NOTE *Clinical Pathology*

Intestinal Smooth Muscle Cells Locally Enhance Stem Cell Factor (SCF) Production against Gastrointestinal Nematode Infections

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ABSTRACT. Smooth muscle cells can produce stem cell factor (SCF) in the normal state for the preservation of mast cells, but it is still unknown whether smooth muscle cells can enhance SCF production in response to the pathological stimuli. The present study showed that smooth muscle cells in mast cell-increased regions around worm cysts of intestinal nematodes significantly enhanced SCF gene expression compared with mast cell non-increased regions in same sample. SCF gene expression in mast cell non-increased regions in nematode-infected mice showed almost the same level as in non-infected control groups. These results indicate that smooth muscle cells can locally enhance SCF gene expression, and may have a role in local immunological reactions as growth factor-producing cells.
KEY WORDS: laser capture microdissection, mast cell, smooth muscle cell, stem cell factor.

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Mast cells play an important role in the induction of pathological changes in type I allergic reactions, and in the expulsion of various gastrointestinal nematodes [2, 4, 5, 8, 9, 13]. In these pathological statuses, mast cell numbers increase in the regions infiltrated by various inflammatory cells. It is thought that Th2 cytokines, such as IL-4, -9, -13, and stem cell factor (SCF) derived from these inflammatory cells, mediate mast cell increase [2, 4, 13]. However, *W* and *Sl* mice, which are deficient for SCF and its ligand c-kit receptor (KIT)s respectively, cannot increase mast cell number in response to nematode infection even though many inflammatory cell exist [1, 5, 11]. Thus, various cytokines and growth factors mediate mast cell growth in pathological statuses, and SCF-KIT signal transduction plays an essential role in mast cell proliferation in gastrointestinal nematode infection. Gastrointestinal nematode infections also increase the number of mast cells within the smooth muscle layer neighboring worms and worm cysts [8, 9]. In a previous report, the mast cell increase within the intestinal muscle layer was suggested to be due to the increase in the systemic production of Th2 cytokines [8]. However, there are many mast cells around the cysts of nematode *Heligmosomoides polygyrus* (*Hp*) within the intestinal muscle layers whereas few mast cells were present within the muscle layer without cysts [9]. This finding indicates that local factors appear to influence mast cell proliferation in intestinal smooth muscle layers, and the accumulation of CD4+ T cells that produce Th2 cytokines around worm cysts is thought to be one the local mechanisms of mast cell proliferation [9]. However, local production of SCF, an important growth factor for mast cell proliferation, has not been investigated in smooth muscle layers during *Hp* infection.

Recent studies have reported that bronchial and blood vessel smooth muscle cells have the ability to produce SCF in the normal state [6, 7]. These findings suggest that intestinal smooth muscle cells have the ability to enhance SCF production in response to immunological stimuli. However, those studies did not show whether or not smooth muscle cells can enhance SCF production or its gene expression in response to the inflammatory reaction *in vivo*. One reason is that the relatively small size of mast cell increased smooth muscle regions in the jejunum, neighboring the worms and worm cysts, makes it difficult to detect and extrapolate SCF expression patterns in the tissues using whole muscle tissues. Laser capture microdissection (LCM), which permits microscopic sections of specific tissue regions for mRNA analysis, provides a technology that makes it possible to assess SCF gene expression between the mast cell-increased regions and mast cell non-increased regions [9]. Because SCF gene expression is strongly correlated with the production of SCF protein [12], the investigation of the levels of SCF gene expression using the LCM technique is suitable for investigating the local production of SCF.

In the present study, I investigated the local levels of SCF mRNA expression using LCM techniques in the smooth muscle layer gastrointestinal nematode infection.

