 and IL-33, but not IL-2 and IL-25, or IL-2 alone (Fig. 3E). In contrast, progenitors constitutively made high levels of IL-6 under all conditions tested (Fig. 3E). As *Hp* elicited progenitors in WT mice expressed low levels of c-kit, we determined whether their absence in Kit^W/Kit^{W-v} mice following infection was caused by an inherent hematopoietic deficiency or by the absence of appropriate tissue cytokine stimuli. Treatment of *Hp*-infected Kit^W/Kit^{W-v} mice with rIL-25 led to an increase in the frequencies of CD45⁺Lin⁺ cells in the Peyer patches, including enhanced frequencies of (CD45⁺)Sca-1⁺ progenitors (Fig. 3F), as has been previously reported (16). Thus, (Lin⁺CD45⁺)CD34⁺Sca-1⁺ progenitors are not absent per se in Kit^W/Kit^{W-v} mice and can be elicited in MC deficient mice by IL-25.

IL-25 Production Is Abrogated in MC-Deficient Mice and Is Required for Th2 Responses and Reduced Parasite Fecundity. IL-25, IL-33, and TSLP are critical tissue-derived cytokines that are required for progenitor expansion, DC conditioning, and Th2 priming. We investigated whether reduced Th2 priming and progenitor expansion in MC-deficient mice could be a consequence of dysregulated tissue-derived cytokine production during the early stages of *Hp* infection. IL-25, IL-33, and TSLP mRNA levels were significantly increased vs. naive levels by days 2 to 4 p.i. in *Hp*-infected WT mice but not in MC-deficient Kit^W/Kit^{W-v} mice (Fig. 4A). Furthermore, all three cytokines could be detected at protein level in homogenates of WT, but not Kit^W/Kit^{W-v}, duodenum at day 6 p.i. via ELISA (Fig. 4B). In line with these findings, expression of cytokine protein in gut homogenates was also found to be attenuated in a second MC-deficient mouse strain (Kit^W-Sh) following infection with *Hp* (Fig. S4A) or *Tm* (Fig. S4B), suggesting a conserved role for MCs in the regulation of tissue-derived cytokines, and therefore Th2 priming. The data further indicate that the abrogation of type 2 cytokine-secreting MC progenitor expansion following helminth infection in Kit^W/Kit^{W-v} mice is likely caused by the lack of IL-25.

TSLP has recently been shown to elicit basophilia independent of the canonical cytokine IL-3 (24), and MHC-II-expressing basophils have Th2 priming ability in some helminth infections (25). Thus, we aimed to ascertain if differences in basophils also contribute to the suppressed Th2 priming in MC-deficient mice—either as a result of inherent hematopoietic abnormalities or as a consequence of decreased TSLP expression. In contrast to MCs, basophil numbers were found to be unaffected in the spleens, MLNs, and lamina propria of uninfected Kit^W/Kit^{W-v} mice or during the first 1 wk of *Hp* infection (Fig. S5A–C). Ag presentation capacity was also not altered in Kit^W/Kit^{W-v} mice, as splenic basophils were found to express MHC-II independent of infection or MC status (Fig. S5D). Thus, although basophils have Ag-presenting capacity, it is unlikely that changes in basophils contribute to the phenotype observed in MC-deficient mice.

As IL-25 was found to be the earliest and most abundant transcript during *Hp* infection, we assessed whether treatment with IL-25 alone was sufficient to restore Th2 responses in MC-deficient mice. Application of rIL-25 to Kit^W/Kit^{W-v} mice during

