Immunity-mediated regulation of fecundity in the nematode *Heligmosomoides polygyrus* – the potential role of mast cells

K. HASHIMOTO*, R. UCHIKAWA, T. TEGOSHI, K. TAKEDA, M. YAMADA *and* N. ARIZONO

Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kyoto 602-8566, Japan

(*Received 8 September 2009; revised 20 October 2009; accepted 22 October 2009; first published online 22 December 2009*)

SUMMARY

Previous studies have shown that host immunity regulates the fecundity of nematodes. The present study was aimed at clarifying the reversible nature of fecundity in response to changes of immunological status and to determine which effector cells are responsible for compromising fecundity in *Heligmosomoides polygyrus*. Enhanced fecundity was observed in immunocompromised SCID and *nu/nu* mice compared to those in the corresponding wild-type mice, with significantly fewer numbers of intrauterine eggs produced in the wild-type than in the immunodeficient mice. When 14-day-old adult worms from BALB/c mice were transplanted into naïve BALB/c mice, their fecundity increased significantly as early as 24 h post-transplantation, but not when they were transferred into immune mice, suggesting the plastic and reversible nature of fecundity in response to changes in host immunological status. In mast cell-deficient W/W^v mice, nematode fecundity was significantly higher than in mast cell-reconstituted W/W^v or +/+ mice. The serum levels of the mast-cell protease mMCP1 were markedly increased in the wild-type as well as the mast cell-reconstituted W/W^v, but not in the W/W^v, SCID, or *nu/nu* mice during infection. These findings raise the interesting possibility that certain activities of mast cells, either directly or indirectly, regulate parasite fecundity during infection.

Key words: *Heligmosomoides polygyrus*, fecundity, mast cell, W/W^v mouse.

INTRODUCTION

Infection with intestinal nematodes induces the expansion of T helper (Th) 2 cells, which play a pivotal role in protection against parasitic nematodes (Finkelman *et al*. 2004; Artis and Grencis, 2008). It has been shown that the Th2 cytokines interleukin (IL)-4 and IL-13 protect against parasites via Stat6 activation, which promotes the expulsion of worms, possibly through mechanisms such as the activation of mast cells, increased intestinal mucus secretion, and enhancement of intestinal smooth muscle contraction (Finkelman *et al*. 2004; Khan and Collins, 2004). Although the downstream biological effects of host immune responses on nematodes have not been fully elucidated, one such effect seems to be the modulation of nematode fecundity. Even if the suppression of fecundity is not directly linked to worm expulsion, it will still ultimately reduce the transmission of nematodes. Urban *et al*. (1991, 1995) showed that administration of anti-CD4 antibody to mice infected with *Heligmosomoides polygyrus* resulted in an increase in worm fecundity, while administration of IL-4 rapidly decreased it, suggesting that $CD4^+$ T cells and/or IL-4 regulate not only the survival of, but also the fecundity of intestinal nematodes. Similarly, in humans infected with *Necator americanus*, a highly significant negative correlation between total IgE level and parasite mass and fecundity was observed (Pritchard *et al*. 1995). Furthermore, in *Strongyloides ratti* infections, density-dependent effects on parasite survivorship and fecundity have been reported to be mediated by the host immune response, although the fecundity of *S. ratti* was not affected by a history of either con- or hetero-specific infection (Paterson and Viney, 2002; Bleay *et al*. 2009). Viney *et al*. (2006) further showed that the immunization-dependent reduction in *per capita* fecundity of *S. ratti* was fully reversed upon immunosuppression. Despite these studies, it is largely unknown which types of intestinal cells or molecules directly mediate the modulation of nematode fecundity and whether the immunological effect on nematode fecundity is exerted independently from its effect on the expulsion of nematodes from the intestine.

Thus, the present study was aimed at clarifying the role of T cells and mast cells in the modulation of fecundity in *H. polygyrus* adult worms using experimental infections in T and B cell-deficient SCID mice, athymic *nu/nu* mice, and mast cell-deficient W/W^v mice. The plasticity in nematode fecundity in immunocompetent mice was also studied by worm-transplantation experiments.

* Corresponding author: Tel: +81 75 2515326. Fax: +81 75 2515328. E-mail: ka-hashi@koto.kpu-m.ac.jp

Parasitology (2010), **137**, 881–887.
doi:10.1017/S0031182009991673

MATERIALS AND METHODS

Animals, nematode infection, and autopsy

Specific-pathogen-free (SPF) male C.B-17/Icr-*scid*/*scid*Jcl, C.B-17/lcr-+/+Jcl, BALB/cAJcl-*nu*/*nu*, and BALB/cAJcl-*nu*/+ mice were purchased from Clea Japan Inc (Tokyo, Japan). SPF male WBB6 F1 W/W^v, WBB6 F1 +/+, BALB/c, and C57BL/6 mice were purchased from SLC Inc. (Shizuoka, Japan). The animals were inoculated orally with 200 *H. polygyrus* infective-stage (L3) larvae via a feeding tube and were allowed to feed *ad libitum* throughout the experiment. All animals were sacrificed 3 weeks after infection.

