Interleukin (IL)-33 is dispensable for *Schistosoma mansoni* worm maturation and the maintenance of egg-induced pathology in intestines of infected mice

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**Abstract**

**Background:** Schistosomes are trematode worms that dwell in their definitive host's blood vessels, where females lay eggs that need to be discharged into the environment with host excreta to maintain their life-cycle. Both worms and eggs require type 2 immunity for their maturation and excretion, respectively. However, the immune molecules that orchestrate such immunity remain unclear. Interleukin (IL)-33 is one of the epithelium-derived cytokines that induce type 2 immunity in tissues. The aim of this study was to determine the role of IL-33 in the maturation, reproduction and excretion of *Schistosoma mansoni* eggs, and in the maintenance of egg-induced pathology in the intestines of mice.

**Methods:** The morphology of *S. mansoni* worms and the number of eggs in intestinal tissues were studied at different time points post-infection in *S. mansoni*-infected IL-33-deficient (IL-33−/−) and wild-type (WT) mice. IL-5 and IL-13 production in the spleens and mesenteric lymph nodes were measured. Tissue histology was performed on the terminal ilea of both infected and non-infected mice.

**Results:** Worms from IL-33−/− and WT mice did not differ morphologically at 4 and 6 weeks post-infection (wpi). The number of eggs in intestinal tissues of IL-33−/− and WT mice differed only slightly. At 6 wpi, IL-33−/− mice presented impaired type 2 immunity in the intestines, characterized by a decreased production of IL-5 and IL-13 in mesenteric lymph nodes and fewer inflammatory infiltrates with fewer eosinophils in the ilea. There was no difference between IL-33−/− and WT mice in the levels of IL-25 and thymic stromal lymphopoietin (TSLP) in intestinal tissues.

**Conclusions:** Despite its ability to initiate type 2 immunity in tissues, IL-33 alone seems dispensable for *S. mansoni* maturation and its absence may not affect much the accumulation of eggs in intestinal tissues. The transient impairment of type 2 immunity observed in the intestines, but not spleens, highlights the importance of IL-33 over IL-25 and TSLP in initiating, but not maintaining, locally-induced type 2 immunity in intestinal tissues during schistosome infection. Further studies are needed to decipher the role of each of these molecules in schistosomiasis and clarify the possible interactions that might exist between them.

**Keywords:** IL-33, *Schistosoma mansoni*, Worm maturation, Type 2 immunity, Egg-induced pathology
**Background**

Schistosomes are blood-dwelling trematode worms that affect over 250 million people in the world, of whom 201.5 million live in sub-Saharan Africa [1]. Among the several schistosome species that exist, three, namely *Schistosoma haematobium*, *S. japonicum* and *S. mansoni*, are the main cause of schistosomiasis in humans [2]. The first of these causes urogenital schistosomiasis and the latter two cause hepato-splenic schistosomiasis [2]. *Schistosoma haematobium* eggs live in perivesical veinplexuses, and *S. japonicum* and *S. mansoni* live in mesenteric veins, where females lay hundreds to thousands of eggs per day [3]. About half of these eggs are washed into the liver by blood flow from the mesenteric veins; of the remainder, one-third to one-half succeed in reaching the intestinal lumen to be discharged into the environment with the host’s feces, while the remaining eggs are trapped in intestinal tissues where they die, either killed by the host’s immune system or of natural death [4, 5].

Through their excretory–secretory products (ESP), such as the interleukin (IL)-4-inducing principle of *S. mansoni* eggs (IPSE/α1) [6, 7] and omega-1 (ω1) [8] from *S. mansoni* and their homologs from *S. haematobium* [9] and *S. japonicum* [10], tissue-trapped eggs elicit strong and vigorous type 2 cell-mediated immunity that induces periportal granuloma formation and leads to fibrosis [11, 12], pathological characteristics of a patent schistosome infection. While this immune response is thought to be beneficial for the host, especially in the liver where it may protect hepatic cells from toxic effects of egg-derived ESP, it also plays a major role in the development of liver pathology [13]. In contrast, in addition to being protective for and yet smiting the host with the pathology, granulomas in intestines play a beneficial role for the parasite, favoring the escape of eggs from the host through the intestinal wall [14, 15].

Eggs are not the sole inducers of type 2 immunity in schistosomiasis, as studies have reported type 2 immune responses during prepatent schistosomiasis infection before egg deposition by female worms begins [16, 17]. The type 2 immunity during the prepatent schistosome infection was later found to be essential for the maturation of the worms, as the injection of IL-4, the T-helper 2 (Th2) polarizing cytokine, in schistosome-infected recombination activating gene (RAG)-deficient mice, in which schistosome worms fail to mature and reproduce due to the lack of functional CD4+ T cells, restored the worm maturation process and egg deposition [18].