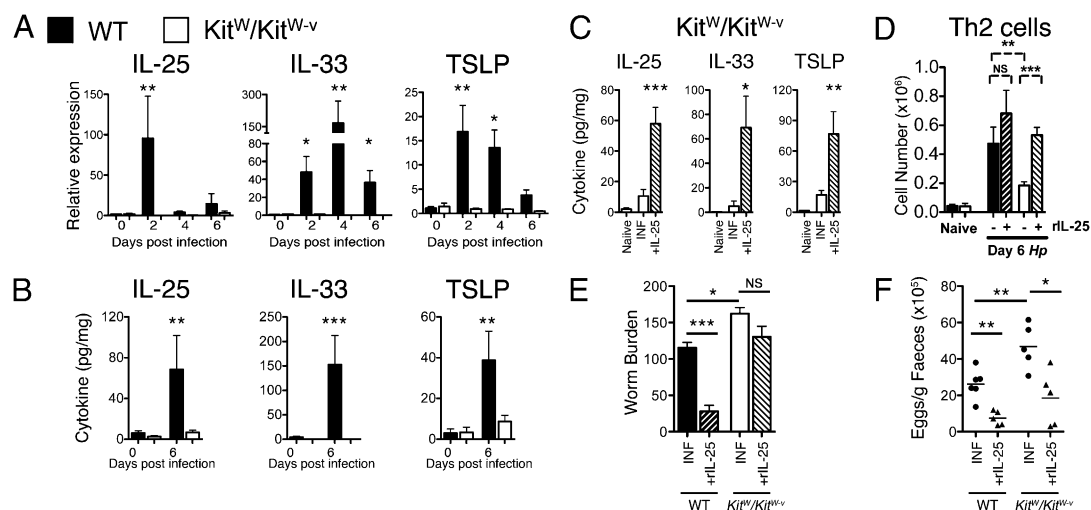


Fig. 4. rIL-25 treatment restores Th2 priming and antiparasitic responses in MC-deficient mice. (A) IL-25, IL-33, and TSLP mRNA expression at days 0, 2, 4, and 6 after *Hp* infection and (B) protein levels (pg/mg total protein) in duodenal homogenates at days 0 and 6 p.i. of WT (black bars) and *Kit^W/Kit^{W-v}* mice (white bars). (C) Concentration of cytokine per mg total protein in duodenal homogenates of naive and day 6 *Hp* (INF) infected *Kit^W/Kit^{W-v}* mice with or without rIL-25 treatment (checked bars). (D) Total numbers ($\times 10^6$) of Th2 cells ($CD4^+GATA-3^+IL-13^+$) in the MLN of *Hp* infected WT (black bars) and *Kit^W/Kit^{W-v}* mice (white bars) with or without rIL-25 treatment. (E) Adult *Hp* worm burdens assessed at day 21 p.i. and (F) median egg output of control and treatment groups. Experiment was repeated three times ($n = 6$ mice per group $\pm$ SEM), and data were combined for A–E (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

the first 4 d of infection led to a dramatically elevated production of IL-33 and TSLP in the intestine (as well as IL-25 itself) at day 6 p.i. in comparison with vehicle-treated control mice (Fig. 4C), whereas no significant further increase in cytokine expression was detected in WT mice (Fig. 4B and C). rIL-25 treatment also restored numbers of Th2 cells ($CD4^+GATA-3^+IL-13^+$) in *Kit^W/Kit^{W-v}* mice to levels comparable with WT (Fig. 4D). *Hp* infection in mice is typically chronic, and therefore, although treatment of MC-deficient mice with rIL-25 was sufficient to restore adaptive type 2 immunity, it did not result in a significant reduction in

adult worm burdens in *Kit^W/Kit^{W-v}* mice at day 21 p.i. (Fig. 4E). Interestingly, however, the application of additional exogenous rIL-25 to IL-25-competent WT mice led to an almost complete expulsion of adult worm burdens, suggesting that high amounts of IL-25 may induce worm expulsion during a normally chronic *Hp* infection (Fig. 4E). Moreover, shedding of parasite eggs into the feces was significantly reduced in both rIL-25-treated WT and MC-deficient groups in comparison with untreated controls, further highlighting the crucial role of early IL-25 in the development of antihelminth immunity (Fig. 4F).

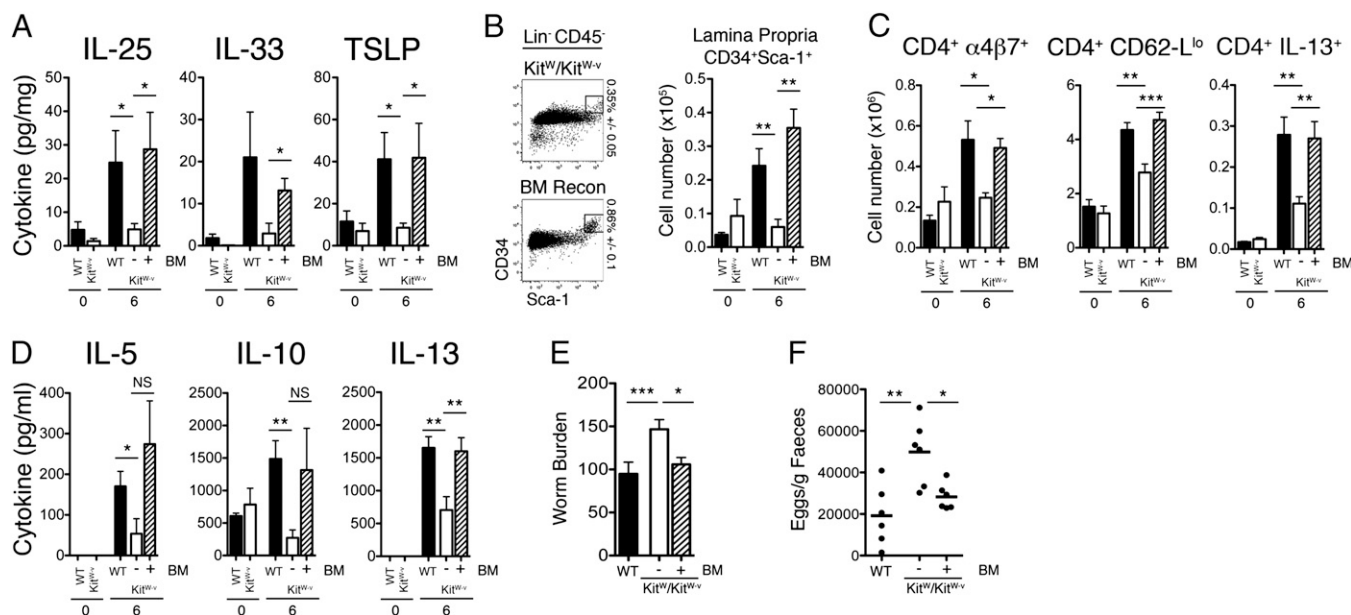


Fig. 5. Repair of MC populations in *Kit^W/Kit^{W-v}* mice via BM transfer restores Th2 priming and reduces *Hp* parasite burden. (A) Protein levels (pg/mg total protein) of IL-25, IL-33, and TSLP in duodenal homogenates of WT (black bars), *Kit^W/Kit^{W-v}* mice (white bars), and BM-reconstituted *Kit^W/Kit^{W-v}* mice (checked bars). (B) Representative plots, total frequencies, and total cell numbers of lamina propria Lin⁺CD45⁺CD34⁺Sca-1⁺ cells. (C) Total MLN cell numbers of $CD4^+ \alpha 4\beta 7^+$, $CD4^+ CD62-L^lo$, and $CD4^+ IL-13^+$ and (D) concentration of IL-5, IL-10, and IL-13 in *Hp* Ag-restimulated MLN cell culture supernatants at days 0 and 6 p.i. (E) Adult worm burdens at day 21 p.i. and (F) median egg output per gram feces in WT, *Kit^W/Kit^{W-v}*, and BM-reconstituted groups. Experiment was repeated three times ($n = 6$ mice per group $\pm$ SEM), and data were combined for panels A–E or shown as a representative experiment (F; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

