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

By George F.J. Newlands, Hugh R.P. Miller, Anne MacKellar, and Stephen J. Galli

We assessed the effects of the c-kit ligand, stem cell factor (SCF), in the jejunal mucosal mast cell hyperplasia that occurs during infection with the intestinal nematodes, Nippostrongylus brasiliensis or Trichinella spiralis in rats. Compared with vehicle-treated rats, rats treated with SCF (25 µg/kg/d, intravenous [IV] for 14 days) during N. brasiliensis infection exhibited significantly higher levels of the rat mucosal mast cell (MMC)-associated protease II (RMCP II) in the jejunum and serum on day 8 of infection, but not on days 10 or 15 of infection. By contrast, in comparison to rats treated with normal sheep IgG, rats treated with a polyclonal sheep antirat SCF antibody exhibited markedly decreased numbers of jejunal MMCs, levels of jejunal RMCP II, and serum concentrations of RMCP II during infection with either nematode, particularly at the earlier intervals of infection (≤ day 10). Taken together, these findings indicate that SCF importantly contributes to MMC hyperplasia and/or survival during N. brasiliensis or T. spiralis infection in rats, but that levels of endogenous SCF are adequate to sustain near maximal MMC hyperplasia during infection with these nematodes. Notably, treatment of rats with SCF somewhat increased, and treatment with anti-SCF significantly decreased, parasite egg production during N. brasiliensis infection. This finding raises the interesting possibility that certain activities of intestinal MMCs may contribute to parasite fecundity during infection with this nematode.

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AT LEAST TWO distinct mechanisms regulate mast cell proliferation, differentiation, and survival in murine rodents. One of these is mediated by a ligand for c-kit, which is a tyrosine kinase receptor that is encoded by the c-kit protooncogene. The c-kit ligand is a growth factor that is produced by many cells, including fibroblasts and other stromal cells, some epithelial cells, and vascular endothelial cells; this growth factor has multiple activities that influence the development of c-kit+ lineages, including hemopoietic progenitor cells and mast cells, as well as primordial germ cells, melanocytes, and certain other cell types. The c-kit ligand has been named Steel factor, kit-ligand, mast cell growth factor and stem cell factor (SCF), the designation that will be used herein. Several lines of evidence indicate that, under physiological conditions in vivo, SCF is critical for the development and survival of all mouse and rat mast cells, including connective tissue-type mast cells, such as serosal mast cells, and intestinal mucosal mast cells (MMCs). The other mechanism that regulates mast cell development in mice and rats is mediated by T cells and possibly other sources of interleukin-3 (IL-3), IL-4, IL-9, and IL-10, cytokines that can promote and/or copromote the proliferation and/or survival of certain murine mast cell populations, including the MMC-like bone marrow-derived mast cells that can be generated in vitro.

In murine rodents, infection with intestinal nematodes induces a striking hyperplasia of intestinal MMCs, which is accompanied by increases in the levels of MMC-associated proteases in the intestinal tissues and increases in the concentrations of these proteases in the blood. Previous work indicates that both SCF- and T-cell-dependent mechanisms contribute to the intestinal MMC hyperplasia associated with nematode infection. In comparison to normal mice or rats, mice or rats with mutations that markedly diminish c-kit receptor tyrosine kinase activity, such as W/W mice or Ws/Ws rats, exhibit no or greatly diminished hyperplasia of intestinal MMCs in response to infection with N. brasiliensis or T. spiralis. Both intestinal MMC hyperplasia and the spontaneous expulsion of the parasites are diminished in T spiralis--infected mice that have been treated with an antibody to the c-kit receptor. Moreover, treatment of normal rats with Escherichia coli-derived recombinant rat SCF, which represents virtually the entire extracellular ligand domain of SCF and possesses high biologic activity, induces a significant hyperplasia of gastrointestinal MMCs, as well as connective tissue-type mast cells. On the other hand, athymic "nude" mice fail to develop MMC hyperplasia in response to nematode infection, whereas this response is restored in nude animals treated by adoptive transfer of T cells. Moreover, in normal mice, treatment with either anti-IL-3 or anti-IL-4 antibodies or, even more effectively, treatment with both antibodies, significantly suppresses intestinal MMC hyperplasia in response to N. brasiliensis infection. Finally, in vitro evidence indicates that SCP and IL-3 can have synergistic effects in promoting the proliferation of rat mast cells with phenotypic similarities to MMCs.