Emerging evidence indicates that the induction of type 2 immunity in tissues is not solely dependent on IL-4, with type 2 immunity shown also to be induced through the activation of group 2 innate lymphoid cells (ILC2) by the epithelium-derived cytokines IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). Activated ILC2, in turn, produce abundant amounts of type 2 effector cytokines IL-4, IL-5, IL-9 and IL-13 [19–22] and, through the expression of the class II major histocompatibility complex (MHC II) [23] and OX40L [24], interact with CD4+ T cells to potentiate such type 2 immune responses. ILC2 were also found to initiate the adaptive type 2 immunity in an IL-4-independent manner by inducing IL-13-dependent activation and migration of dendritic cells to the draining lymph nodes where they polarize naive CD4+ T cells into Th2 cells [25].

However, the role of ILC2-activating cytokines IL-25, IL-33 and TSLP in schistosomiasis remains less understood. Focusing on the liver pathogenesis during *S. japonicum* infection, two studies showed that IL-33 contributes to the development of pathology via the induction of type 2 immune responses in infected mice [26, 27]. Indeed, studies have shown that IL-33 plays a critical role in the development of liver pathology through the alternative activation of macrophages (M2) [27] and the activation of hepatic stellate cells by ILC2-derived IL-13 [28]. Moreover, Yu et al. [26] found that the injection of exogenous IL-33 into *S. japonicum*-infected mice led to an increased worm burden at the sixth week of infection without affecting their fecundity, suggesting that IL-33 might play a role in the migration and maturation of schistosome worms. Whether endogenous IL-33 plays a role in schistosome maturation and reproduction is not known.

As IL-33 is known to induce and/or amplify M2 polarization of macrophages [27, 29–31] which are essential for the excretion of schistosome eggs [14, 15], we considered the possibility that in addition to contributing to the maturation of schistosome worms through the induction of type 2 immunity during prepatent schistosome infection, IL-33 may also play a role in the accumulation of *S. mansoni* eggs in the intestinal tissues. We hypothesized that IL-33 deficiency would impair the maturation of *S. mansoni* worms and possibly also lead to the accumulation of more eggs in the intestinal tissues [14, 32, 33], and that type 2 immunity would be impaired in the absence of IL-33. In the study reported here, we show that IL-33 is in fact dispensable for the maturation of *S. mansoni* worms and that its absence does not affect much the number of eggs accumulated in the intestinal tissues. Also, our findings support the notion that IL-33 might be most potent in initiating, but not maintaining, type 2 immunity in tissues and that to maintain type 2 immunity once initiated, IL-33 may need the synergy of IL-25 and TSLP and/or of CD4+ Th2-derived effector cytokines.
Methods
Parasite, mice and infection
A Puerto Rican strain of *S. mansoni* was maintained in the laboratory by passage between Biomphalaria glabrata snails and ICR mice. BALB/cCrSlc (hereinafter referred to as BALB/c) mice were purchased from Japan SLC (Hamamatsu, Shizuoka, Japan) and maintained in specific pathogen-free conditions at Nagasaki University animal facilities. Provided by Professor Satoshi Uematsu (Osaka City University Graduate School of Medicine, Osaka, Japan), IL-33−/− mice on the BALB/c background were bred and maintained in the same conditions as for WT BALB/c mice at Nagasaki University animal facilities. All mice were provided with water and food *ad libitum*. Female mice aged 8–12 weeks were subcutaneously infected [34] with 50 and 35 freshly shed *S. mansoni* cercariae for 9 and 12 weeks, respectively. Mice were sacrificed every 3 weeks from week 6 post-infection (6 wpi) onwards, except for worm morphology assessment where mice were also sacrificed at 4 wpi. To assess the production of IL-25, IL-33 and TSLP in intestinal tissues during *S. mansoni* infection, WT BALB/c mice were infected with *S. mansoni* cercariae as described above and sacrificed weekly from week 0 to week 4 post-infection, then every 2 weeks to 12 wpi.

Worm morphology and number
Adult *S. mansoni* worms were obtained by portal vein perfusion and fixed with 4% neutral buffered formalin (NBF) [35], following which their morphology was assessed under an inverted light microscope at 40× magnification and their number counted. Briefly, the portal vein was cut at its base under the liver, then the left cardiac ventricle was perfused with 30 mL of saline citrate (7.5 g of sodium citrate and 8.5 g of sodium chloride in milliQ) [34], followed by perfusion with 30 mL of phosphate buffered saline (PBS). The mesenteric veins were thoroughly checked for manual retrieval of worms that failed to wash out during perfusion.