Repair of MC Populations via Bone Marrow Transfer Restores Type 2 Immunity and Antiparasitic Responses in MC-Deficient Mice. To further define the role of MCs in the priming and orchestration of the Th2 response during *Hp* infection, we aimed to restore the MC compartment of mucosal tissues in *Kit^W/Kit^{W-v}* mice. It has previously been reported that repair of mucosal MC deficiency in the small intestine via the transfer of in vitro generated bone marrow (BM)-derived MCs is not possible because of the inability of relatively immature in vitro generated MCs to properly migrate into the lamina propria and intraepithelial compartment (26). Therefore, we restored the MC compartment via transfer of whole WT (*Kit^{+/+}*) BM, which, in contrast, has been described to fully reconstitute the small intestine and restore normal MC function (27). *Hp*-infected *Kit^W/Kit^{W-v}* mice that had been repaired for MC deficiency via adoptive transfer of WT BM showed a significant restoration of the levels of IL-25, IL-33, and TSLP in the duodenum following *Hp* infection in comparison with infected, nonreconstituted control *Kit^W/Kit^{W-v}* mice at day 6 p.i. (Fig. 5A). The increased expression of IL-25 correlated with a significant increase in the number of (Lin⁻/CD45⁻)CD34⁺Sca-1⁺ progenitors in the lamina propria of *Hp*-infected mice that had received WT BM (Fig. 5B). Furthermore, an increase in the total cell numbers of CD4⁺αβ⁺ and CD4⁺CD62-L^{lo} activated T cells and CD4⁺IL-13⁺ Th2 cells was observed in BM-repaired, *Hp*-infected *Kit^W/Kit^{W-v}* mice, reaching levels comparable with WT mice (Fig. 5C). BM reconstituted mice had markedly enhanced *Hp* Ag-specific production of IL-5, IL-10, and IL-13 in the draining lymph node (Fig. 5D) and significantly reduced adult worm burdens and parasite egg output to levels comparable with WT controls (Fig. 5E and F).

Treatment with MC-Stabilizing Agent Cromolyn Sodium Suppresses Type 2 Immunity. To confirm that MCs play a role in the orchestration of type 2 immunity to helminths, and to rule out the contribution of other abnormalities in *Kit*-mutant MC-deficient mice, we inhibited MC degranulation in otherwise normal C57BL/6 mice by using the MC stabilizing agent cromolyn sodium (CS), as previously described (5, 28). *Hp*-infected WT mice had abrogated serum mMCP-1 levels following CS treatment (Fig. 6A). Critically, treatment with CS recapitulated the phenotype seen in *Kit^W/Kit^{W-v}* and *Kit^{W-SH}* mice, as CS-treated mice had significantly reduced GATA-3⁺IL-13⁺ Th2 cell levels (Fig. 6B), IL-2, IL-4, and IL-13 production (Fig. 6C), tissue-derived cytokine production, and (Lin⁻CD45⁻)CD34⁺Sca-1⁺ progenitor expansion (Fig. 6D and E), which were comparable with levels observed in *Kit^W/Kit^{W-v}* mice. Furthermore, the contribution of MCs was found to be IgE-independent, as serum IgE, B-cell activation, IgE class switching, and surface-bound IgE⁺ basophils were all not detectable during the early stages of *Hp* infection (Fig. S6), whereas IL-25 induction and progenitor expansion occurred normally following *Hp* infection in RAG^{-/-} mice (Fig. 6F).

Discussion

The orchestration of type 2 immunity, characterized by the production of IL-4, IL-5, IL-9, and IL-13 from both innate and adaptive (i.e., Th2) sources, is critical for host defense during infection with parasitic helminths that colonize mucosal tissues. Understanding the early events that determine induction of type 2 responses is also of major clinical relevance for the prevention of Th2-associated immunopathologic processes. Herein, we used three independent models targeting MCs (*Kit^W/Kit^{W-v}*, *Kit^{W-SH}*, and CS treatment of WT mice) and report that innate IgE-independent MC-derived signals are required for optimal priming of the adaptive Th2 response, as well as expansion of a recently reported subset of innate type 2 cytokine-producing cells, in response to gastrointestinal helminth infections (schematic overview provided in Fig. S7). MC targeting resulted in abrogated Th2 priming and type 2 cytokine production in the MLN and a reduction in antiparasitic responses in MC-deficient mice, as determined by increased worm burdens and fecundity, that could

