

Enhanced Protection against *Heligmosomoides polygyrus* in IL-2 Receptor β -Chain Overexpressed Transgenic Mice with Intestinal Mastocytosis

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ABSTRACT. IL-2 receptor β -chain overexpressed transgenic (Tg2R β) mice lack NK cells, but the development of other lymphocyte subsets and macrophages remained apparently intact. These mice also exhibit intestinal mastocytosis. Helminth infection induces various immune responses, such as mast cells, goblet cells, eosinophils and IgE, mediated by Th2 cytokines. IL-4 is also important in the regulation of resistance and susceptibility to *Heligmosomoides polygyrus* infection. However, there are contradictory results about the relation between resistance to *H. polygyrus* and intestinal mastocytosis. The present study showed that Tg2R β mice suppressed worm fecundity with mastocytosis without an increase of the levels of goblet cells, eosinophils and IgE compared with control mice. These results clearly indicated that mast cells have the ability for to protect against *H. polygyrus* infection. However, additional studies are required to evaluate protective effector mechanisms against *H. polygyrus*.

KEY WORDS: *Heligmosomoides polygyrus*, IL-2 receptor β -chain overexpression, mast cell, transgenic mice.

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IL-2 receptor β -chain overexpressed transgenic (Tg2R β) mice lack NK cells, but the development of other lymphocyte subsets and macrophages remained apparently intact [14]. We found that Tg2R β mice represent intestinal mastocytosis and globule leukocytes without any inflammation in a normal state (Fig. 1). Although the mechanisms of mast cell hypertrophy and the relationship of NK cell deficiency and mast cell increment have been unknown in this transgenic mouse, this is the only mouse that represents intestinal mastocytosis without any pathological stimuli. So, this transgenic mouse is a useful animal model for investigating mast cell function. Helminth infections induce various immune responses such as marked IgE antibody production, peripheral blood eosinophilia, intestinal mucosal mastocytosis and a hyperplasia of intestinal goblet cells, and these immunological responses have the protective effects against helminth [4, 7, 8]. However, protection against each parasite is through considerably different mechanisms [3, 6]. Contradictory results have been reported about the relation between resistance to *Heligmosomoides polygyrus* and intestinal mastocytosis [1, 2, 5], and the specific effector mechanisms mediating worm expulsion remain unknown [1, 15]. In the present study, we investigated the protective ability against *H. polygyrus* of Tg2R β mice, in which mast cells are increased without inflammation.

To obtain Tg2R β mice, male Tg2R β and female C57BL/6J (B6) were mated, and each offspring was verified to be a transgenic mouse by a PCR method, as described previously [10, 14]. Transgenic and wild-type litter mate mice were used. Autoclaved pellets (MF; Oriental Yeast, Tokyo, Japan) and water were supplied *ad libitum*. All animals

were handled under the regulations for animal welfare of Yamaguchi University. Groups of more than five mice of the same age and approximately the same weight were used for each experiment. *H. polygyrus* was maintained by serial passage in mice, as described previously [9]. Both groups of mice were inoculated orally with 300 infective-stage *H. polygyrus* larvae (L3), as described previously [9]. Parasite egg number was determined in feces collected from each *H. polygyrus*-infected mouse. Mice were anaesthetized with ether and euthanized by exsanguination from the heart using a heparinized syringe. Fresh blood was used for determination of the eosinophil count, and then plasma was used for determination of the total amounts of IgE. The jejunum was removed and fixed in Carnoy's fluid [9]. Mast cell counts were performed on 4- μ m alcian blue-stained sections. Goblet cell counts were performed on 4- μ m hematoxylin and

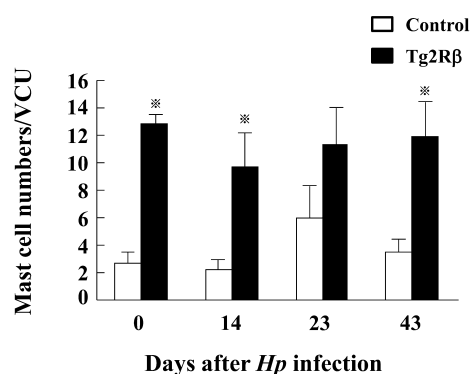


Fig. 1. Numbers of intestinal mast cells in the control (□) and Tg2R β (■) mice after infection with *H. polygyrus*. Each group consisted of 5 to 8 mice. Data shown are means $\pm$ SEM. *: Significantly different from the values of the control mice (* $P < 0.05$).