Tissue eggs and eggs per worm pair numbers
Livers and intestines were harvested and digested with 4% KOH at 37 °C for 14 h. Briefly, livers were weighed and digested with 10 mL of 4% KOH, and intestines were cleansed of fecal matters, opened longitudinally, thoroughly washed with PBS, weighed and then digested as described for livers [34]. After digestion, samples were centrifuged for 5 min at 2000 rpm and room temperature. Eggs were counted in 50 µL of thoroughly mixed pellet suspension under the light microscope at 40× magnification and related to the organ weight. The number of eggs per worm pair was obtained by dividing the total number of tissue eggs per mouse by the number of worm pairs from the same mouse.

Egg isolation and production of *S. mansoni* soluble egg antigen
Eggs were isolated from the livers of *S. mansoni*-infected ICR mice and frozen at −30 °C until use. Briefly, 8 weeks after infection with 200 *S. mansoni* cercariae, livers of the infected mice were removed after the portal perfusion, washed with PBS, minced with sterile scissors and then homogenized in 1× PBS using the IKA T25 digital Ultra Turrax homogenizer (IKA-Werke GmbH & Co., Staufen, Germany). The homogenates were centrifuged for 5 min at 1500 rpm and room temperature, digested twice at 37 °C with shaking at 120 rpm, first with 1 mg/mL of Actinase E (Funakoshi, Tokyo, Japan) for 3 h, then with 0.1 mg/mL of Actinase E (Funakoshi) and 0.5 mg/mL of collagenase (Wako Pure Chemicals, Osaka, Japan) for 2 h. Obtained egg suspensions were filtered through a series of sieves (425, 180, 106 and 45 µm) [34]. The eggs retained on the smallest sieve (45 µm) were washed onto a Petri dish [29]. By swirling the dish, mature eggs were concentrated in the center of the dish and collected with a wide-bore pipette tip [34]. After settling, the supernatant was discarded and eggs were dry frozen at −30 °C until use.

For the production *S. mansoni* soluble egg antigen (SmSEA), frozen eggs were thawed on ice, resuspended in ice-cold PBS containing 10 µg/mL of leupeptin, then homogenized on ice using a handheld sterile glass Teflon homogenizer. The homogenate was subjected to five freeze (−80°C) and thaw (on ice) cycles, incubated at 4 °C overnight with rotation, then centrifuged for 1 h at 30,000 g and 4 °C. The supernatant was collected in new tubes on ice, dialyzed in PBS three times at 4 °C for 2 and 4 h and overnight, respectively, by using a Slide-A-Lyzer Dialysis Cassette (Thermo Fisher Scientific, Rockford IL, USA) as per the manufacturer's instructions. The protein concentration was determined by the Bicinchoninic acid (BCA) method (Pierce BCA Protein Assay, Thermo Fisher Scientific). The solution was filter-sterilized through a 0.2-µm filter, aliquoted and stored at −30 °C until use.

Cell stimulation and cytokine measurement
Immune cells were isolated from spleens and mesenteric lymph nodes (MLNs) of infected and non-infected wild-type (WT) and IL-33−/− mice and stimulated with SmSEA. Briefly, spleens were crushed and filtered through a 70-µm-mesh cell strainer, washed with Hank’s balanced salt solution (HBSS), then resuspended in complete RPMI medium (containing 10% fetal bovine
in a 5% CO₂ incubator at 37 °C for 72 h. The plates were
coated with 5 µg/mL of SmSEA in a 5% CO₂ incubator at 37 °C for 72 h. The plates were
then stored at −30 °C until use. The concentrations of IL-25, IL-33 and
IL-13 were measured in the culture supernatants by an enzyme-linked immunosorbent assay (ELISA)
as per the manufacturer’s instructions (DuoSet ELISA; R&D Systems, Minneapolis, MN, USA).

**Statistical analysis**

Data normality was determined by the Shapiro–Wilk test. Using GraphPad Prism version 8.4.2 for Windows
(GraphPad Software, San Diego, CA, USA), we performed Welch’s t-test or the Mann-Whitney U-test to
compare IL-33−/− and WT mice. Statistical significance
was set at \( P < 0.05 \). Unless otherwise stated, all data are
presented as mean with standard error of the mean and
are representative of at least two independent experiments
with similar results.