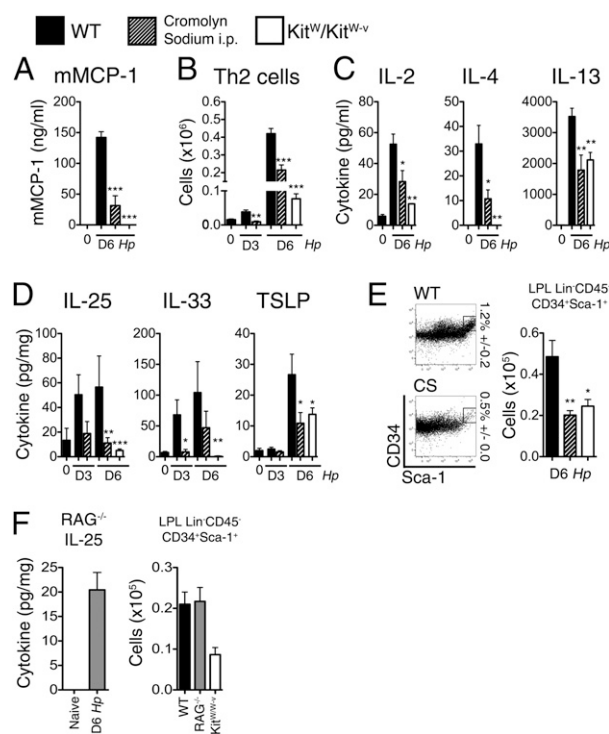


Fig. 6. CS treatment suppresses type 2 immunity during *Hp* infection. (A) Serum mMCP-1 levels in WT mice at days 0 and 6 p.i. with or without CS treatment. (B) Total MLN cell numbers of CD4⁺GATA-3⁺IL-13⁺ (Th2) cells, (C) concentration of IL-2, IL-4, and IL-13 in *Hp* Ag-restimulated MLN cell culture supernatants, (D) protein levels (pg/mg) of IL-25, IL-33, and TSLP in duodenal homogenates, and (E) total cell numbers of lamina propria Lin⁻CD45⁻CD34⁺Sca-1⁺ progenitor cells in WT (black bars), CS-treated WT (hatched bars), and *Kit^W/Kit^{W-v}* mice (white bars) at days 0 and 6 p.i. (F) Levels of IL-25 and progenitor cells in RAG^{-/-} mice at days 0 and 6 p.i. Experiment was repeated three times (A–E) and two times (F; $n = 4–8$ mice per group $\pm$ SEM), and data were combined (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

be restored upon repair of MC deficiency, highlighting a critical role for MCs in the onset of type 2 immunity.

We further identify a role for MCs in regulating the tissue-derived cytokines IL-25, IL-33, and TSLP during the early stages of gastrointestinal helminth infection. All three cytokines have been reported to be essential for optimal Th2 responses and expulsion of adult worms following helminth infection (8–10); however, little is known to date about the cells and signals that regulate their expression. The abrogated expression of these tissue-derived cytokines in MC-deficient mice or following CS treatment, and subsequent restoration of expression by repair of MC deficiency, implicates MCs as an important regulator of these cytokines during the early stages of mucosal Th2 responses. Typically, MCs have been considered important effector cells in the later stages of helminth infections and in the pathogenesis of allergic asthma; however, our data are in line with recent reports ascribing critical roles to myeloid cells, such as basophils, in orchestrating Th2 immunity (25, 29, 30). Although the precise mechanism by which MCs regulate Th2 immunity remains unclear, we found that inhibition of MC degranulation with CS was sufficient to suppress type 2 cytokine production, suggesting MCs provide crucial signals in the tissues. It has previously been reported that epithelial cells are the major sources of IL-25 during helminth infection (31), and by using several independent experimental approaches, we were unable to detect production of IL-25, IL-33, or TSLP by MCs following stimulation with *Hp* Ags. Thus, we hypothesize that one mechanism by which MCs modulate early priming events is via the release of adjuvant

signals in response to helminth infection that activate epithelial cell production of tissue-derived cytokines (Fig. S7).

IL-25 and IL-33 have recently been shown to be required for the induction of a number of unique c-kit-expressing innate cell types, which express type 2 cytokines and contribute to the priming and/or enhancement of the adaptive Th2 response (16–20). In particular, Saenz et al. reported the IL-25-dependent induction of a type 2 cytokine-secreting progenitor cell (MPP^{type2}) in the mucosal tissues with the potential to differentiate into MCs (16). Herein, we report that (Lin⁺CD45⁺)c-kit^{int}CD34⁺Sca-1⁺ progenitor cells are found in the lamina propria and Peyer patches of the small intestine during *Hp* infection, which can be differentiated into MCs ex vivo. MCs were required for the expansion of this population via their regulation of IL-25 and IL-33 production in helminth-infected tissues, as this population could be restored in helminth-infected *Kit^W/Kit^{W-v}* mice following repair of MC deficiency (which led to restoration of IL-25 and IL-33 production) or via treatment with rIL-25 alone. Isolated progenitors were able to produce type 2 cytokines following stimulation with IL-2 and IL-33 as previously reported (17, 18). Regulation of epithelial IL-25 expression by tissue-resident MCs may help maintain mucosal equilibrium, as IL-25 is known to be important in limiting responses to intestinal flora (15, 32), and MCs are important regulators of tissue homeostasis (3). These findings could have implications in a diverse range of diseases in which both MCs and IL-25, IL-33, or TSLP are associated with disease pathogenesis, such as allergic asthma (33), virus-induced airway hyperreactivity (34), arthritis (35), skin inflammation (36), and tumor progression (37).