Taken together, this evidence indicates that the cytokine-dependent regulation of MMC hyperplasia during nematode infection in murine rodents, while quite complex, impor-
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stantly involves both SCF-- and T-cell--derived cytokines, such as IL-3 and IL-4. In the present study, we assessed whether the administration of exogenous rrSCF is amounts sufficient to induce MMC hyperplasia in normal (uninfected) rats, influenced either the jejunal MMC hyperplasia or the changes in levels of jejunal or serum MMC-associated RMCP II in rats infected with N. brasiliensis. We also assessed whether treatment of normal or nematode-infected rats with a polyclonal sheep antibody to rat SCF altered MMC numbers, RMCP II levels, or, in N. brasiliensis--infected rats, parasite fecundity.

MATERIALS AND METHODS

Rats

Random-bred female Wistar rats, weighing 250 to 300 g and maintained under conventional conditions at Moredun Research Institute, were used in all studies.

Parasites

Rats were infected with 2,500 to 3,000 N. brasiliensis third-stage larvae by subcutaneous injection or with 750 to 1,000 T. spiralis muscle larvae per os. For both of these procedures, the rats were anesthetized with Halothane (May and Baker, Dagenham, Essex, UK).

Stem Cell Factor

Recombinant rat stem cell factor (rrSCF) (Amgen, Inc., Thousand Oaks, CA) modified by the covalent attachment of polyethylene glycol was administered intravenously to anesthetized rats, at 25 μg/kg of body weight/day in 1 mL physiological saline, for 14 consecutive days; control rats were sham treated with physiological saline alone. The protocols were the same as those described previously.

Antibodies

Polyclonal anti-rrSCF antibodies were produced by inoculating sheep intramuscularly into both hind legs with 100 μg of rrSCF, suspended in 4 mL of Complete Freund’s Adjuvant. This procedure was repeated 4 and 8 weeks after the initial injection, except that Incomplete Freund’s Adjuvant was used for the subsequent inoculations. Blood samples were taken 3 weeks after the final inoculation and the serum tested by enzyme-linked immunosorbent assay (ELISA) for anti-rrSCF activity. Immune sera that gave absorbances more than fivefold greater than the background absorbance of preimmunization serum when tested at dilutions of up to 1/80,000 by ELISA were used to prepare specific antibody. The anti-SCF antibody preparation virtually abolished the effects of rrSCF on the survival and proliferation of in vitro-derived rat mast cells, but had no detectable effect on the IL-3--dependent proliferation of these cells (submitted for publication).

An immuno-affinity column was prepared by coupling 1.6 mg of rrSCF to CNBr-activated Sepharose-4B (Pharmacia Biotech, St Albans, Herts, UK) in accordance with the manufacturer’s instructions. Aliquots of serum (5 mL) were applied to the column, which had been equilibrated with phosphate buffered saline (PBS), and the column was subsequently eluted with 0.1 mol/L citric acid pH 2.2 + 0.5 mol/L NaCl to recover bound specific antibody. For control purposes, sheep immunoglobulin was prepared by precipitation of normal sheep serum with 50% saturated ammonium sulphate followed by gel filtration chromatography of the redissolved precipitate on Sephacryl S-200 (Pharmacia Biotech). Immunoglobulin preparation concentrations were concentrated by vacuum dialysis in a collodion thimble apparatus (Sartorius, Epsom, Surrey, UK) against PBS.

Treatment With Anti-SCF Antibodies

Rats were inoculated intraperitoneally with 1 mg sheep anti-rrSCF or normal sheep IgG in 1 mL PBS. Normal rats were treated daily for each of 4 or 7 days to assess the effects of treatment on resting mast cell populations. Because of the tissue and pulmonary migration of the L3 and L4 larval stages of N. brasiliensis, rats infected with this parasite were treated on day 3 of infection when the L3 larvae first reached the intestine and then on days 5, 7, 10, and 12. A further experiment was performed with N. brasiliensis--infected rats to determine whether treatment with anti-SCF had an effect on mast cell populations already expanded in response to parasitic infection. In this experiment, rats were treated with anti-SCF daily on days 10 to 13 after infection. For rats infected with T. spiralis, treatment commenced on day 0 and continued on days 3, 5, 7, and 10. Normal rats were killed 24 hours after, and parasitized rats 24 or 48 hours after, the final inoculation by exsanguination under deep Holothane anesthesia followed by cervical dislocation.

