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Intestinal protection against *Strongyloides ratti* and mastocytosis induced by administration of interleukin-3 in mice

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SUMMARY

Information about interleukin-3 (IL-3) effects *in vivo* is limited compared with the *in vitro* effects. We found that a repetitive injection of a low dose of recombinant IL-3 induced protection against intestinal worms of *Strongyloides ratti* in C57BL/6 mice. When mice were injected i.p. with different doses of recombinant IL-3 twice a day from day −5 to day −1 and infected orally with larvae recovered from the head of infected rats on day 0, worm recovery from the small intestine was markedly reduced by a total of 10^4 U IL-3 or more on day 2 post-infection. The number of intestinal mucosal mast cells (MMC) was increased by the protective dose of IL-3. The IL-3 treatment, however, was ineffective in protecting mice against tissue migrating larvae, as assessed by recovery from the head. The protective effect of IL-3 on intestinal worms was observed within 6 hr post oral infection, suggesting little concern with antigen-specific immune responses. The effective dose of IL-3 treatment increased the number of MMC progenitors five times in the spleen and the mesenteric lymph nodes. An MMC-specific protease, MMCP-1, was secreted 200 times more than in controls in the intestinal lumen by the IL-3 treatment. The IL-3 treatment induced no protection or mastocytosis in mast cell-deficient W/W^v mice. These results suggest that the IL-3-induced intestinal protection against *S. ratti* is mediated by MMC.

INTRODUCTION

Interleukin-3 (IL-3) is a multi-colony stimulating factor, having effects on differentiation and proliferation of several haematopoietic cells and their progenitor cells, including mast cells *in vitro*.[1] Differentiation and proliferation of haematopoietic cells are generally supported by more than two cytokines. Mucosal mast cells (MMC), which are different from connective tissue type mast cells, are capable of differentiating and proliferating from the progenitor cells only by IL-3, *in vitro*[2] or *in vivo*,[3] although IL-10 was recently found to enhance the growth of mast cell lines and mast cell progenitors *in vitro* when added with IL-3 and/or IL-4.[4] In comparison with the *in vitro* effects, information about the biological effects of IL-3 *in vivo* is limited. Administration of IL-3 to mice stimulates the same types of haematopoietic cells as are stimulated *in vitro*, such as eosinophils, neutrophils, monocytes, megakaryocytes and mast cells,[5] but fails to stimulate T or B lymphopoiesis.[6]

Concerning the host defence effects, administration of IL-3 aggravates experimental cutaneous leishmaniasis in mice, probably because of the increase in the circulating mononuclear cells which the parasites multiply in,[7] or protects mice from acute herpes simplex virus infection, probably by inducing interferon production.[8] We have demonstrated that a repetitive injection of IL-3 to nude mice already infected with an intestinal nematode, *Strongyloides ratti*, induces the worm expulsion with marked intestinal mastocytosis.[9] The IL-3-induced protection against *S. ratti* is probably mediated by intestinal MMC. If this is the case, intestinal MMC induced in mice before infection should also protect them against the parasite. To examine the mechanism of the IL-3-induced protection, C57BL/6 mice were treated with different doses of recombinant IL-3 before infection and then infected orally with *S. ratti* larvae recovered from the heads of infected rats. The results in the present paper support the opinion that IL-3 induces intestinal MMC, which work on protection against *S. ratti* worms in the small intestine.

Abbreviations: MMCP, mouse mast cell protease; MMC, mucosal mast cells; VCU, villus crypt units.