**Results**

**IL-33 deficiency does not affect S. mansoni worm maturation and the number of eggs in intestinal tissues**

Schistosome worms are characterized by their dependence
on the host immune system, particularly type 2 immunity, for their maturation, reproduction and egg
excretion \[15, 18, 35, 37\], indicating the importance of
type 2 immunity in the biology of schistosomes. Because IL-33 is known to induce type 2 immunity independently
of IL-4 \[38\] and in light of the results of a recent study
\[26\] which reported increased S. japonicum worm numbers after injection of exogenous IL-33 into infected
mice, we decided to investigate whether IL-33 deficiency
would compromise the maturation of S. mansoni worms
by comparing the morphology and number of worm
pairs between IL-33−/− and WT mice. We therefore
infected IL-33−/− and WT BALB/c mice with S. mansoni
cercariae and sacrificed them at indicated post-infection

time points (Fig. 1a). As shown in Fig. 1b, in terms of
morphology, there was no difference between worms
recovered from IL-33−/− and those recovered from WT
mice. Although the number of worm pairs tended to be
higher in IL-33−/− mice during the course of infection,
this difference was statistically significant only at the
ninth week of infection \( (U = 0.5, P = 0.0065; \text{Fig. 1c}) \). The
number of eggs per worm pair, calculated by dividing
the raw number of worm pairs by the raw number of tis-
sue (liver and intestinal) eggs, seemed to be comparable
between IL-33−/− and WT mice during the whole course
of infection \( (t_{(5)} = 0.184, P = 0.861; t_{(9)} = 1.972, P = 0.081; \text{Fig. 1c}) \).
To evaluate whether IL-33 deficiency may affect the
accumulation of eggs in the intestinal tissues, we com-
pared the number of eggs in intestinal tissues between
IL-33−/− and WT mice every 3 weeks from week 6 to
week 12 post infection. Similar to the observations
on the number of worm pairs, the number of eggs in intes-
tinal tissues tended to be higher in IL-33−/− mice than
in WT mice (Fig. 1d), but no statistical difference was found between both mouse genotypes ($U = 3, P = 0.2; U = 18, P = 0.281; U = 5, P = 0.285$ at 6, 9 and 12 wpi, respectively). Because several previous studies had reported a pathogenic role for IL-33 in egg-induced liver pathology by an increased number of liver tissue eggs [26, 27], we investigated whether IL-33 deficiency would affect the number of eggs in liver tissues of IL-33$^{-/-}$ compared to WT mice. We found that although IL-33$^{-/-}$ mice tended to have higher number of eggs than WT mice (Additional file 1: Figure S1b), there was no statistical difference in liver egg numbers between both mouse genotypes ($t_{(4)} = 2.097, P = 0.097; t_{(9)} = 0.397, P = 0.7; t_{(4)} = 1.298, P = 0.2653$ at 6, 9 and 12 wpi, respectively). Taken together, these data indicate that IL-33 is dispensable for the maturation of S. mansoni worms and that its absence may have a negligible effect on the accumulation of eggs in the intestinal tissues.

### IL-33 deficiency is associated with transitory impairment of type 2 immunity in mesenteric lymph nodes of S. mansoni-infected mice

Compared with IL-25 and TSLP, IL-33 is known to be the stronger molecule for inducing type 2 immunity through the activation of ILC2 and macrophages [39, 40]. Therefore, we assessed whether IL-33 deficiency would impair type 2 immunity in intestines. We isolated immune cells from MLNs of S. mansoni-infected IL-33$^{-/-}$ and WT mice, stimulated them with SmSEA for 72 h and then measured IL-5 and IL-13 cytokines by ELISA. As expected, IL-33 deficiency impaired the production of IL-5 and IL-13 in the MLNs of infected mice in response to stimulation with SmSEA at 6 weeks of infection ($t_{(8)} = 6.595, P = 0.0002$ for IL-5 and $U = 2, P = 0.008$ for IL-13; Fig. 2a, b). However, this impairment was not sustained during the course of infection as it disappeared at subsequent infection time points ($U = 6, P = 0.6857; U = 1, P = 0.4$ for IL-5 and $U = 3, P = 0.2; U = 1, P = 0.4$ for IL-13).
for IL-13 at 9 and 12 wpi, respectively; Fig. 2a, b). To verify whether this impairment was limited to the intestines or was systemic, we isolated immune cells from spleens of infected mice and measured IL-5 and IL-13 in the supernatants after 72 h of stimulation with S. mansoni soluble egg antigens for 72 h at 37 °C, 5% CO2. IL-5 and IL-13 cytokines were measured in cell culture supernatants by enzyme-linked immunosorbent assay (ELISA). a IL-5 from MLNs. Comparison by unpaired two-tailed t-test with Welch’s correction for 6 wpi. b IL-13 from MLNs. c IL-5 from spleens. d IL-13 from spleens. Comparison by unpaired two-tailed t-test with Welch’s correction for 6 wpi. Data are representative of two independent experiments with similar results and are presented as the mean with SEM. Significance (P value) is indicated above connector bars between appropriate groups. Mouse groups were compared using the Mann-Whitney test at the P < 0.05 level of significance.