Mast cell reconstitution of W/W^v *mice*

Bone marrow cells were extracted from the femurs of WBB6-F1 +/+ mice and suspended in α-MEM (ICN Biomedical, Aurora, OH) supplemented with 10% (v/v) fetal bovine serum (ICN Biomedical), 100 U/ml penicillin, and 100 μg/ml streptomycin. Bone marrow cells (2×10^7 cells in 0·2 ml) were transferred into 12 W/W^v mice by injecting them into the tail vein. Twenty weeks after the transfer, the mast cell-reconstituted W/W^v mice were subjected to *H. polygyrus* infection.

Egg and worm count

Fecal pellets were collected from individual mice at 10, 14, 17, and 21 days post-infection. The number of eggs per gram of feces (EPG) was determined by counting the number of eggs in 100 mg feces, which was dissolved in 1 ml of 10% (v/v) neutral formalin. Worm burdens were determined 3 weeks after infection. In brief, after euthanizing the animals, the duodenum and the small intestine were removed and opened longitudinally. The worms were recovered by a saline incubation method, and the number of worms was counted under a dissecting microscope. The EPG per female adult worm (EPGPF) was defined as the number of eggs per gram of colonic feces divided by the number of female worms in the small intestine. To do this, all the fecal material was collected from the colon, weighed, and dispersed in 10% (v/v) neutral formalin, and the number of eggs was counted under a microscope.

The number of intrauterine eggs of adult female worms was examined at 3 weeks post-infection. In brief, 10 adult female worms per mouse were collected and fixed in 10% (v/v) neutral formalin for 2 days. These worms were re-suspended in 250 μl of 10% (v/v) neutral formalin and subjected to a 15-sec burst of sonication (Tomy Seiko Ltd, Tokyo), which resulted in the release of practically all of the intrauterine eggs unbroken. The number of eggs released was counted under a microscope.

Ex vivo *egg deposition by female worms*

After counting the total number of worms to determine the worm burden, female worms were collected randomly to make up a total of 80 female worms per experimental group. The female worms were individually placed in 180 μl of phosphate-buffered saline (PBS) in a 96-well plate and incubated at 37 °C. After a 2-h incubation, 20 μl of 30% (v/v) formalin were added to each well, and the number of eggs deposited in each well was counted under an inverted-stereotype microscope. The mean numbers of eggs deposited per female worm and the S.E. of 80 female worms were determined.

Adult worm transplantation

Twenty BALB/c mice were divided into 4 groups: 8 donor mice, 4 immune-recipient mice, 4 naïve-recipient mice, and 4 non-transplantation-control mice. The donor, immune-recipient, and non-transplantation-control mice were infected with 200 L3 larvae of *H. polygyrus* 14 days before the transplantation experiment. Eleven days post-infection (3 days before transplantation), the immune-recipient mice were administered orally with pyrantel pamoate (Pfizer, NYC) at a dose of 150 mg/kg. After this treatment, the eggs became undetectable 1 day before the transplantation experiment. A preliminary experiment showed that the same dose of anthelminthics had totally eliminated the adult worms from the intestine within 24 h.

One-day before and on the day of transplantation, the EPGs in the 8 donor mice were determined. For worm transplantation, each donor mouse was sacrificed, and adult worms were collected as quickly as possible by the saline incubation method. The naïve-recipient mice and immune-recipient mice were anaesthetized by ether, a small skin incision was made in the abdomen, and an in-dwelling intravenous cannula was inserted through the stomach into the duodenal lumen. The worms recovered were injected into the duodenum through the cannula, and the abdominal incision was sutured. In order to minimize the damage to the worms, the number of worms transferred to the recipient mice was not counted; however, all the worms recovered from a donor mouse were transferred immediately into a recipient mouse, which enabled us to monitor the changes in the EPG of the same population of worms in the recipient and donor mouse. The non-transplantation control mice received a sham operation, which included the abdominal incision and the insertion of a cannula, but not worm transplantation. Two mice died during these procedures. Thus, each group of mice consisted of 3 immune-recipient mice, 4 naïve-recipient mice, and 3 non-transplantation-control mice.

https://doi.org/10.1017/S0031182009991673 Published online by Cambridge University Press