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MATERIALS AND METHODS

Animals and parasitological techniques

C57BL/6 mice were raised from the original pairs obtained from Japan SLC Co. (Shizuoka, Japan) in the Animal Facilities of Akita University School of Medicine and used at 7–12 weeks old. Male WBB6F1-W/W^v mice (7 weeks old) and Wistar rats were obtained from Japan SLC Co. Two pairs of Wistar rats were mated to rear young rats until 4 weeks old. All animals

were treated in accordance with the Guidelines for Animal Experimentation, Akita University. *S. ratti* was maintained by serial passage in Wistar rats. Infective larvae were obtained by faecal culture on filter papers. The 4-week-old rats were infected s.c. with 4000 larvae and killed 36 hr after infection to recover tissue migrating larvae from the head, as described elsewhere.[10] The larvae recovered from the heads of these rats were used for infecting mice orally. *S. ratti* adult worms were obtained from the small intestine of mature rats infected s.c. with 4000 larvae on day 9 and given orally to mice.[11] The method for recovering and counting intestinal worms was as reported elsewhere.[12]

Preparation of recombinant IL-3

Recombinant IL-3 was prepared from the culture supernatants of a myeloma cell line transfected with murine IL-3 cDNA, X63BMG 14-17, kindly provided by Dr H. Karasuyama (University of Tokyo, Japan), as reported elsewhere.[12] The sterile stock preparation of recombinant IL-3 in a serum-free medium contained less than 0·05 ng/ml lipopolysaccharides, as assessed by a Limulus test kit, LIMUTESTER (Funakoshi, Tokyo, Japan), and was usually diluted 40 times with sterile Hanks' balanced salt solution before use. One unit of IL-3 was defined as the amount of factor required to support half-maximal [^{3}H]thymidine incorporation by 2×10^4 FDC-P2 cells in 0·2 ml cultures.[12]

Treatment with IL-3 and infection of mice

In a typical experiment, mice were injected i.p. with 0·1 ml IL-3 solution or medium twice a day from day −5 to day −1 to receive a total of 10^3–10^5 U IL-3 or no IL-3. The day after the last injection (day 0), male C57BL/6 or W/W^v mice were infected orally with 300 larvae recovered from the heads of infected rats using a stomach tube[13] and killed 43 or 44 hr post-infection to count intestinal worms. The treated mice were killed 6 or 21 hr post oral infection in one experiment. The mice treated with a total of 10^5 U IL-3 were infected orally with 400 adult worms obtained from the intestine of infected rats and killed 43 hr after infection. In another experiment, to see the IL-3 effects on tissue migrating larvae, the IL-3-treated mice as described above were infected s.c. with 1600 or 2000 larvae from a faecal culture and killed 36 hr after infection. Recovery of larvae from the head was assessed by the method described elsewhere.[10]

Detection of intestinal mast cell protease

Female C57BL/6 mice were treated with a total of 10^4 U IL-3 or medium as described above and killed 1 day after the last injection. The upper half of the small intestine was immersed in 3 ml of cold saline on ice and kept for 5 min without shaking. After removing the small intestine, the saline was centrifuged at 300 ***g*** for 10 min. The supernatant was submitted to ELISA. An ELISA kit for measuring mouse mast cell protease-1 (MCCP-1), which is specific for intestinal MMC,[14] was a kind gift from Dr H. R. P. Miller (University of Edinburgh, U.K.).

Assay of mast cell progenitors

Male C57BL/6 mice were treated with a total of 10^4 U IL-3 or medium and killed 1 day after the last injection to obtain cell preparations. The frequency of mast cell progenitors per million cells was determined by the modified method of Crapper & Schrader.[15] Briefly, four concentrations of spleen or mesenteric lymph node cells were cultured in RPMI-1640 (Gibco, Grand Island, NY) containing 10% fetal calf serum (Boknnek, Toronto, Canada), 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin, 5% WEHI-3B conditioned medium concentrated 10 times and 1·1% methylcellulose 1500 (Nacalai Tesque, Kyoto, Japan) in 96-well plates (Sumitomo, Tokyo, Japan) for 2 weeks at 37° in 5% CO_2. WEHI-3B-conditioned medium was prepared as described elsewhere.[16] The number of negative wells of mast cell colony in a plate was obtained with each concentration of cells and the frequency of progenitors was assessed by the limiting dilution method.[17]

Histology

Mast cells in the small intestine were detected by a method described previously.[16] A piece of small intestine was immersed in Carnoy's fixative, sectioned as a paraffin block and stained with Alcian blue and Safranin-0. The number of intestinal mast cells in the epithelium and villous lamina propria was counted together in 30–50 villus crypt units (VCU).[18]

Statistical analysis

Probability of significant differences between groups ($P < 0·05$) was determined by Mann–Whitney's *U*-test.