Hematologic Studies

Blood samples were obtained by tail-snip under halothane anesthesia with the blood collected into heparinized tubes. Total red blood cell and leukocyte counts were performed on a model ZM Coulter counter, hemoglobin concentrations were measured on a Coulter hemoglobinometer and packed cell volume by micromethemoglobin. Blood films were stained with Leishman’s stain for differential cell counts in which 100 leukocytes were counted per blood film.

Material Collected Postmortem

Peritoneal cells were collected by lavage of the peritoneal cavity with 20 mL of PBS containing 0.1% wt/vol bovine serum albumin (BSA) (PBS/BSA). Small intestine (mid-jejenum) was also collected for analysis and the tissues were either stored frozen at -20°C, or fixed by immersion in 4% wt/vol paraformaldehyde in PBS for 6 hours followed by 70% vol/vol ethanol overnight 14 before processing to paraaffin wax, before further processing. Serum was also stored at -20°C. Peritoneal lavage samples were sedimented by centrifugation at 1,000 g for 20 minutes at +4°C. The cells were resuspended in 1 mL PBS/BSA, recentrifuged at 1,000 g for 5 minutes and finally resuspended in 1 mL PBS/BSA for estimation of mast cell numbers. Mast cell counts were performed by diluting a 10-μL aliquot of the cell suspension with 90 μL of a mast cell stain containing 0.5% wt/vol methylene blue in 50% vol/vol propylene glycol. Blue staining mast cells were counted using an Improved Neubauer hemocytometer. Concentrations of Rat Mast Cell Proteases I or II (RMCP I or II) were measured in serum and tissue homogenates by ELISA as described previously.

Assessment of Rat Antisheep Antibodies

Because ruminant proteins are likely to have been included in the rats’ diet, and the experimental rats may have mounted an immune response against sheep proteins, an ELISA was developed to quantify the antibody response against sheep immunoglobulin. ELISA plates (Dynatech M129B) were coated with a solution of normal sheep immunoglobulin, prepared as described above, in 0.1 mol carbonate buffer pH 9.6 (50 μL/well). The plate was incubated at +4°C overnight and then washed six times with PBS containing 0.05% vol/vol Tween 20 (Sigma, Poole, Dorset, UK) before loading with samples. Sera from the animals that had been treated with anti-rrSCF or normal sheep IgG, and pretreatment serum samples from
the same rats, were prepared in serial dilutions in PBS and loaded, in triplicate, onto the plate (50 μL/well) and then incubated at room temperature for 1 hour. The ELISA plate was washed six times with PBS/Tween 20 and a sheep antirat IgG-horseradish peroxidase conjugate (Sigma), optimally diluted with PBS/RW20, was applied. The plate was again incubated at room temperature for 1 hour, washed six times as before and the color reaction developed, using orthophenylenediamine in triplicate, onto the plate (50 μL/well) and then incubated at room temperature for 10 minutes and the reaction was stopped with 1 M H2SO4. The plate was read on a Titertek Multiskan MC ELISA plate reader (Titertek, UK) at 492 nm. Samples that had absorbances, which were not subtractable from background, were considered to be positive.

Histological sections (5 μm thick) were stained with Toluidine blue (0.5% w/vol, pH 0.5) and mast cells in the jejunal mucosa (epithelium and lamina propria) were enumerated on a Leitz Dialux microscope with a ×25 objective lens and ×12.5 eyepieces equipped with a 100 mm² graticule. A minimum of five fields were counted per section.

Data Presentation and Statistical Analysis

Unless otherwise specified, all data are presented as the mean ± SEM. The two-tailed Student’s t-test was used to analyze data that were normally distributed, whereas the Mann-Whitney U-test was used as the nonparametric test. The time course of responses in different groups of rats were examined for statistical significance by using analysis of variance (ANOVA). Data for RMCP I content confirm previous findings showing that the concentration of RMCP I in the rat jejenum is several orders of magnitudes less than that of RMCP II, and these values changed to a lesser extent than values for RMCP II as a result of N brasiliensis infection (Table 1).