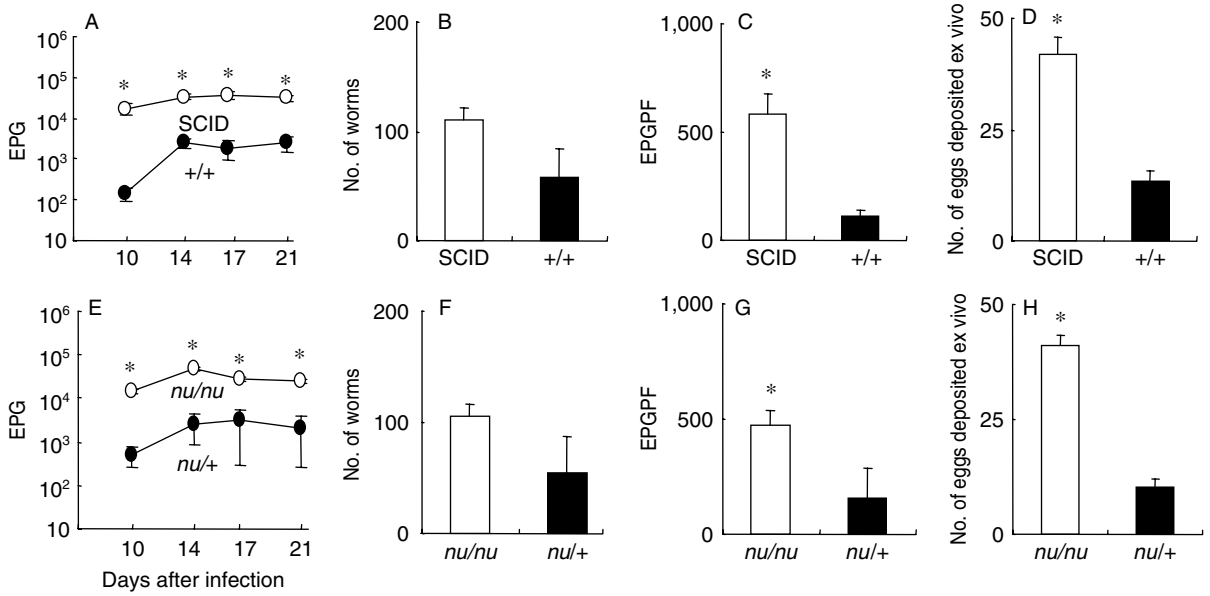

Fig. 1. Fecundity of *Heligmosomoides polygyrus* adult worms in SCID and the corresponding +/+ mice (A–D), and in athymic *nu/nu* and euthymic *nu/+* mice (E–H). (A, E) EPG. (B, F) Worm burdens at 21 days post-infection. (C, G) EPGPF at 21 days post-infection. (D, H) Number of eggs deposited by a female worm during 2-h incubation *ex vivo*. Each group consists of 5 animals except in the *nu/+* mice ($n=4$). Data shown are means ± S.E. * Significantly different from the corresponding control strain of mice ($P<0{\cdot}05$).

Quantification of serum mMCP-1

Blood was taken from the femoral artery, and sera were stored at −80 °C until use. The serum mMCP1 levels were measured with an mMCP-1 ELISA kit (Moredun Scientific Ltd. Scotland, UK) according to the manufacturer's instructions.

Statistical analysis

The Student's *t*-test (two-tailed) was employed. Where the *t*-test was not applicable, the Mann-Whitney U test was used. *P*-values less than 0·05 were considered significant.

RESULTS

Compromised nematode fecundity in immunocompetent mice

Urban *et al*. (1991, 1995) showed that $CD4^+$ T cells and IL-4 regulate the survival as well as the fecundity of *H. polygyrus*. To confirm this, we re-examined the fecundity of *H. polygyrus* in SCID and *nu/nu* mice. As shown in Fig. 1A and E, the EPG levels were consistently higher in the SCID and *nu/nu* mice than in the corresponding +/+ or *nu/+* mice. The worm burdens examined 3 weeks post-infection did not differ significantly between the SCID and +/+ or between the *nu/nu* and *nu/+* mice, although the mean numbers of worms were somewhat larger in the SCID and *nu/nu* mice than in corresponding mice. EPGPF levels at 3 weeks post-infection were significantly higher in the SCID and *nu/nu* mice than in the corresponding control mice.

EPGPF is influenced by the accuracy of EPG, which might vary considerably according to the water content of feces. Thus, the number of eggs deposited by individual female worms *ex vivo* was also examined (Fig. 1D, H). The number of eggs deposited within 2 h *ex vivo* was also significantly higher in the worms that recovered from SCID and *nu/nu* than in the corresponding mice, confirming the compromised fecundity of nematodes in the immunocompetent mice.