**Fig. 2** IL-33 deficiency is associated with transitory impairment of type 2 immunity in mesenteric lymph nodes (MLN) of infected mice. Female IL-33−/− and WT BALB/c mice (4–8 animals per group) were subcutaneously infected with 50 and 35 S. mansoni cercariae for 9 and 12 weeks, respectively, and sacrificed at 6, 9 and 12 wpi. Immune cells were isolated from MLNs and spleens of infected and non-infected (control) mice and stimulated with S. mansoni soluble egg antigens for 72 h at 37 °C, 5% CO2. IL-5 and IL-13 cytokines were measured in cell culture supernatants by enzyme-linked immunosorbent assay (ELISA).

**IL-33 deficiency transiently attenuated egg-induced pathology in intestines of S. mansoni-infected mice**

Although schistosome worms also induce type 2 immunity [16, 17], eggs remain the most potent inducers of type 2 immunity and the main cause of the pathology in the liver and intestines of infected definitive hosts [11–13]. A pathogenic role for IL-33 in liver pathology during schistosome infections has been reported [26–28]. Although none of these studies reported on the role of IL-33 in the development of intestinal pathology, studies related to inflammatory bowel diseases have found controversial roles for IL-33 in the development and/or exacerbation of these diseases, with some reporting a protective role for IL-33 [41] and others incriminating it in the development or exacerbation of these diseases [42, 43]. Given this uncertainty, we investigated whether IL-33 deficiency would compromise the development of egg-induced pathology in intestinal tissues of infected mice. As shown in Fig. 3a, b, IL-33 deficiency was transiently associated with attenuated type 2 inflammatory responses in the terminal ilea of IL-33−/− mice compared to WT mice, characterized by less infiltration of intestinal tissues by inflammatory cells and a wall thickness similar to that of non-infected mice at the sixth week of infection (t(3) = 5.897, P = 0.010; Fig. 3b). Moreover, the inflammatory infiltrates in IL-33−/− mice contained fewer eosinophils than those in WT mice at the sixth week of infection (Fig. 3a). Both mouse genotypes did not differ in terms of granuloma number (U = 3, P > 0.999 and U = 2, P = 0.8 at 9 and 12 wpi, respectively; Fig. 3c) and area (U = 0, P = 0.2 and U = 3, P > 0.999 at 9 and 12 wpi, respectively; Fig. 3d). Taken together, these data suggest that IL-33 may be more important in initiating—but not maintaining—type 2 immunity at mucosal barriers than needed systemically and that it is not needed for the maintenance of schistosome egg-induced pathology in intestines. These results prompted us to speculate that IL-25 and TSLP expression might be upregulated in this setting to compensate for the absence of IL-33 at later infection time points in intestines.

**There is no change in IL-25 and TSLP production in the absence of IL-33 in intestines of infected mice**

Individually or synergistically, IL-25, IL-33 and TSLP are known to induce tissue type 2 immune responses in different homeostatic and pathologic conditions [39, 40, 44, 45]. The existence of possible interactions between these cytokines has also been suggested [40, 45]. We therefore reasoned that, due to IL-33 deficiency, there might be compensatory changes in IL-25 and/or TSLP production in S. mansoni-infected IL-33−/− mice compared to WT mice. As shown in Fig. 4, in the small intestinal tissues there was no statistically significant difference in the levels of IL-25 (U = 3, P > 0.999; t(4) = 0.185, P = 0.863; t(4) = 1.169, P = 0.309 for non-infected and infected mice at 6 and 9 wpi, respectively; Fig. 4a) and TSLP (U = 1, P = 0.666; t(4) = 0.296, P = 0.781; t(4) = 1.205, P = 0.294 for non-infected and infected mice at 6 and 9 wpi, respectively; Fig. 4b, c).
respectively; Fig. 4b) between IL-33−/− and WT mice, indicating that there is no compensatory changes in IL-25 and TSLP production in the absence of IL-33. Although the levels of IL-25 and TSLP expression in intestinal tissues homogenates tended to increase with S. mansoni infection compared to non-infected mice, infected mice did not produce enough of these cytokines to reach a statistically significant difference (Fig. 4a, b).