Materials and Methods

Mice and Helminth Infections. Specific pathogen-free MC-deficient mice (*Kit^W/Kit^{W-v}* and *Kit^{W-sh}*) and WBB6 (*Kit^{+/+}*) background controls were bred at the animal facilities of the Charité University Medicine. C57BL/6 control mice were purchased from Charles River GmbH. Mice were infected with either 250 viable L3 of *Heligmosomoides polygyrus bakeri* or 200 embryonated *Trichuris muris* eggs as described in the *SI Materials and Methods*. In some experiments mice received 0.4 µg rIL-25 in PBS i.p. (R&D Systems) on d 1–4 postinfection. Alternatively, MC-deficient mice were reconstituted with *Kit^{+/+}* (WT) bone marrow via intravenous transfer of 1×10^7 purified mononuclear bone marrow cells and rested for 6 wk prior to parasite infection. In some experiments, C57BL/6 mice received 100 mg/kg cromolyn sodium (Sigma Aldrich) in PBS i.p. twice a day as previously described to inhibit mast cell degranulation in the small intestine (28). Isolation of lymphocytes, assessment of cytokine production, and flow cytometry were performed as indicated in the *SI Materials and Methods*. All experiments were performed in accordance with the National Animal Protection Guidelines and approved by the German Animal Ethics Committee.

Statistics. Experiments were performed as indicated, combined for analyses where appropriate, and expressed as mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism software using Kruskal Wallis analysis for worm burdens and one-way ANOVA or two-tailed Mann–Whitney test for other analyses. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 values were considered to be statistically significant.

ACKNOWLEDGMENTS. We thank Rohit Saluja, Sina Heydrich, Sascha Petz, Marion Müller, and Bettina Sonnenburg for excellent technical assistance. We also thank Christian Schwartz and David Voehringer (University Clinic Erlangen) for personal communications. This work was supported by German Research Foundation Grants SFB650 and HA2542/3-1 (both to S.H.) and SFB650 and SPP1394 (both to M. Metz and M. Maurer); and a grant from the Medical Program of the Broad Foundation (to S.H.).

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Supporting Information

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SI Materials and Methods

Parasite Infections and Quantification. *Heligmosomoides polygyrus bakeri* (*Hp*) life cycle was maintained via serial passage in C57BL/6 mice and mice were infected via oral gavage with 200 infective L3 in 200 μ l distilled water. Secondary infections were administered following two oral treatments of infected mice with Pyrantel Pamoate and a three week resting period. *Trichuris muris* (*Tm*) were maintained under sterile conditions at 4°C prior to infection with 200 embryonated eggs via oral gavage. *Hp* and *Tm* adult parasite burdens were assessed via longitudinal dissection of either small intestine (*Hp*) or the caecum and proximal colon (*Tm*) and enumerated using a binocular microscope. The shedding of infective *Hp* eggs was assessed via collection and weighing of faecal pellets and resuspension in saturated NaCl solution. Samples were then counted in duplicate using MacMaster chambers and total eggs/g feces calculated and expressed as the median counts of eggs enumerated on three independent days per mouse.

Lymphocyte Isolation and Culture. Lymphocytes from the mesenteric lymph node (MLN) were sterilely obtained via passage through a 70 μ m nylon mesh and cultured in RPMI 1640 media containing 10% fetal calf serum (FCS), 20 mM L-glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin. Cells were plated at a density of 1×10^6 cells/well and cultured with media alone or restimulated with 20 μ g/ml *H. polygyrus* antigen (*HpAg*), 50 μ g/ml *T. muris* E/S (*TmAg*) or 1 μ g/ml α CD3 and α CD28 mAb for 48 hours at 37°C/5% CO₂ and supernatants assessed for cytokine production via ELISA. For intracellular cytokine staining 5×10^6 cells/well were stimulated with 1 μ g/ml PMA and 1 μ g/ml Ionomycin for 30 min followed by the addition of Brefeldin A (5 μ g/ml) (Sigma Aldrich) and a further 3-h incubation. Cellular proliferation was analysed via a further 20-hour culture in fresh media containing 1 μ Ci methyl-[³H]-thymidine (Amersham Pharmacia) and subsequent detection using a β -counter (Perkin-Elmer).

Cytokine Quantification. The production of IFN- γ , IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, and IL-17A in culture supernatants was determined using Cytometric Bead Assay (CBA, BD Bioscience) as per manufacturers instructions. Production of IL-25, IL-33 and TSLP were assessed in tissue homogenates - briefly, frozen segments of small intestine were processed using a tissue homogeniser (MP Biomedicals) in a buffer containing protease inhibitor cocktail (Roche). Samples were centrifuged at 20,000 $\times$ g for 10 min to remove debris and the total protein concentration in supernatants was determined via BCA test (Pierce). Samples were then assessed in duplicate via DuoSet ELISA kit for IL-25,

IL-33, and TSLP (R&D systems) and the concentration (pg/ml) normalised to total protein content (pg/mg total protein).

Isolation of Lymphocytes from Lamina Propria and Peyer's Patches. Duodenum and jejunum were stripped of mesenteric fat and Peyer's Patches, opened longitudinally and washed thoroughly in HBSS containing 2% FCS. Intestinal tissue was incubated in HBSS 2% FCS containing 2mM EDTA to remove epithelial cells and remaining tissue minced. Intestinal tissue and isolated Peyer's Patches were incubated for 1 h at 37°C in RPMI medium containing 10% FCS and 0.1 U/ml Collagenase D and 200 U/ml Collagenase VIII. The digested tissue was filtered and centrifuged and the subsequent cell pellet resuspended and layered onto a 40% / 70% Percoll gradient and centrifuged at 800 $\times$ g for 20 min. The interface containing lymphocytes was removed and used for flow cytometric analysis.