To see if the significantly reduced fecundity of the nematodes in the immunocompetent mice was due to impeded egg development, the number of intrauterine eggs with shells was counted in adult female worms that had recovered 21 days post-infection. The numbers of intrauterine eggs per female worm were 99·8 ± 9·7 and 54·1 ± 18·8 in the SCID mice and wild-type mice, respectively (number of host animals = 4, $P<0{\cdot}05$), suggesting that egg development was suppressed in the wild-type mice.

Plasticity of nematode fecundity in immunocompetent mice

It is of interest to know whether the compromised fecundity of nematodes in certain microenvironments can be changed by transferring the worms into a new microenvironment. In a preliminary experiment, expecting the compromised fecundity to be markedly increased in the SCID-mouse intestine, a fixed number of 2-week-old adult worms recovered from +/+ mice was transferred into the duodenum of naïve +/+ and naïve SCID mice. However, the

https://doi.org/10.1017/S0031182009991673 Published online by Cambridge University Press

Table 1. The number of eggs per gram of feces (EPG) of *Heligmosomoides polygyrus* adult worms before and after transplantation into naïve and immune mice

(Two-week-old adult worms in BALB/c mice were recovered and transplanted into naïve BALB/c mice (naïve 1–4), and immune mice (immune 1–3) which had been infected with 200 L3 larvae 2 weeks previously and de-wormed 3 days before receiving the worm transplantation. The non-transplantation-control mice (control 1–3) had been infected with 200 L3 larvae 2 weeks previously and received a sham operation on the day of worm transplantation. To monitor the EPG of the same population of worms from the pre-transplantation to the post-transplantation period, all the worms collected from each donor mouse were transferred into a single recipient mouse.)

EPG in donor mouse			EPG in recipient mouse		
Days before transplantation			Days after transplantation		
−1	0	Mouse no.	1	3	7
4600	19 200	Immune-1	9800	8400	13 400
21 200	11 400	Immune-2	12 400	14 600	7200
12 600	15 000	Immune-3	11 400	11 200	10 600
8800	6400	Naive-1	38 000	51 200	35 200
14 000	14 400	Naive-2	47 400	58 200	40 600
22 000	6200	Naive-3	60 000	29 800	48 400
12 000	22 000	Naive-4	77 600	36 000	24 600
7 000	15 200	Control-1	21 400	12 600	15 600
11 400	15 600	Control-2	25 200	25 000	9800
16 800	19 000	Control-3	22 600	21 600	18 400

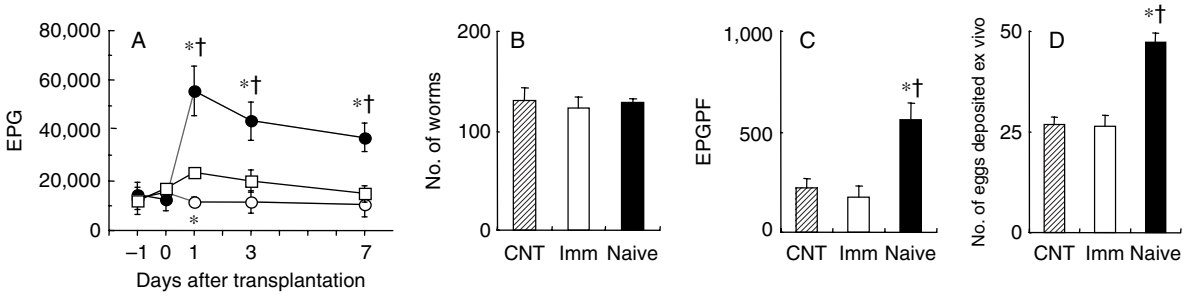

Fig. 2. Alteration of the fecundity of *Heligmosomoides polygyrus* adult worms after transplantation into naïve and immune mice. See Table 1 for the details of the worm transplantation experiment. (A) EPG in immune-recipient mice (open circles), naïve-recipient mice (filled circles), and control mice (open squares). The EPG in the donor mice or control mice 1 day before and on the day of transplantation are indicated as day −1 and 0, respectively. (B) Worm burdens in the control (CNT), immune-recipient (Imm), and naïve-recipient (Naïve) mice 7 days post-transplantation. (C) EPGPF in the control (CNT), immune-recipient (Imm), and naïve-recipient (Naïve) mice 7 days post-transplantation. (D) Number of eggs deposited by a female worm during 2-h incubation *ex vivo*: control (CNT), immune-recipient (Imm), and naïve-recipient (Naïve) mice 7 days post-transplantation. Data shown are means ± S.E. * Significantly different from the control mice ($P<0{\cdot}05$). † Significantly different from the immune-recipient mice ($P<0{\cdot}05$).