Previous studies reported an increase in IL-33 levels in the sera of individuals with S. japonicum infection compared to non-infected individuals [27]. In mice, this increase, which starts around week 4 of infection, reaches its peak around 8 wpi [26], corresponding with the oviposition period. This timeline may indicate that schistosome eggs are the major inducers of IL-33 release in schistosome infection settings. However, due to the functional redundancy of IL-33 with respect to IL-25 and TSLP [46], we investigated the kinetics of production of these cytokines during an S. mansoni infection. Thus, we infected only WT BALB/c mice with S. mansoni cercariae and checked for the release of IL-25, IL-33 and TSLP in their intestinal tissues. While the levels of IL-33 remained constantly higher even in naïve mice, levels of IL-25 and TSLP tended to increase with oviposition (Additional file 2: Figure S2), indicating that S. mansoni eggs may induce the release of IL-25 and TSLP, but not of IL-33, in the intestines of infected mice.

Discussion

Studies have shown that both schistosome worms and eggs induce type 2 immunity, which is essential for their maturation, reproduction and egg excretion [14–18]. Deficiency in CD4+ Th2 cells and their effector cytokines IL-4 and IL-13 has been shown to substantially decrease or completely abrogate egg excretion as a consequence of impaired worm maturation or failed signaling by type
Lungs have been shown to be the major site of attrition for migrating schistosomula, achieved through different mechanisms, including the penetration of schistosomula into alveoli and their expulsion through the pulmonary tract, and immune-mediated killing of schistosomula [52, 53]. Lungs are also one of the tissues where constitutive IL-33 is abundantly expressed in mice [54]. Schistosomula that enter the alveoli may damage the bronchoalveolar epithelium, inducing the release of IL-33 which, in turn, may initiate an anti-schistosomula immune response, thus contributing to the attrition of schistosomula. This process can result in IL-33−/− mice appearing to be more permissive or susceptible to S. mansoni infection, explaining the apparent lower number of worm pairs in WT mice compared to IL-33−/− mice (Fig. 1c).

We did not determine worm length, the proportion of single worms (males or females), the proportion of females in pairs [18, 55] or the number of eggs in the feces [14, 15]. However, based on the known morphology of worms [35, 56] and known intestinal tissue egg numbers [14, 32, 33], we believe that our study design is an appropriate alternative approach to looking at the effect of IL-33, as a potent initiator of type 2 immunity necessary for schistosome worm maturation, on the maturation of S. mansoni worms and the accumulation of eggs in intestinal tissues in the case of failed expulsion [14, 32, 33].

Yu et al. [26] reported that the injection of S. japonicum-infected mice with exogenous IL-33 increased the number of worms recovered at the sixth week of infection and also exacerbated the liver pathology by increasing the number and size of liver granulomas. This finding may simply mean that as endogenous IL-33 plays a role in the development of egg-induced liver pathology [26–28], injecting exogenous IL-33 would exacerbate its pathogenic effects. In the present study, we did not find any statistically significant difference in the number of eggs per worm pair between IL-33−/− and WT mice (Additional file 1: Figure S1a). These results corroborate those reported by Yu et al. [26] as they did not find a difference in the number of eggs per female worm. This result indicates that IL-33 alone may play a negligible role in worm maturation and the expulsion of eggs.

While studies related to inflammatory bowel diseases have reported controversial roles for IL-33 in the development and/or exacerbation of these diseases, with some reporting a protective role for IL-33 [31, 41] and others incriminating it in the development or exacerbation of these diseases [42, 43], to the best of our knowledge there has been no report on the role of this cytokine in intestinal pathology during schistosomiasis. Although IL-33 seemed dispensable for S. mansoni worm maturation and the excretion of their eggs, we sought to determine
whether it may play a significant role in the development of egg-induced pathology in the intestines of infected mice, as it does in the liver [26–28]. We found that the absence of IL-33 transiently impaired type 2 immunity in the small intestines of IL-33−/− mice, but not in their spleens, characterized by impaired production of IL-5 and IL-13 cytokines in MLNs in response to stimulation with SmSEA (Fig. 2) and attenuated egg-induced inflammation in the ilea of IL-33−/− mice at 6 wpi (Fig. 3a, b). These results are in line with findings by Vannella et al. [46] who, focusing on the role of alarmin cytokines IL-25, IL-33 and TSLP in the development and maintenance of type 2 cytokine-driven inflammation and fibrosis in lungs and liver, found that ablation of these cytokines singly had no significant ameliorating effect on liver pathology. However, when all three cytokines were ablated, a significant improvement of the pathology could be observed in the early phase of the infection, indicating functional redundancy among these cytokines. The trends observed in the present study are in the same direction, with the absence of IL-33 not affecting the development of pathology (Fig. 3a) nor the number and size of granulomas in the intestines of IL-33−/− mice at time points beyond the 6 wpi (Fig. 3c, d). However, the difference between the study by Vannella et al. [46] and our study is that we started our observations at 6 wpi, when egg-induced type 2 immunity is still at its start, while Vannella et al. started their observations at 9 wpi when egg-induced type 2 immunity has already reached its peak. We believe that Vannella et al. [46] may have found a significant difference between IL-33 deficient mice and WT at an earlier stage of the infection, as observed in our study.