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eosin-stained sections. The number of mast cells or goblet cells in the villous crypt unit (VCU) of the jejunal mucosa was determined according to the previously reported method [9]. The number of eosinophils in peripheral blood was counted after staining with Hinkelmann's solution, as reported previously [11]. Total amounts of IgE in plasma were measured with a Mouse IgE EIA Kit (Yamasa Shoyu Co., Ltd., Chiba, Japan) according to the manufacturer's instructions. This system was capable of detecting as little as 5 ng/ml of IgE. Each sample was diluted at least 10-fold with the dilution buffer supplied with the kit to avoid the influence of plasma. The student's *t*-test (two-tailed) was used to evaluate the significance of differences; a *P* value less than 0.05 was considered significant.

Eggs were first found in the feces of both groups of mice infected with *H. polygyrus* at 10 days postinfection. The number of eggs per gram of feces (EPG) in the control mice was increased, peaked at day 18 postinfection and then decreased at 20 days postinfection. After day 22 of *H. polygyrus* infection, the EPG of the control mice decreased to almost the same level as that of the Tg2R β mice. On the other hand, the EPGs of the Tg2R β mice were shown to be at almost the same low levels throughout the experimental period, and the EPGs of the Tg2R β mice were significantly lower than that of the control mice on days 10, 14, 16, 18 and 20 postinfection (Fig. 2). The number of mast cells was extremely high in the Tg2R β mice without infection and remained high throughout the experimental period after *H. polygyrus* infection. On the other hand, the number of mast cells increased in the control mice on day 23 postinfection compared with that before *H. polygyrus* infection. On days 14 and 43 postinfection, the numbers of mast cells in the control mice were almost the same as the level before *H. polygyrus* infection. On days 0, 14 and 43 postinfection, the numbers of mast cells were significantly higher in the Tg2R β mice than in the control mice (Fig. 1). In both groups, the number of intestinal goblet cells was increased 2 week after *H. polygyrus* infection compared with before *H. polygyrus* infection. However, the number of goblet cells in both groups decreased to the levels before infection at 43 days after *H. polygyrus* infection. There were no significant differences in the numbers of goblet cells between the Tg2R β and control mice throughout the experimental period (Fig. 3). In both groups, the number of eosinophils in peripheral blood was increased at days 23 postinfection compared with before *H. polygyrus* infection. However, the number of eosinophils in both groups decreased to the level before infection at 43 days after *H. polygyrus* infection. There were no significant differences in the numbers of eosinophils in peripheral blood between the Tg2R β and control mice throughout the experimental period (Fig. 4). Before *H. polygyrus* infection, the levels of total plasma IgE were very low in both groups, but they were increased at day 14 postinfection. Then, the levels of total plasma IgE were maintained throughout the experimental period. There were no significant differences in the levels of total plasma IgE between the Tg2R β and control mice throughout the exper-

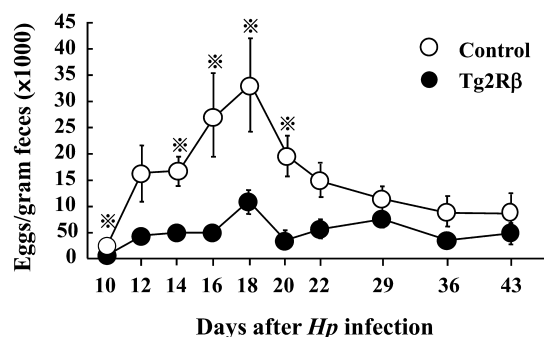


Fig. 2. Numbers of *H. polygyrus* eggs in the control (○) and Tg2R β (●) mice. Each group consisted of 5 to 8 mice. Data shown are means $\pm$ SEM. *: Significantly different from the values of the control mice (**P*<0.05).