EPGs 4 days after the worm transplantation (100 female and 100 male worms/mouse) showed no significant differences between those in the +/+ (32 725 ± 12 027) and SCID recipients (42 450 ± 4313). The EPGPF 7 days after the worm transplantation also showed no significant differences: 423·6 ± 113·4 and 725·3 ± 345·2 in the +/+ recipients and SCID recipients, respectively, suggesting that the mucosal environment of the naïve +/+ mice was not much different from that of the SCID mice in terms of its effect on fecundity.

Thus, in the next experiment, 2-week-old adult worms recovered from BALB/c mice were transferred into the duodenum of naïve BALB/c and immune BALB/c mice. To determine the changes in fecundity in the same population of worms, all the worms recovered from 1 donor mouse were transferred into 1 recipient mouse, and EPG levels were examined from 1 day before to 7 days after transplantation. When 14-day-old worms were transferred into naïve mice, the EPG increased approximately 3-fold as early as 24 h after worm transplantation (Table 1 and Fig. 2A). In contrast, the EPG levels did not change significantly when the worms were transferred into immune recipient mice. One day after transplantation, the EPG levels in the immune-recipient mice were slightly but significantly lower than those in the control mice, probably

https://doi.org/10.1017/S0031182009991673 Published online by Cambridge University Press