Flow Cytometry. Single cell suspensions were surface stained with fluorescent conjugated antibodies against Sca-1, α 4 β 7, OX40-L, Fc ϵ RI, CD3, CD4, CD11c, CD19, CD34, CD44, CD45, CD49b, CD62-L, CD103, CD117 (c-kit), and a Lineage cocktail (eBioscience). Intracellular stains for GATA-3, IL-13, or respective isotype controls were performed following a fixation and permeabilisation protocol (eBioscience). Samples were acquired using LSR II or Fortessa flow cytometers (BD Biosciences) and analysed using FlowJo software (Treestar).

Real-Time PCR. RNA was isolated from frozen small intestinal tissue sections via homogenization in RNA lysis buffer and subsequent processing of tissue supernatants using the innuPREP RNA kit following manufacturers instructions (Analytik Jena). Two micrograms of RNA was reverse transcribed to cDNA using a High Capacity RNA to cDNA kit (Applied Biosystems). The relative expression of β -actin, IL-25, IL-33, and TSLP was then determined via real-time PCR using 5 ng of cDNA and FastStart Universal SYBR Green Master Mix (Roche). Primer pairs used for gene amplification were as follows: β -actin, forward 5' TC-TTGGGTATGGAATCCTGTGGCA 3' and reverse 5' TCTCC-TTCTGCATCCTGTCAGCAA 3'; IL-17E (IL-25), forward 5' ACAGGGACTTGAATCGGGTC 3' and reverse 5' TGGTAA-AGTGGGACGGAGTTG 3'; IL-33, forward 5' AGGAAGA-GATCCTTGCTTGGCAGT 3' and reverse 5' ACCATCAGCT-TCTTCCCATCCACA 3'; TSLP, forward 5' AGCAAGCCAG-CTGTCTCCTG 3' and reverse 5' TGTGCCATTTCTGAG-TACCGTCA 3'. Relative gene expression was determined via normalization to the housekeeping gene (β -actin) and to strain specific naïve controls using the $2^{-\Delta\Delta C_t}$ method.

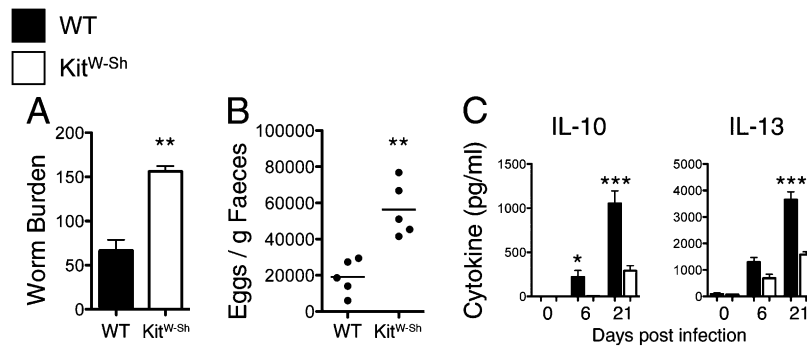


Fig. S1. Mast cell (MC)-deficient *Kit^{W-Sh}* mice have impaired Th2 responses and increased parasite burden following *Hp* infection. (A) Adult worm numbers assessed at day 21 postinfection (p.i.) in WT (black bars) and *Kit^{W-Sh}* mice (white bars) and (B) median parasite egg output per gram feces of three independent measurements on days 14, 16, and 18 p.i. (C) Cytokine production of mesenteric lymph node (MLN) cells restimulated with *Hp* antigen (Ag) at days 0, 6, and 21 p.i. All data represent values of five mice per group $\pm$ SEM and are representative of two independent experiments (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

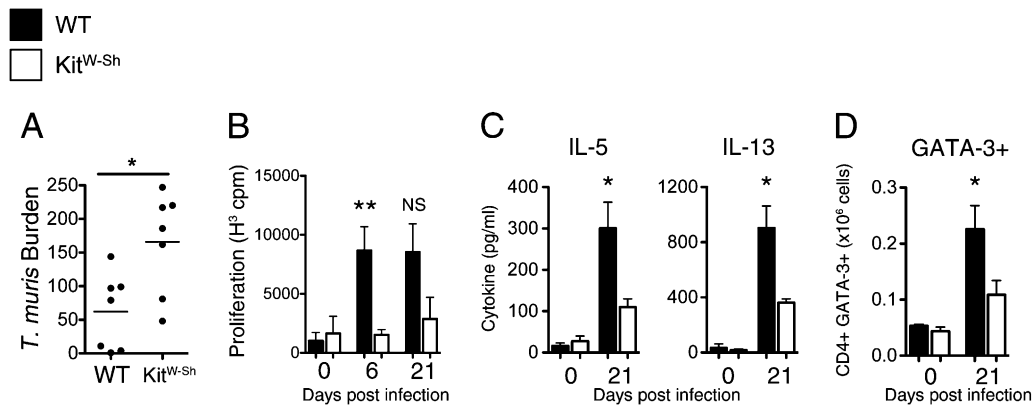


Fig. S2. Th2 responses and antiparasitic immunity is impaired in MC-deficient *Kit^{W-Sh}* mice following *Tm* infection. (A) Adult worm burdens in the cecum and colon at day 21 p.i. (B) Proliferation of *Tm* E/S Ag stimulated MLN cells after 0, 6, and 21 d p.i. (C) *Tm* E/S-specific IL-5 and IL-13 cytokine production in MLN cells and (D) total cell number CD4⁺ GATA-3⁺ in MLN. All data represent values from seven or eight mice per group $\pm$ SEM and are representative of pooled data from two independent experiments (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