RESULTS

C57BL/6 mice were injected i.p. with IL-3 twice a day for 5 days and then infected orally with *S. ratti* larvae recovered from the heads of infected rats. The IL-3 treatment induced a dose-dependent reduction of worm recovery and an increase of the number of the intestinal MMC on day 2 post-infection (Fig. 1). Treatment with a total of 10^4 U IL-3 was sufficient to induce a clear difference in protection and mastocytosis from the control. The timing of injection was then studied to see if a shorter period

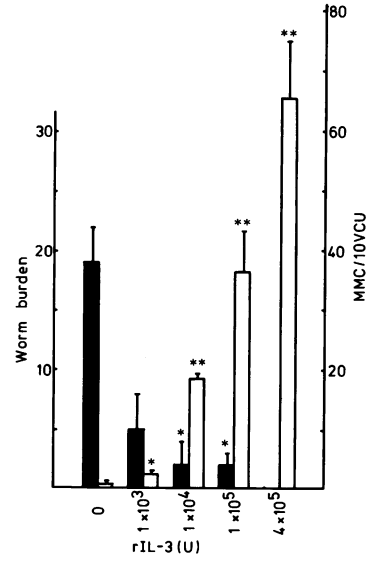

Figure 1. Dose-dependent protection and mastocytosis by IL-3 treatment. C57BL/6 mice were treated with different doses of IL-3 or medium from day −5 to day −1, then infected orally with 300 larvae recovered from the head of donor rats on day 0 and killed 44 hr after infection. Means ± SEM of intestinal worm burden (■) and number of MMC (□) were obtained from five mice each. $*P < 0·01$, $**P < 0·001$.

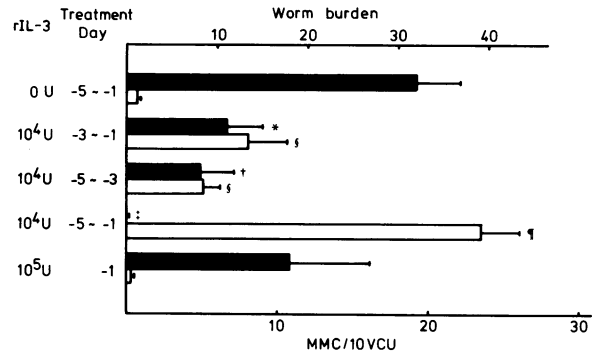

Figure 2. Effective timing of IL-3 treatment on protection and mastocytosis. C57BL/6 mice were treated with IL-3 or medium from day −5 to day −1, day −5 to day −3, day −3 to day −1 or on day −1, then infected orally with 300 larvae recovered from the head of infected rats on day 0 and killed 43 hr after infection. Means ± SEM of intestinal worm burden (■) and number of MMC (□) were obtained from four or five mice. $*P<0.04$, $\dagger P<0.02$, $\ddagger P<0.01$, $\S P<0.002$, $\P P<0.001$.

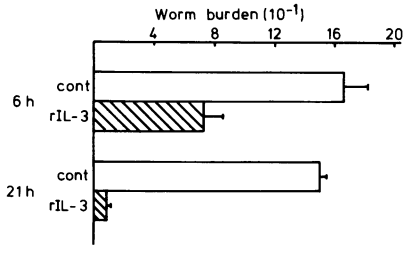

Figure 3. Rapid expression of protective effects by IL-3 treatment. C57BL/6 mice were treated with IL-3 or medium from day −5 to day −1 and then infected orally with 320 larvae recovered from the head of infected rats on day 0. Worm burdens were assessed 6 and 21 hr after infection. Mean ± SEM from five mice. $P<0.02$ in 6 hr and $P<0.004$ in 21 hr.

of treatment is effective or not. Mice were injected i.p. with IL-3 twice a day from day −5 to day −1, day −5 to day −3 and day −3 to day −1, to receive a total of 10^4 U. Control mice were injected with medium from day −5 to day −1. All mice were infected orally with *S. ratti* on day 0, and assessed for intestinal protection and number of MMC on day 2 post-infection. IL-3 treatment for 5 days was more effective in inducing protection and mastocytosis than treatments with the same dose for 3 days by the two different methods (Fig. 2). Furthermore, treatment with a total of 10^4 U IL-3 for 5 days showed higher protection than that with a total of 10^5 U IL-3 on only day −1 (Fig. 2), suggesting that effective induction of the protection and mastocytosis by IL-3 takes several days. IL-3 was injected twice a day for 5 days in all the other experiments thereafter.