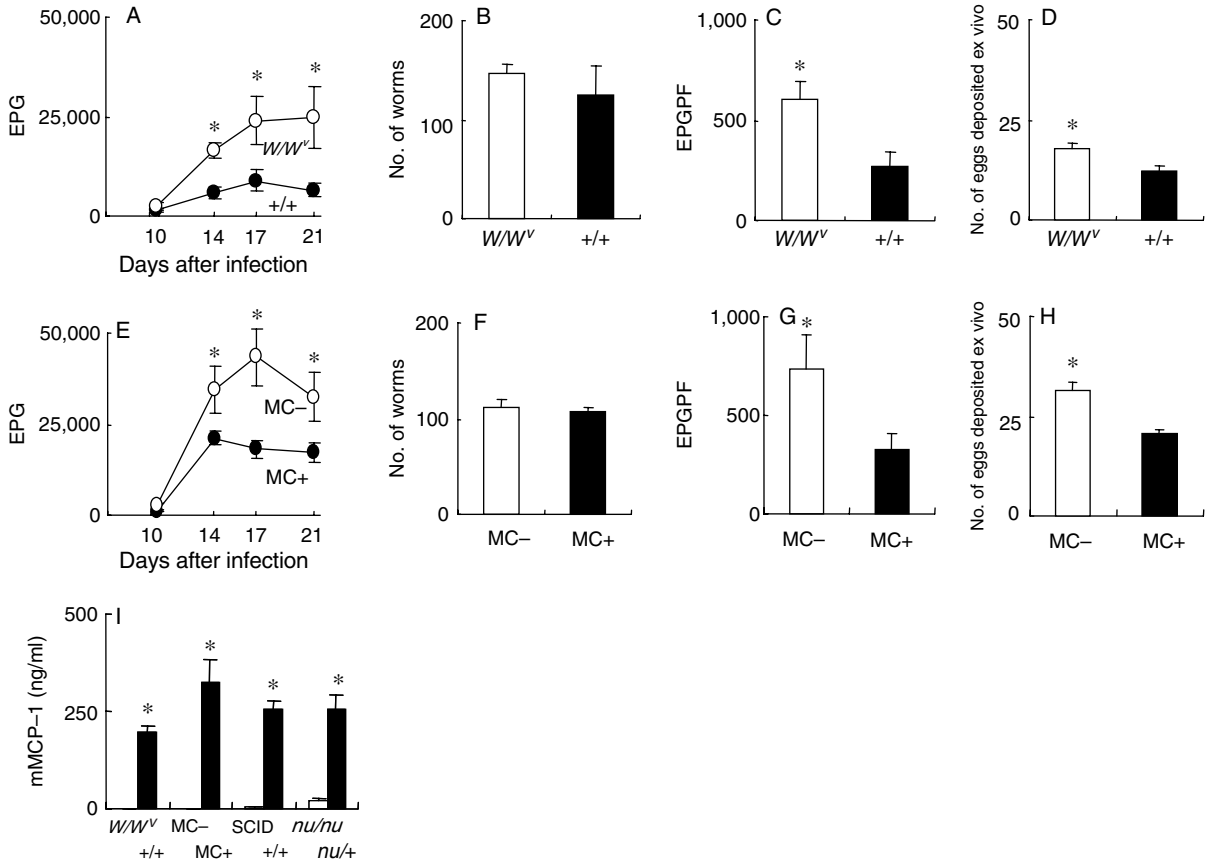

Fig. 3. Fecundity of *Heligmosomoides polygyrus* adult worms in mast cell-deficient W/W^v and mast cell-reconstituted W/W^v mice. (A–D) W/W^v and +/+ mice ($n=4$, respectively). (E–H) Mast cell-reconstituted W/W^v mice (MC+) ($n=7$) and age-matched non-reconstituted W/W^v mice (MC−) ($n=4$). (A, E) EPG. (B, F) Worm burdens at 21 days post-infection. (C, G) EPGPF at 21 days post-infection. (D, H) Number of eggs deposited by a female worm during 2-h incubation *ex vivo*. (I) mMCP-1 levels in the sera (ng/ml) of W/W^v and +/+ mice, MC+ and MC− mice, SCID and +/+ mice, and *nu*/*nu* and *nu*/+ mice at 21 days post-infection. The mMCP-1 levels in uninfected mice were lower than 10 ng/ml (not shown). The number of animals corresponds to those in (A–D) and (E–H) of this figure and those in Fig. 1. Data are means and S.E. * Significantly different from the corresponding strain of mice ($P<0{\cdot}05$).

reflecting mild damage suffered by the worms during the transplantation procedure. Seven days post-transplantation, the EPG levels were still significantly higher in the naïve-recipients than in the immune-recipient mice. The EPGPF and *ex-vivo* egg deposition were also significantly higher in the worms from the naïve mice than those from the immune mice (Fig. 2C, D). These results clearly showed that even if the egg production of nematodes is suppressed in an immune environment, it is reversible and rebounds quickly if the worms are transferred into a non-immune environment.

Effector cells that affect nematode fecundity

The effector cells or molecules that affect the fecundity of worms on local mucosa have not been clarified. To determine whether mast cells are one such effector of fecundity, mast cell-deficient W/W^v and corresponding +/+ mice were infected with 200 L3 larvae of *H. polygyrus*. The nematodes that infected the W/W^v mice showed significantly higher levels of EPG, EPGPF, and *ex vivo* egg production than those in the +/+ mice (Fig. 3A–D). To confirm that the differences were due to the presence or absence of mast cells, nematode fecundity was further examined in mast cell-reconstituted W/W^v mice that had received a wild-type bone-marrow-cell transplantation. The mast cell-reconstituted W/W^v mice showed significantly lower levels of EPG, EPGPF, and *ex-vivo* egg deposition compared to the non-reconstituted W/W^v mice (Fig. 3E–H). Marked elevation of the mast cell-protease mMCP-1 in the serum was observed during infection in the mast-cell-reconstituted W/W^v mice and wild-type mice, but not in the non-reconstituted W/W^v mice (Fig. 3I). mMCP-1 levels were also measured in the SCID and *nu*/*nu* mice. As expected, the mMCP-1 levels during infection were significantly lower in the SCID and *nu*/*nu* mice than in the corresponding +/+ and *nu*/+ mice (Fig. 3I). These results suggest that the activation of mast cells plays a crucial role in the regulation of the fecundity of nematodes, although it did not affect

https://doi.org/10.1017/S0031182009991673 Published online by Cambridge University Press

the survival of worms at least until 3 weeks post-infection.

DISCUSSION

These results show that the fecundity of *H. polygyrus* females was significantly suppressed in wild-type and euthymic *nu*/+ mice compared to that in SCID or *nu*/*nu* mice. The result that the number of intrauterine eggs was significantly lower in the female worms from the wild-type than in those from the SCID mice further indicated that egg development, rather than egg deposition, was suppressed to some extent in wild-type mice. L3 larvae of *H. polygyrus* invade into the intestinal mucosa and migrate into the submucosa juxtaposed to the propria muscularis, where they develop into adult worms and finally emerge into the lumen (Monroy and Enriquez, 1992). Thus, it is possible that tissue reactions to larvae, which may be more intense in immunocompetent than in immunodeficient mice, subsequently influence the fecundity of the adult worm when it emerges into the gut lumen. In fact, the EPG levels of 10-day-old worms, which have just emerged into the gut lumen, were significantly higher in the SCID and *nu*/*nu* mice than in the corresponding immunocompetent mice. However, the results of the transplantation experiments in the 14-day-old adult worms clearly showed that the mucosal-surface or luminal environment (naïve *vs* immune) is a more important determinant of nematode fecundity, at least between 2 and 3 weeks post-infection, than the effect of the submucosal tissue reaction to larval stages. It seems that the suppression of fecundity is a host defence strategy, but it is unclear whether it occurs in parallel with or prior to the expulsion of worms. In fact, although the worm burdens showed no significant difference between the +/+ and SCID mice, or between the *nu*/+ and *nu*/*nu* mice, the worm burdens in some +/+ and *nu*/+ mice were markedly smaller than those in the SCID and *nu*/*nu* mice at 3 weeks post-infection, suggesting partial worm expulsion in some, but not all, mice at this time-point. However, our results showed a dramatic and rapid increase of EPG in the 14-day-old worms just after transplantation into the naïve wild-type mice while the worm burden remained the same, indicating that the down-regulation of fecundity does not necessarily occur in parallel with the clearance of worms from the intestine and may not be irrecoverable. The reversibility of the immune-mediated inhibition of the fecundity of *H. polygyrus* supports similar observations in *S. ratti* (Viney *et al.* 2006).