Despite IL-33 sharing functional redundancy with IL-25 and TSLP [46], the former remains the most potent of all three cytokines in inducing type 2 immunity [39, 40, 57]. In addition to inducing type 2 immunity by itself, IL-33 can also potentiate the type 2 immunity induced by IL-25 and TSLP [58]. Of all the cells that respond to IL-33, ILC2 and Th2 are the most important as through their production of abundant amounts of the type 2 cytokines IL-4, IL-5 and IL-13, they play the most important role in cell-mediated effector type 2 immunity [59, 60], characterized by, among others, the accumulation of M2 macrophages and eosinophils in affected tissues. Although dispersed in all tissues, ILC2 are more abundant in the lungs and intestinal tissues [61], where they are the first to be activated by IL-33, subsequently migrating to local draining lymph nodes to initiate adaptive type 2 immunity [25, 62]. Thus, it is understandable that the absence of IL-33 in IL-33−/− mice at the early stage of the patent infection might have left ILC2 inactivated, leading to impaired type 2 immunity [59], as seen in the present study (Fig. 2a, b). In addition to acting through ILC2 and Th2 cells, IL-33 also acts directly on eosinophils, inducing their activation and expansion [63, 64]. Therefore, its absence in IL-33−/− mice can explain the small number of eosinophils in the inflammatory infiltrates at 6 wpi (Fig. 3a, b) [65]. However, due to the persistence of egg-derived ESP [6–10] as eggs keep accumulating in the tissues, and to the fact that IL-25 and TSLP can induce type 2 immunity independently of IL-33 [51, 66–68], alternative mechanisms leading to the activation of both innate and adaptive type 2 immunity, including the taking over of ILC2 activation by IL-25 and TSLP and Th2-dependent effector pathways, might have been activated to compensate the absence of IL-33 (Figs. 2, 3). Together, these alternative mechanisms may have led to improved type 2 immunity at time points beyond 6 wpi.

Results from various studies have pointed to the existence of possible interactions between IL-25, IL-33 and TSLP [40, 45]. In one study, anti-IL-33 treatment and TSLP receptor deficiency blocked the infection-induced expression of IL-25 in lung epithelial cells, and ex vivo treatment of ILC2 with TSLP increased their expression of IL-25 and IL-33 receptors [45]. In another study, the authors noted that IL-25 shared with IL-33 many activities on macrophages without having additive effects, pointing toward the possible existence of common downstream signaling pathways for their biological activities [40]. This led us to postulate that IL-33 deficiency might be associated with a modified production of IL-25 and TSLP in the intestines of S. mansoni-infected IL-33−/− mice. However, our results show no modification of intestinal production of IL-25 and TSLP as their levels in intestinal tissue homogenates did not differ between mouse genotypes (Fig. 4a, b), meaning that although they can, individually or synergistically, induce type 2 immunity, the absence of one may not affect the others in the schistosome infection settings or intestines. The nature of the interactions and conditions of their occurrence between IL-25, IL-33 and TSLP pointed out in the above-mentioned studies [40, 45] remain to be clarified.