Specific pathogen-free, female BALB/c mice (Clea Japan Inc., Tokyo, Japan) were used. Autoclaved food pellets (MF; Oriental Yeast, Tokyo, Japan) and tap water were supplied *ad libitum*. All animals were handled under the regulations for animal welfare of Yamaguchi University. Groups of more than four mice of the same age, and approximately the same weight were used for each experiment. *Hp* was maintained by serial passage in mice. BALB/c mice were inoculated orally with 300 infective-stage HP larvae (L3), as described previously [10]. Fourteen days after the priming with Hp inoculation, mice were treated with the antihelminthic drug, pyrantel pamoate. One group of mice was then re-inoculated with Hp orally with 300 L3 at 50 days after the first Hp inoculation (HP), and the other group

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served as non-inoculated controls (RX). Gut tissues were collected from 5 mice per groups on day 4 after the second inoculation. Tissues were taken from the jejunum of the mice, slit longitudinally, prepared using the Swiss-roll technique, embedded in Tissue-Tek OTC compound (Sakura, Tokyo, Japan), frozen on dry ice-acetone, and stored at –80°C. For LCM, 4-mm serial tissue sections were cut from frozen blocks using a cryostat (Sakura). One section was stained with Giemsa (Sigma Aldrich, St. Louis, MO, U.S.A.) to detect worm cysts and mast cells (Figs. 1 and 2). Adjoining tissue sections of Giemsa-stained sections were dehydrated and stained with HE (Sigma Aldrich). LCM was performed on smooth muscle cells in stained sections on a PixCell II (Arcturus Engineering, Mountain View, CA, U.S.A.) [9]. Representative regions for sampling of each experimental group are shown in Figs. 1 and 2. Captured cells were transferred to CapSureTM LCM Caps (Arcturus Engineering). The LCM cap was inserted into a 0.5-m*l* microcentrifuge tube containing RNA isolation kit (Stratagene Cloning Systems, La Jolla, CA, U.S.A.). Total RNA was then reverse transcribed as previously described [9]. A real-time PCR kit (PE Applied Biosystems, Foster City, CA, U.S.A.) specific for SCF or rRNA was used to quantitate differences in gene expression. All data were normalized to constitutive rRNA values and a PE Applied Biosystems StepOne^{TM} Real-Time PCR System (PE Applied Biosystems) was used for target mRNA amplification.

As shown in Fig. 3, SCF gene expressions were significantly increased in mast cell-increased regions of HP mice (HP-mast) compared with the mast cell non-increased regions in the same sample (HP-cont). In both HP and RX mice, SCF gene expressions in mast cell non-increased regions (HP-cont and RX-cont) were almost the same as those in non-infected control mice (Cont). While SCF gene expressions of mast cell-increased regions in RX mice (RX-mast) were almost the same as those in mast cell non-increased regions in the same sample (RX-cont).

The present results show that SCF gene expression was significantly increased in mast cell-increased regions compared with the mast cell non-increased regions in the same sample, and SCF expression levels in non-increased regions were the same as those in the non-infected group (Fig. 3). This suggests that the intestinal smooth muscle cells enhanced SCF production against nematode infection locally, and this enhanced SCF production may have led to an increase in mast cell numbers [12]. Several researchers have hypothesized that smooth muscle cells are SCF producing cells, because mast cells increase within the bronchial smooth muscle layers, and within the interstitial tissues beside the blood vessels of lung, in patients sensitized to common allergens [6, 7]. These results demonstrate

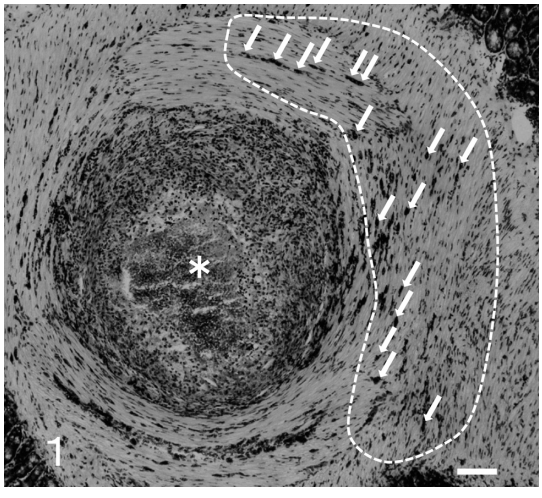

Fig. 1. A mast cell-increased the region in the smooth muscle layer of an *Hp*-infected mouse. The center of a worm cyst is filled with inflammatory cells (*), and arrows show the mast cells. The hatched line indicates the region of the samples. Giemsa stain. bar=100 μm.