RESULTS

Effects of rSCF<sup>164</sup> Treatment on Jejunal MMC Numbers and Mast Cell-Associated Proteases in N brasiliensis-Infected Rats

Infection of rats with N brasiliensis causes an initial depletion of MMC from the jejunum, followed by a massive MMC hyperplasia. The data in Table 1 confirm that mast cells were depleted from the jejunum at day 8 of infection (0 ± 0 MMC/0.2 mm²; n = 3) compared with control values in noninfected rats (26 ± 1.2 MMC/0.2 mm²; n = 5, P < .02; Table 1). By day 15 of infection, mast cell densities in the jejenum of vehicle-treated rats were increased ninefold over control values (P < .0001; Table 1). The concentrations of RMCP II reflected the MMC counts in the jejunum, with a 5.8-fold increase on day 15 when compared with control values in rats not treated with rSCF<sup>164</sup> (P < .02; Table 1).

Treatment of parasitised rats with rSCF<sup>164</sup> beginning on day 0, resulted in a significant (~85%) elevation of mean jejunal mast cell density at baseline, a result that is in good agreement with our previous report. However, the mast cell densities at 8, 10, or 15 days of infection in the rSCF<sup>164</sup>-treated or -untreated rats were not significantly different. There were more mast cells in the specimens from the SCF-treated infected group compared to the control values (P < .05). The peak of serum RMCP II values occurred earlier, on day 8, in the rSCF<sup>164</sup>-treated group. The day 8 values in the SCF-treated infected group (individual values = 3,250, 4,250 and 6,450 ng/mL, mean ± SEM = 4,650 ± 945 ng/mL) were substantially higher than the corresponding values in the vehicle-treated infected rats (individual values = 1,200, 1,650, and 2,150 ng/mL, mean ± SEM = 1,670 ± 274 ng/mL). While the number of observations...
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found on days 3, 6, 9, or 12 of infection when compared with serum samples obtained from the same group of rats before infection.

Effects of treatment with anti-SCF in normal (uninfected) rats. When compared with rats treated with normal sheep IgG, 4 days of treatment with polyclonal sheep anti-rrSCF depleted serosal mast cells in the peritoneal cavity by greater than 56% (P < .05) and decreased the concentration of the major serosal mast cell-associated protease (RMCP I) in the cell pellet obtained at peritoneal lavage by 65% (Table 2). These levels of depletion did not increase by prolonging the treatment with anti-rrSCF for 7 days (data not shown).

No mast cells at all were detected after anti-SCF treatment in sections of jejunum (P < .001 v values for control rats) and both RMCP I and RMCP II concentrations were depleted at this site (P < .05 or P < .0001, respectively, Table 2). The depression of jejunal mast cell densities and RMCP II concentrations were reflected in the systemic levels of RMCP II, which were depleted by 96% in the serum of anti-SCF-treated rats (Table 2).

Anti-SCF treatment for 4 days also resulted in a 45% reduction in peripheral blood white blood cells, as assessed in rats killed for hematological analysis on day 5 (P < .02) (Table 3). From the differential cell counts, this appeared to represent an effect on all types of leukocytes (Table 3). The proportion of neutrophils counted in both the anti-SCF and normal IgG-treated control groups was depressed compared with pretreatment values (Table 3), possibly as a result of the stress induced by daily handling for inoculation and blood sampling and/or in response to the foreign protein.

Infection with N brasiliensis. N brasiliensis–infected rats were first treated with anti-SCF or normal sheep IgG on day 3 of infection when the worms begin to emerge into the gastrointestinal tract. As expected, mast cell counts in both control and treated groups were low on day 6 of infection (Fig 2). This result is in accord with previous work indicating that N brasiliensis infection produces an initial reduction in

was relatively small at this time point (n = 3/group), there were no overlapping values in the two groups, and the difference between the groups was significant at the P < .05 level. Together with the data in Table 1, this result shows that, at day 8 of infection, rrSCF<sup>™</sup> treatment results in both increased jejunal RMCP II content and increased secretion of this mast cell protease. Moreover, by ANOVA, serum levels of RMCP II over the entire time course of the infection were significantly higher in the rrSCF<sup>™</sup>-treated than in the control group not treated with the cytokine (P = .001).