Studies in humans and mice have reported an increase of IL-33 levels in the sera of individuals and animals infected with S. japonicum [27]. Also, these increased levels of IL-33 in serum were found to peak around the 8th week of infection in mice [26], corresponding to the peak of egg-induced immune responses, suggesting that through their ESP, eggs may be the main inducers of IL-33 release in schistosome infections. Indeed, Hams et al. [50, 51] reported that injection of S. mansoni eggs or the recombinant form of their derived components, namely w1, induced the production of IL-25 and IL-33 in the lungs and fat tissue, respectively. Whether eggs in intestinal tissues induce the production of IL-33, IL-25 and TSLP is not known. We measured the levels of
these alarmin cytokines in the intestinal tissue homog-
genates during S. mansoni infection in WT BALB/c mice and
found that IL-33 levels remained constantly higher,
even in non-infected mice (Additional file 2: Figure S2).
In contrast, IL-25 and TSLP levels fluctuated over
the course of infection, peaking around the tenth week
of infection, with TSLP at much lower levels than IL-25
(Additional file 2: Figure S2). The start of an increase
in levels of IL-25 and TSLP tended to correspond to that of
oviposition, suggesting that the latter might be inducing
the release of IL-25 and TSLP, but not of IL-33. Flam-

et al. [62] recently reported that IL-33 expression was
high in the small intestines of naïve mice, corroborat-
ing our findings, and indicating that IL-33 is constantly
expressed in high amounts in mouse intestinal tissues.

Conclusions
To the best of our knowledge, this is the first study to
look at the role of IL-33 in the maturation of S. mansoni
worms, as well as at the effect of its absence on the accu-
mulation of eggs in the intestinal tissues. It is also the
first study to report on the role that IL-33 may play in the
maintenance of egg-induced type 2 immunity in intest-
tines of S. mansoni-infected mice. The results show that
IL-33 is dispensable for the maturation of S. mansoni and
that its absence may have a negligible effect on the num-
er of eggs accumulating in intestinal tissues when they
fail to exit the intestines. Furthermore, due to transient
impairment of type 2 immunity observed in the intestines
but not spleens, this study highlights the importance of
IL-33 over IL-25 and TSLP in initiating, but not main-
taining, locally induced type 2 immunity in intestinal tis-
sues in schistosome infections. These results corroborate
previously reported findings that IL-25, IL-33 and TSLP
may be sharing a partial functional redundancy in their
ability to maintain tissue-induced type 2 immunity. Their
combined or sequential ablation might be the best option
to decipher the role of each of them in schistosoma-
sis and clarify the possible interactions that might exist
between them.

Supplementary Information
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Additional file 1: Figure S1. IL-33 deficiency does not affect the number
of eggs produced per S. mansoni worm pair and the liver egg burden
in infected mice. Female IL-33−/− and WT BALB/c mice (4–8 animals per
group) were subcutaneously infected with 50 and 35 S. mansoni cercariae
for 9 and 12 weeks, respectively, and sacrificed at 6, 9 and 12 wpi to
determine the number of worm pairs and the number of liver

tissue eggs. a Number of eggs per worm pair, b number of eggs per gram
of liver tissue. Experiments were replicated at least three times. Data are
represented as 2 independent experiments with similar results and are
presented as mean with SEM. Groups were compared using unpaired
two-tailed t-test with Welch’s correction, with statistical significance set at
P < 0.05

Additional file 2: Figure S2. Oviposition in S. mansoni infection induces
intestinal production of IL-25 and TSLP but not of IL-33. Female WT BALB/c
mice (3 animals per time point) were subcutaneously infected with 50
and 35 S. mansoni cercariae for 9 and 12 weeks, respectively, and sacrificed
weekly from week 0 (non-infected) to week 4, then every 2 weeks up
to week 12 of infection. Small intestines were homogenized with the
gentleMACS Octo Dissociator, and the cytokines were measured in the
homogenate supernatants by ELISA. Data are presented as the mean
with standard deviation. Cytokines were measured in only one mouse at
12 wpi.

Abbreviations
BCA: Bicinchoninic acid; ESP: Excretory–secretory product; HBSS: Hank’s
balanced salt solution; IL: Interleukin; ILC2: Group 2 innate lymphoid cell;
M2: Alternatively activated macrophage; MLN: Mesenteric lymph node; NBF:
Neutral buffered formalin; PBS: Phosphate buffered saline; SmSEA: Schistosoma
mansoni soluble egg antigens; Th2: T-helper 2 cell; TSLP: Thymic stromal lym-
phopoietin; wpi: Weeks post-infection; WT: Wild type; ω1: Omega-1.

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Authors’ contributions
JPKM and SH conceived the study; JPKM and RN designed the experiments;
JPKM performed the experiments, analyzed data and wrote the manuscript;
SU provided the critical materials; RN and SH supervised the study; SH
acquired the funding. JPKM and SH revised the manuscript. All authors read
and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published
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The study protocol was approved by the Nagasaki University Committee for
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Competing interests
The authors declare that they have no competing interests.
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