Although host suppression of *H. polygyrus* fecundity requires $CD4^+$ T cells (Urban *et al.* 1991), the molecules or cells that mediate the suppression of fecundity remain unknown. Lawrence and Pritchard (1994) reported that there are some genetic differences in the responsiveness of mouse strains to infection with *H. polygyrus*, resulting in 'fast' or 'slow' responder strains, where egg output has been shown to be correlated to the level of mastocytosis, i.e. lower EPG correlates with a high mast cell response. The possibility that certain activities of intestinal mast cells contribute to the modulation of parasite fecundity during infection has also been raised by the observations that at the peak of egg output of *N. brasiliensis* infection, parasite egg production was ~3·5-fold greater in congenic +/+ rats than in mast cell-deficient *Ws*/*Ws* rats (Arizono *et al.* 1993), and treatment of the rats with stem cell factor (SCF) increased, and treatment with anti-SCF significantly decreased parasite egg production during *N. brasiliensis* infection (Newlands *et al.* 1995). These results suggest that mast cell activation has favourable effects on parasite fecundity during infection. However, the present study in mast cell-deficient mice showed a contradictory result. In the W/W^v mice, EPG and EPGPF were ~2-fold greater than those in +/+ or mast cell-reconstituted W/W^v mice and were comparable to the levels in the SCID and *nu*/*nu* mice. Moreover, the mMCP-1 levels in the SCID and *nu*/*nu* mice during infection were also significantly lower than those in the corresponding normal mice, apparently showing a compromised mast-cell response in immunodeficient mice. These results suggest that mast cell activation created a microenvironment that was unfavourable to the manifestation of maximum fecundity in nematodes, although the evidence for mast cell involvement is still circumstantial, since the W/W^v mice are not entirely healthy and also exhibit other haematopoietic abnormalities such as anaemia, which is also corrected by bone-marrow transplantation (Kitamura *et al.* 1978). The reason for the contradictory results in *N. brasiliensis* and *H. polygyrus* is not clear, but it is possible that the effect of mast cells on fecundity differs between nematode species, just as the effect of mast cells on worm rejection is different between nematode species. Mast cells play an important role in the clearance of *Trichinella spiralis* and *Strongyloides* spp., whereas rejection of *N. brasiliensis* depends on the effects of IL-4/IL-13 on non-bone marrow-derived cells, such as changes in intestinal epithelial function, increased intestinal mucus secretion, and the enhancement of intestinal smooth muscle contractility (Finkelman *et al.* 2004; Khan and Collins, 2004). On the other hand, it is also possible that the effect of mast cells on nematode fecundity differs between host species, as mast cell mediators are different not only between mucosal and connective tissue-type mast cells, but also between human, mouse, and rat mast cells (Galli and Tsai, 2008). Mast cells secrete a variety of inflammatory mediators such as prostaglandins, leukotrienes, histamine, and eosinophil chemotactic factors. The roles of these factors in the modulation of nematode fecundity should be clarified in future studies.

https://doi.org/10.1017/S0031182009991673 Published online by Cambridge University Press

It is possible that multiple molecules contribute to the modulation of nematode fecundity. Mucins may interfere with some biological functions of parasites, as it is assumed that they trap parasites and inhibit their motility and feeding capacity (Miller, 1987; Rothwell, 1989). Although goblet cell hyperplasia and mucus responses are T cell-dependent, W/W^v mice show normal goblet cell hyperplasia during *H. polygyrus* infection (Hashimoto *et al.* 2009), suggesting that the mucus response is not relevant to the modulation of fecundity. On the other hand, the effects of antibody to the nematode surface coat have been reported in a *C. elegans* model system of nematode infection. Exposure to surface-reactive IgG during the entire developmental stage of *C. elegans* resulted in a significant reduction in worm length and fecundity, suggesting that the binding of specific antibodies to the parasite surface coat affects the biological functions of nematodes (Nowell *et al.* 1999). The role of antibodies in the modulation of parasite fecundity remains to be elucidated.

Taken together, the present study showed that the immunological status of parasite niches markedly affects nematode fecundity, if not to an irrecoverable extent, and this might be at least partly mediated by the activation of mast cells.

ACKNOWLEDGEMENTS

Part of this work was supported by a grant-in-aid for scientific research (B16390127) from the Japan Society for the Promotion of Science.