Effects of Treatment of Rats With Anti-SCF

Development of an antibody response to sheep IgGs in rats treated with the polyclonal sheep anti-SCF antibodies. On day 6 of treatment with either sheep anti-SCF or normal sheep IgG, T spiralis–infected rats had an antisheep IgG antibody titre of 320, as assessed by ELISA, when compared with pretreatment sera from the same group of rats (P < .05). Interestingly, although the assay of sera from day 12 of treatment gave somewhat higher absorbances than that from the day 6 groups, the titre remained at 320 (P < .05). To determine whether this was a genuine antisheep IgG response or simply a reflection of upregulated IgG production as a result of infection, the sera from N brasiliensis–infected rats that had not been treated with sheep IgGs were also assayed. No significant difference in absorbance was

![Fig 1. Serum concentrations of RMCP II in uninfected rats treated with rrSCF<sup>™</sup> (●) or vehicle alone (□) compared with N brasiliensis–infected animals treated with rrSCF<sup>™</sup> (●) or vehicle controls (▲) (n = 4 to 8 for each interval in each group except for the day 8 values; n = 3 for this interval in both groups).](image)

Table 2. Effects of Anti-SCF Treatment for Four Days on Mast Cell Populations in Normal Rats

<table>
<thead>
<tr>
<th></th>
<th>Anti-SCF Mast Cells/0.2 mm&lt;sup&gt;2&lt;/sup&gt;</th>
<th>RMCP I (μg/ml)*</th>
<th>RMCP II (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>6.5 ± 1.4</td>
<td>0.84 ± 0.22</td>
<td>343 ± 4.6</td>
</tr>
<tr>
<td>Peritoneal mast cells (x10&lt;sup&gt;3&lt;/sup&gt;/lavage)</td>
<td>5.1 ± 1.0</td>
<td>98 ± 8.6</td>
<td>ND</td>
</tr>
<tr>
<td>Serum (ng/mL)</td>
<td>NA</td>
<td>NA</td>
<td>157 ± 15</td>
</tr>
</tbody>
</table>

Each of the two groups (+ or − anti-SCF treatment) contained five rats that were killed 24 hours after the last injection of anti-SCF or normal sheep IgG.

Abbreviations: NA, not applicable; ND, not determined.

* RMCP I was measured per lavage in peritoneal mast cells, and is expressed for peritoneal mast cells only as μg/lavage.

† P < .001 by two-tailed Student’s t-test v values for anti-SCF–untreated animals.

‡ P < .05.

§ P < .0001.
Table 3. Hematologic Parameters of Rats Treated for Four Days With Sheep Anti-SCF (anti-SCF) or With Normal Sheep IgG (Control) Compared With Pretreatment (Day 0) Controls

<table>
<thead>
<tr>
<th></th>
<th>WBC ($\times 10^9/L$)</th>
<th>RBC ($\times 10^9/L$)</th>
<th>Hb (g/dL)</th>
<th>Hct (%)</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 control</td>
<td>1.1 ± 0.05</td>
<td>6.7 ± 0.1</td>
<td>15.4 ± 0.3</td>
<td>43.5 ± 0.5</td>
<td>14.9 ± 1.5</td>
<td>78 ± 2.0</td>
<td>6.1 ± 0.7</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>anti-SCF Day 0</td>
<td>0.8 ± 0.04*</td>
<td>6.3 ± 0.2</td>
<td>14.6 ± 0.6</td>
<td>39.4 ± 1.1</td>
<td>5.0 ± 1.1</td>
<td>85 ± 2.5</td>
<td>7.8 ± 1.9</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>Day 5 control</td>
<td>1.1 ± 0.16</td>
<td>6.1 ± 0.4</td>
<td>14.9 ± 0.8</td>
<td>38.8 ± 1.6</td>
<td>5.2 ± 1.6</td>
<td>88 ± 1.8</td>
<td>5.0 ± 1.4</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each of the groups contained five rats that were killed 24 hours after the last injection of anti-SCF or normal sheep IgG. Thus, n = 10 for day 0 values (after blood was drawn on day 0, 5 of the 10 rats received anti-SCF treatment, and the other 5 received normal sheep IgG treatment), whereas n = 5 for the day 5 values.