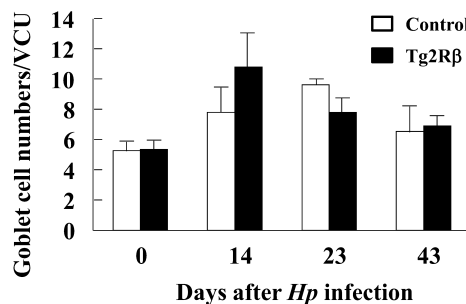


Fig. 3. Numbers of intestinal goblet cells in the control (□) and Tg2R β (■) mice after infection with *H. polygyrus*. Each group consisted of 5 to 8 mice. Data shown are means $\pm$ SEM. *: Significantly different from the values of the control mice (**P*<0.05).

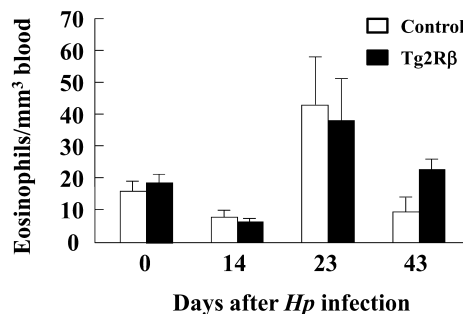


Fig. 4. Numbers of peripheral eosinophils in the control (□) and Tg2R β (■) mice after infection with *H. polygyrus*. Each group consisted of 5 to 8 mice. Data shown are means $\pm$ SEM. *: Significantly different from the values of the control mice (**P*<0.05).

imental period (Fig. 5).

The present study showed that Tg2R β mice suppressed worm fecundity with mastocytosis and without an increase of the goblet cells, eosinophils, and IgE compared with con-

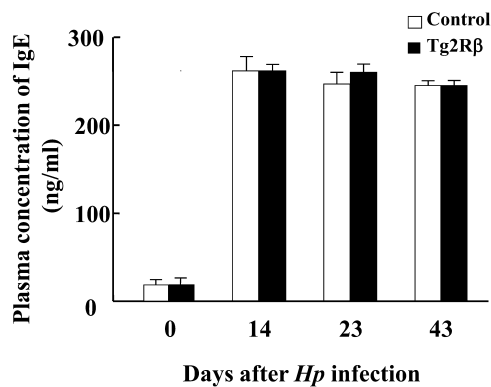


Fig. 5. Plasma IgE levels in the control (□) and Tg2R β (■) mice after infection with *H. polygyrus*. Plasma IgE levels were determined by ELISA. Each group consisted of 5 to 8 mice. Data shown are means $\pm$ SEM. *: Significantly different from the values of the control mice (* P <0.05).

trol mice. These results suggest that the protective effects against *H. polygyrus* were induced by mastocytosis in the Tg2R β mice and showed for the first time that increased mast cells suppressed *H. polygyrus* fecundity (Figs. 1 and 2). Recently, it was shown that mast cell-deficient *W/W^v* mice enhanced the fecundity of *H. polygyrus* [5], and we also obtained the same results (data not shown). These results suggested that the host protective ability against *H. polygyrus* is correlated with the number of intestinal mast cells and that mast cells were an effector for *H. polygyrus*.

The present results showed that Tg2R β mice which have a defect in numbers of NK cells accompanied with the increase in number of mast cells without significant change in levels of the other parameters. These results suggested that the decrease in the number of NK cells might induce growth of intestinal mast cells. Further investigations are needed to resolve the mechanisms of mast cell growth in Tg2R β mice. These mice are useful model for investigating the interaction between mast cells and NK cells.

We were unable to evaluate the effects of eosinophils, goblet cells and IgE on *H. polygyrus*. Previous reports showed that IL-5 knockout mice, in which eosinophils could not be induced with a helminth infection, enhanced the fecundity of *H. polygyrus* and that these mice could induce mast cells and other leukocytes [12, 13]. These reports suggested that eosinophils also have the ability to protect against *H. polygyrus* infection. Further investigations are needed to elucidate the protective effector mechanisms against *H. polygyrus*.

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