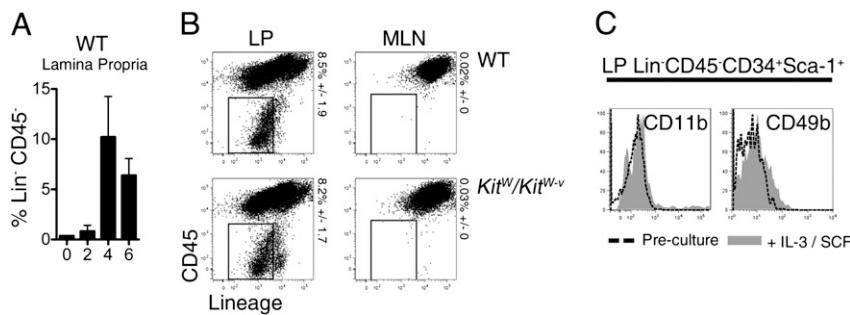


Fig. S3. Phenotypic analysis and quantification of *Hp* infection-induced c-kit^{int} progenitors. (A) Kinetics of the frequency of Lin⁻CD45⁻ cells in the lamina propria of *Hp*-infected WT mice. (B) Representative plots showing Lin⁻CD45⁻ cell populations in the MLN and lamina propria of WT and *Kit^W/Kit^{W-v}* mice. (C) Expression of CD11b and CD49b on sorted CD45⁻Sca-1⁺ before culture (dotted line) or 6 d following culture with 10 ng/mL IL-3 and 50 ng/mL stem cell factor. Data are combined from two independent experiments performed with five mice per group $\pm$ SEM.

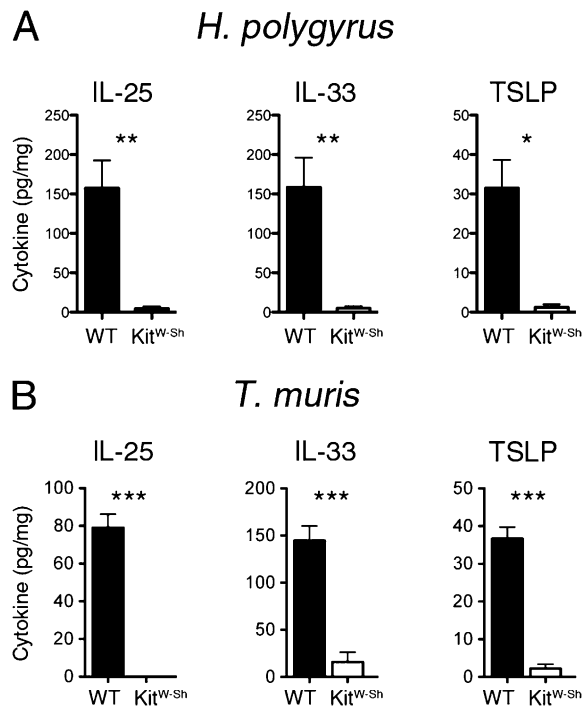


Fig. S4. MC-deficient *Kit^{W-Sh}* mice have reduced production of early IL-25, IL-33, and TSLP in the intestinal tissue following *Hp* and *Tm* infection. Levels of IL-25, IL-33, and TSLP in (A) duodenal tissue 6 d after *Hp* infection and (B) colon tissue 6 d after *Tm* infection as assessed by ELISA and normalized to total tissue homogenate protein content. Data are compiled from three independent experiments with five mice per group $\pm$ SEM (* P < 0.05, ** P < 0.01, and *** P < 0.001).

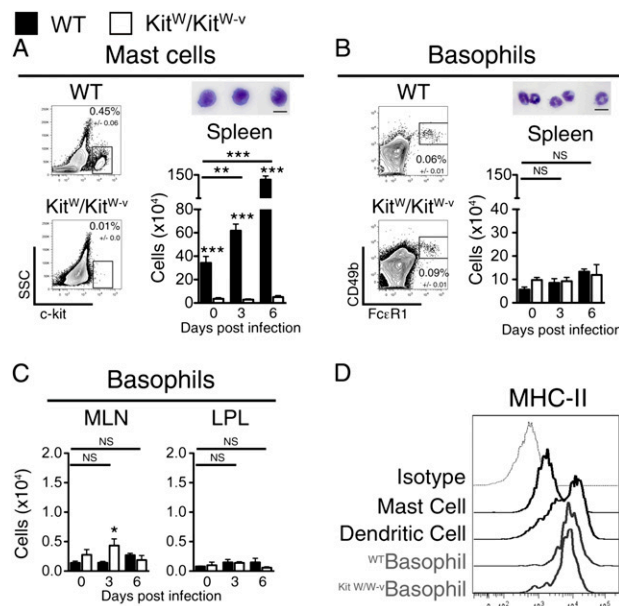


Fig. S5. *Kit^{W/Kit^{W-v}}* mice lack MCs but have comparable basophil numbers in naive and *Hp*-infected mice. Total numbers and representative cytopspins of (A) viable splenic (CD45^{dim}) SSC^{lo} c-kit⁺ MCs and (CD45^{dim} c-kit⁺) CD49b⁺FcεRI⁺ basophils in (B) the spleen and (C) MLN and lamina propria of WT and *Kit^{W/Kit^{W-v}}* mice during *Hp* infection. (D) Expression of MHC-II on WT MCs (SSC^{lo} c-kit⁺), dendritic cells (CD11c⁺), WT or *Kit^{W/Kit^{W-v}}* basophils (CD49b⁺FcεRI⁺), and isotype control (whole lymphocytes) in the spleen of *Hp*-infected mice. Data are compiled from two independent experiments performed with six mice per group $\pm$ SEM (* P < 0.05, ** P < 0.01, and *** P < 0.001).