C57BL/6 mice treated with a total of 10^4 U IL-3 were infected orally with *S. ratti* and killed 6 or 21 hr post-infection. The number of worms recovered from the small intestine was reduced by 56% at 6 hr and 94% at 21 hr from that obtained from control mice (Fig. 3). This observation suggests the presence of antigen non-specific effector mechanisms induced by IL-3 to protect mice from intestinal *S. ratti*. The oral infection of *S. ratti* is performed by larvae recovered from the head of

Table 1. Protection against adult worms of *S. ratti* by IL-3 treatment

Total IL-3	Adult worms recovered
10^5 U	0 ± 0
0 U	27 ± 6

C57BL/6 mice were treated with IL-3 or medium, then infected orally with 400 adult worms recovered from the small intestine of infected rats and killed 43 hr after the infection. Mean ± SEM from six mice. $P<0.002$ between the groups.

Table 2. Ineffectiveness of IL-3 treatment on migrating larvae of *S. ratti*

Total IL-3	Larvae recovered from head	MMC/10VCU
10^4 U	247 ± 22	34·3 ± 3·9
0 U	215 ± 36	6·7 ± 1·7
10^5 U	87 ± 18	
0 U	87 ± 21	

C57BL/6 mice were treated with IL-3 or medium, then infected s.c. with 2000 (for 10^4 U IL-3) or 1600 (for 10^5 U IL-3) infective larvae and killed 36 hr after infection. Mean ± SEM from six mice.

infected rats, therefore the rapid protection caused within a few hours post-infection indicates that *S. ratti* worms are expelled before maturation. To see if the IL-3 treatment is effective against mature worms, mice treated with a total of 10^5 U IL-3 were infected orally with adult worms recovered from the small intestine of infected rats and killed on day 2. Recovery of intestinal adult worms was markedly reduced by the IL-3 treatment (Table 1). IL-3 treatment, therefore, induces protection against not only intestinal larvae but also adult worms.

S. ratti migrates through the subcutaneous tissues and the head before reaching the small intestine. Effects of IL-3 treatment on tissue migrating larvae were examined by assessing larval recovery from the heads of mice after s.c. infection. No significant difference was detected between the number of tissue migrating larvae recovered from mice treated with IL-3 and those with medium. The increased number of intestinal MMC in the IL-3-treated mice indicates that IL-3 expressed its biological activities in the mice but was ineffective in protecting them against tissue migrating larvae (Table 2). To confirm the importance of mast cells in the IL-3-induced protection, mast cell-deficient W/W^v mice were treated with a total of 10^4 U IL-3 and infected orally with *S. ratti*. Intestinal worm recovery was not affected with the IL-3 treatment on day 2 and no intestinal mastocytosis was detected in W/W^v mice treated with either IL-3 or medium (Table 3). Therefore, mast cells are required for IL-3 to express its effects on protecting mice against intestinal *S. ratti*.

Table 3. Ineffectiveness of IL-3 treatment on *S. ratti* infection in W/W^v mice

Total IL-3	Worms recovered	MMC/10VCU
10^4 U	42±7	0±0
0 U	49±5	0±0

W/W^v mice were treated with IL-3 or medium, then infected orally with 300 larvae recovered from the head of infected rats and killed 43 hr after infection. Mean±SEM from five mice.