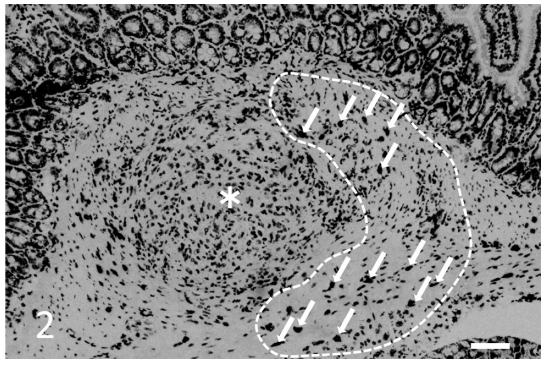

Fig. 2. A mast cell-increased region in the smooth muscle layer of a mouse treated with an antihelminthic drug. The center of a cyst is filled with connective tissues (*), and arrows show mast cells. The hatched line indicates the region of the samples. Giemsa stain. bar=100 μm.

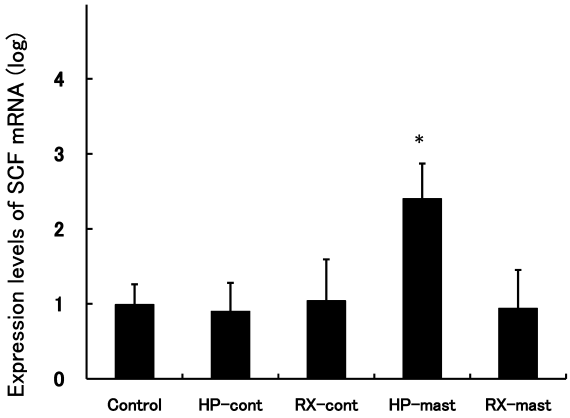

Fig. 3. Elevations in SCF in smooth muscle cell levels were limited to the localities around worm cysts. BALB/c mice (five per treatment group) were primed, and challenged with HP (HP) or without HP (RX). All data are expressed relative to the uninfected control group. The results are expressed as the mean and SEM of each treatment group. The Mann-Whitney test was used to evaluate the significance of the differences (* $P<0.05$).

that cultured smooth muscle cells can produce SCF, however, it has not been known whether smooth muscle cells can enhance SCF production in response to the pathological stimuli that induce increases in mast cell numbers. The present results showed for the first time that smooth muscle cells enhance SCF gene expression in response to a pathological stimulus, a nematode infection. In addition, this enhanced SCF gene expression is not controlled by the systemic humoral factors as reported previously [8], because the smooth muscle cells which increased SCF gene expression were limited to the locality of the worm cysts.

I could find no increase of SCF gene expression in the mast cell-increased region in the anthelminthic treatment group without worms (Fig. 3). The mast cell-increased regions in this group of mice were leftovers of worm cysts caused by the first infection [9]. After anthelminthic treatment, these regions lost the pathologic stimulus of nematodes (Fig. 2). The life span of resident mast cells is weeks to months [3]. Thus, the resident mast cells that infiltrated the inflammatory regions around worm cysts in the first *Hp* infection may still have been alive in these regions at the time of obsevation. The findings also support the hypothesis that smooth muscle cells enhance SCF gene expression against nematode infection. I used an *Hp* re-infected experimental system in the present study. Because many inflammatory cells surrounded the worm cyst in the first *Hp* infection, this *Hp* re-infected experimental system, in which mainly mast cells are induced, was suitable for the purpose of this study [9].

The results of the present study indicate that smooth muscle cells have the ability to enhance SCF production in response to nematode infection suggesting smooth muscle cells not only regulate muscular contraction but also the immunological reaction as growth factor producing cells in nematode infection.

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