REFERENCES

Arizono, N., Kasugai, T., Yamada, M., Okada, M., Morimoto, M., Tei, H., Newlands, G. F., Miller, H. R. and Kitamura, Y. (1993). Infection of *Nippostrongylus brasiliensis* induces development of mucosal-type but not connective tissue-type mast cells in genetically mast cell-deficient *Ws/Ws* rats. *Blood* **81**, 2572–2578.

Artis, D. and Grencis, R. K. (2008). The intestinal epithelium: sensors to effectors in nematode infection. *Mucosal Immunology* **1**, 252–264.

Bleay, C., Wilkes, C. P., Paterson, S. and Viney, M. E. (2009). The effect of infection history on the fitness of the gastrointestinal nematode *Strongyloides ratti*. *Parasitology* **136**, 567–577. doi:10.1017/S0031182009005617

Finkelman, F. D., Shea-Donohue, T., Morris, S. C., Gildea, L., Strait, R., Madden, K. B., Schopf, L. and Urban, J. F., Jr. (2004). Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunological Reviews* **201**, 139–155. doi:10.1111/j.0105-2896.2004.00192.x

Galli, S. J. and Tsai, M. (2008). Mast cells: versatile regulators of inflammation, tissue remodeling, host defense and homeostasis. *Journal of Dermatological Science* **49**, 7–19. doi:10.1016/j.jdermsci.2007.09.009

Hashimoto, K., Uchikawa, R., Tegoshi, T., Takeda, K., Yamada, M. and Arizono, N. (2009). Depleted intestinal goblet cells and severe pathological changes in SCID mice infected with *Heligmosomoides polygyrus*. *Parasite Immunology* **31**, 457–465. doi:10.1111/j.1365-3024.2009.01123.x

Khan, W. I. and Collins, S. M. (2004). Immune-mediated alteration in gut physiology and its role in host defence in nematode infection. *Parasite Immunology* **26**, 319–326.

Kitamura, Y., Go, S. and Hatanaka, K. (1978). Decrease of mast cells in W/W^v mice and their increase by bone marrow transplantation. *Blood* **52**, 447–452.

Lawrence, C. E. and Pritchard, D. I. (1994). Immune response profiles in responsive and non-responsive mouse strains infected with *Heligmosomoides polygyrus*. *International Journal for Parasitology* **24**, 487–494. doi:10.1016/0020-7519(94)90139-2

Miller, H. R. P. (1987). Gastrointestinal mucus, a medium for survival and for elimination of parasitic nematodes and protozoa. *Parasitology* **94** (Suppl), S77–S100.

Monroy, F. G. and Enriquez, F. J. (1992). *Heligmosomoides polygyrus*: a model for chronic gastrointestinal helminthiasis. *Parasitology Today* **8**, 49–54. doi:10.1016/0169-4758(92)90084-F

Newlands, G. F., Miller, H. R., MacKellar, A. and Galli, S. J. (1995). Stem cell factor contributes to intestinal mucosal mast cell hyperplasia in rats infected with *Nippostrongylus brasiliensis* or *Trichinella spiralis*, but anti-stem cell factor treatment decreases parasite egg production during *N. brasiliensis* infection. *Blood* **86**, 1968–1976.

Nowell, M. A., De Pomerai, D. I. and Pritchard, D. I. (1999). *Caenorhabditis elegans* as a biomonitor for immunological stress in nematodes. *Parasite Immunology* **21**, 495–505. 10.1046/j.1365-3024/1999/00249.x

Paterson, S. and Viney, M. E. (2002). Host immune responses are necessary for density dependence in nematode infections. *Parasitology* **125**, 283–292. doi:10.1017/S0031182002002056

Pritchard, D. I., Quinnell, R. J. and Walsh, E. A. (1995). Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. *Parasite Immunology* **17**, 71–75. doi:10.1111/j.1365-3024.1995.tb00968.x

Rothwell, T. L. (1989). Immune expulsion of parasitic nematodes from the alimentary tract. *International Journal for Parasitology* **9**, 139–168.

Urban, J. F., Jr., Katona, I. M. and Finkelman, F. D. (1991). *Heligmosomoides polygyrus*: CD4+ but not CD8+ T cells regulate the IgE response and protective immunity in mice. *Experimental Parasitology* **73**, 500–511. doi:10.1016/0014-4894(91)90074-7

Urban, J. F., Jr., Maliszewski, C. R., Madden, K. B., Katona, I. M. and Finkelman, F. D. (1995). IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. *The Journal of Immunology* **15**, 4675–4684.

Viney, M. E., Steer, M. D. and Wilkes, C. P. (2006). The reversibility of constraints on size and fecundity in the parasitic nematode *Strongyloides ratti*. *Parasitology* **133**, 477–483. doi:10.1017/S003118200600062X