Table 4. Increase of mast cell progenitors by IL-3 treatment in uninfected mice

	Progenitors/10^6 cells	
Total IL-3	Spleen	Lymph node
10^4 U	69·5±0·5	12·5±1·5
0 U	13·5±5·5	2·5±1·5

Spleen and mesenteric lymph node cells were obtained from C57BL/6 mice treated with IL-3 or medium 1 day after the last injection. Frequency of progenitor cells was assessed by two different experiments (mean±SEM).

IL-3 effects on mast cells were further examined in uninfected C57BL/6 mice. Spleen cells and mesenteric lymph node cells were collected from mice treated with a total of 10^4 U IL-3 or medium. Frequencies of mast cell progenitors were assessed by the limiting dilution method using methylcellulose colony assay. The frequency was increased five times by the IL-3 treatment in both spleen and mesenteric lymph node cells (Table 4). The number of intestinal MMC was counted histologically in uninfected mice treated with a total of 10^4 U IL-3 or medium and killed 1 day (day 0), 3 days (day 2) or 6 days (day 5) after the last injection. Days are named, for convenience, in accordance with the method of naming in infection. The number of intestinal MMC increased 40–50 times with IL-3 treatment between days 0 and 2 (no significant difference between them) and decreased on day 5, but more MMC ($P<0·005$) were detected on day 5 than compared with control mice (Table 5). Intestinal MMC contain a granular serine protease, MMCP-1, which is distinct from the other granular serine protease, MMCP-4, of serosal mast cells. Amounts of MMCP-1 in the upper half of the small intestinal lumen and the serum were measured in mice treated with a total of 10^4 U IL-3 or medium on the day following the last injection. The amount in the intestinal lumen was increased 200 times by the IL-3 treatment as well as the increase in the serum (Fig. 4).

Table 5. Kinetics of number of MMC in uninfected mice treated with IL-3

	MMC/10VCU		
Total IL-3	Day 0	Day 2	Day 5
10^4 U	16·5±1·9	20·8±2·5	5·7±0·7
0 U	0·4±0·1	ND	ND

C57BL/6 mice were treated with IL-3 or medium for 5 days. Number of intestinal MMC was assessed 1 day (day 0), 3 days (day 2) and 6 days (day 5) after the last injection. Mean±SEM from four mice. $P<0·005$ in all IL-3 treated versus medium control (0 U).

ND, not determined.

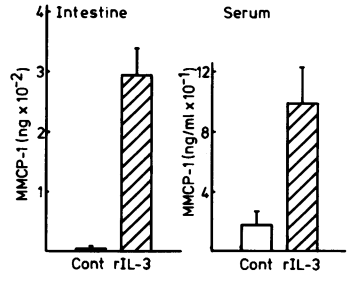

Figure 4. Secretion of MMCP-1 in the intestinal lumen by IL-3 treatment. C57BL/6 mice were treated with IL-3 or medium for 5 days. MMCP-1 in the upper half of the small intestinal lumen and the serum was measured by ELISA on the day after the last injection. $P<0·02$ in the intestine and $P<0·01$ in the serum.

DISCUSSION

This study confirms that repetitive IL-3 injection *in vivo* induces protection against intestinal *S. ratti* in mice. We have reported similar protective effects of IL-3 in athymic nude mice infected with *S. ratti*.[9] Two experimental conditions in this study were different from the previous study, in addition to the strain difference of mice. Firstly, C57BL/6 mice were treated with IL-3 for 5 days before infection, whereas the nude mice were injected with IL-3 for 7 or 10 days after infection.[9,12] Secondly, the amount of IL-3 effective in C57BL/6 mice was about 50 times as little as that used in athymic nude mice. The two different experimental systems resulted in the same protective effects of IL-3 against *S. ratti*.

Because IL-3 is a multi-colony stimulating factor, explanation of the mechanism of the protective effects *in vivo* is complicated. However, the observation that the IL-3 treatment had no effect on protection in mast cell-deficient W/W^v mice indicates involvement of mast cells in the protective effects. In fact, IL-3-induced protective effects correlated with the number of intestinal MMC detected histologically and were expressed within 6 hr after oral infection. The rapid expression of the effects suggests that the effector mechanisms are prepared by repetitive IL-3 injection before infection but are not induced by antigen-specific immune responses after infection. Treatment of mice with a total of 10^4 U IL-3 for 5 days before infection was more effective in inducing the protection than that with the same dose of IL-3 for 3 days or with a total of 10^5 U IL-3 for 1 day. Therefore, induction of effector mechanisms takes several days.