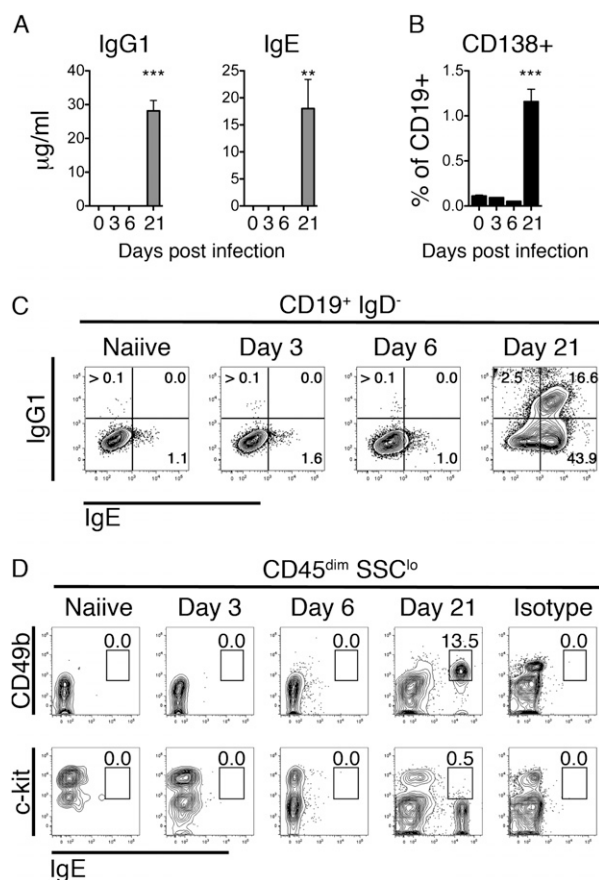


Fig. S6. IgE is detectable only during late-stage *Hp* infection. (A) *Hp*-specific IgG1 and IgE were quantified in the sera of *Hp*-infected mice at days 0, 3, 6, and 21 p.i. (B) Frequency of CD138⁺ cells among CD19⁺ B cells in the spleen, (C) expression of intracellular IgG1 and surface/intracellular IgE on CD19⁺IgD⁺ B cells in the spleen, and (D) frequency of CD49b⁺IgE⁺ and c-kit⁺IgE⁺ cells in CD45dim SSC^{lo}-gated spleen cells at days 0, 3, 6, and 21 p.i. All data represent values of three to five mice per group $\pm$ SEM and are representative of two independent experiments (* P < 0.05, ** P < 0.01, and *** P < 0.001).

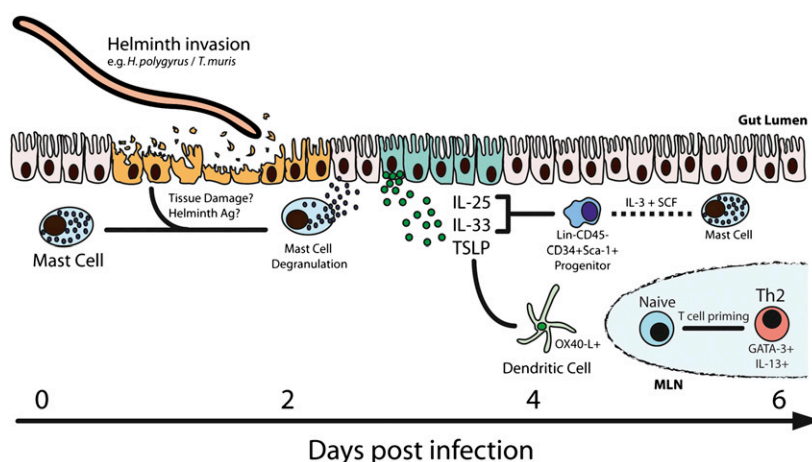


Fig. S7. Proposed model for MC-mediated orchestration of type 2 immunity. Invasion of *Heligmosomoides polygyrus* larval stages or *Tm* leads to the disruption of the epithelial barrier and the release of damage-related stimuli, resulting in the degranulation of intestinal MCs and the release of potent inflammatory mediators (between days 0 and 2 p.i.), that are required for increased expression of IL-25, IL-33, and TSLP in the tissues (days 2–4 p.i.). The absence of MCs in $Kit^{W/Kit^{W-v}}$ and Kit^{W-sh} , or via treatment with the MC stabilizing agent cromolyn sodium, results in the amelioration of IL-25, IL-33, and TSLP cytokine expression. Release of IL-25, IL-33, and TSLP is required for proper conditioning of intestinal dendritic cells and their subsequent migration to the MLN, where they present antigen and prime naive T cells, leading to up-regulation of GATA-3 and Th2-associated cytokines (days 4–6 p.i.). Development of the Th2 response is critical for control of the luminal dwelling adult stages of the parasite. Expression of IL-25 in the tissues also leads to the expansion of an intestinal (Lin[−]CD45[−]CD34⁺Sca-1⁺ progenitor population that could be differentiated into MCs with IL-3 and stem cell factor. IL-25 treatment was sufficient to restore progenitor populations that were absent in MC-deficient mice. Thus, IL-25 plays critical roles in the recruitment and expansion of these progenitors that may also contribute to the immune response via release of type 2 cytokines. Taken together, MCs provide adjuvant signals that regulate tissue-derived cytokines and orchestrate type 2 immunity.