The period is consistent with that for MMC proliferation *in vivo*. A peak of growth factor production precedes a peak of intestinal mastocytosis of 4–6 days in *S. ratti*-infected mice.[19] In the case of aggravation of murine leishmaniasis by IL-3 administration, an increase of circulating mononuclear cells appears to be the reason.[7] The IL-3 treatment in this study was ineffective in recovery of tissue migrating larvae from the head. No or less stimulation of macrophages must have been induced by the IL-3 treatment, because protection against the tissue migrating larvae of *S. ratti* is regulated by macrophages.[20,21]

The number of intestinal MMC was increased by the IL-3 treatment in uninfected mice. A kinetics study shows that the MMC induced by repetitive injection of IL-3 are present in the small intestine for several days without infection. The increased rate of number of intestinal MMC detected histologically was more than that of mast cell progenitors in the mesenteric lymph node or spleen. The observation probably reflects the presence of more progenitor cells in the small intestine than in the mesenteric lymph node or spleen in normal conditions.[22] Committed progenitor cells in the small intestine may proliferate more quickly. An MMC-specific serine protease, MMCP-1, was markedly increased in the intestinal lumen by IL-3 treatment of uninfected mice. Therefore, mediators of MMC can be released without specific IgE antibodies. A similar phenomonon of MMC degranulation without IgE antibodies has been suggested in nude mice infected with *S. ratti* and treated with IL-3.[12] Degranulation of MMC may be caused by IL-3 as well as that of serosal mast cells[23] or MMC may spontaneously secrete their mediators. The intestinal protection induced by IL-3 was observed with a relatively small number of MMC detected histologically. The marked increase of MMCP-1 in the intestinal lumen of the IL-3-treated mice suggests that immature mouse MMC may secrete MMCP-1 as immature rat MMC secrete their proteases.[24] Mediators secreted from those immature MMC, which are not detected histologically, may be effective to protect mice against intestinal *S. ratti* as well as those from mature MMC. Although the effective mediators of MMC on damaging worms have not been identified yet, some possible candidates are mast cell proteases[24] or leukotrienes C4 and B4.[25]

Recently, the presence of several distinct mouse mast cell proteases, MMCP-1 to MMCP-6, has been shown among different subtypes of mast cells.[26] MMCP-2 mRNA was detected in MMC obtained from the intestine of helminth-infected mice, but not in bone marrow-derived mast cells cultured *in vitro* with WEHI-3-conditioned medium, IL-3 or c-kit ligand.[2] Differentiation of cultured mast cells to the MMC phenotype, which expresses MMCP-1 and MMCP-2, is induced by IL-10.[27] The differential expression of serine protease among intestinal MMC and bone marrow-derived cultured mast cells suggests a possibility of functional difference between them, though the functional difference may not be due to the serine proteases. If this is the case, we can easily explain the reason why we have not detected any activities with cultured mast cells in killing or damaging *S. ratti in vitro*. There may be a functional difference in affecting *S. ratti* between the mast cells *in vivo* and those *in vitro*.

The IL-3 treatment was effective in protecting mice against *S. ratti* adult worms as well as larvae infected orally. Therefore, MMC which are differentiated and proliferated by endogenous IL-3 or other cytokines mediated by a similar mechanism, should work on expulsion of *S. ratti* in a usual infection in mice. Intestinal mucosal mastocytosis is generally observed in the intestinal helminth infection. However, mucosal mastocytosis may not be a general effector mechanism of the worm expulsion. Because nude mice concurrently infected with *Nippostrongylus brasiliensis* and *S. ratti* expelled only *S. ratti* but not *N. brasiliensis*, with marked intestinal mucosal mastocytosis by repetitive injection of IL-3,[12] effector mechanisms of protection against intestinal nematodes are probably specific among various parasites infections.

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