* P < .02 by two-tailed Student’s t-test v day 5 control.

Mast cell densities in the intestines and other, distant, sites. However anti-SCF treatment resulted in significant inhibition of mast cell hyperplasia (Fig 2A). This effect was most notable on day 10 of infection (P < .001 v the corresponding value in control rats), but was observed throughout the entire time course of the infection (P < .0005 v control values). The anti-SCF treated rats also exhibited significantly reduced levels of RMCP II in the jejunal mucosa at all time points tested (Fig 3A), as well as over the entire time course of the infection (P < .0005 v control values). This reduced intestinal concentration of RMCP II was paralleled by a highly significant depression of RMCP II levels in the blood at 6 or 10 days after infection (P < .001 at either interval, P < .0005 over the entire course of the infection; Fig 4A). At 14 days after infection, the anti-SCF–treated rats developed MMC hyperplasia and increases in jejunal and serum levels of RMCP II, perhaps partly as a result of the development of an antibody response that diminished the efficacy of the
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sheep anti-SCF antibodies. Simple regression analysis of mast cell densities and RMCP II concentrations in the jejunal mucosa of all N brasiliensis–infected animals gave a highly significant correlation ($R = 0.96$, $P < 0.0001$, Fig 5A).

To determine whether SCF remains a significant factor in MMC hyperplasia or survival even after the onset of the intestinal MMC response, groups of rats were treated with anti-SCF or normal sheep IgG daily from day 10 of infection, when mast cell hyperplasia was well under way (eg, see Fig 2A). The rats treated with anti-SCF showed a 32% depletion of RMCP II from the small intestine on day 14, compared with values for normal sheep IgG-treated controls ($P < 0.01$; Table 4), with a concomitant 32% decrease in mast cell density ($P < 0.03$; Table 4). The 15% decrease in serum RMCP II concentrations in anti-SCF-treated rats was not significant (Table 4), nor were there any significant alterations in hematological parameters as a result of the anti-SCF treatment (Table 5).

*Infection with T spiralis.* Infective T spiralis larvae establish themselves in the intestine within hours of oral challenge, therefore treatment with anti-SCF was first given at the time of infection on day 0. Mast cell hyperplasia occurs sooner during trichinosis than in N brasiliensis–infected rats, and anti-SCF treatment significantly depressed mast cell densities on day 6, 10, and 12 of infection ($P < 0.001$, $P < 0.001$, and $P < 0.005$, respectively; $P < 0.0005$ over the entire course of the infection; Fig 2B) when compared with values in normal IgG-treated controls. Again, this result was paralleled by values for mucosal concentrations of RMCP II, which were significantly reduced 6, 10, or 12 days after infection ($P < 0.005$, $P < 0.001$, and $P < 0.001$, respectively; $P < 0.0005$ over the entire course of the infection; Fig 3B) in anti-SCF–treated rats. The relationship between mucosal RMCP II and mast cell densities, when analyzed by simple regression analysis, was significantly correlated ($R = 0.90$, $P < 0.0001$, Fig 5B). In contrast with responses in Nippostrongylus–infected rats, the systemic secretion of RMCP II was depressed on day 6 ($P < 0.001$), but not at other time points (Fig 4B). As previously reported,10 we found that T spiralis infection, in comparison to N brasiliensis infection, was associated with a greater, and earlier, systemic release of RMCP II (compare Fig 4A and B).

*Effects of treatment with rrSCF164* or anti-SCF on parasite fecundity. The effects of the administration of rrSCF164 or anti-SCF on the fecundity of N brasiliensis was determined by monitoring egg output in the feces of the rats. Egg output was maximal on day 6 of infection in both rrSCF164–treated...
Table 4. Jejunal Mast Cell Densities and RMCP II Concentrations in Intestine and Serum in Rats Treated With Anti-SCF or Normal Sheep IgG (Control) From Day 10 to 13 of N brasiliensis Infection

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 10</th>
<th>Day 14 Control</th>
<th>Day 14 Anti-SCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunal mast cells (cells/0.2 mm³)</td>
<td>ND</td>
<td>ND</td>
<td>65 ± 7</td>
<td>44 ± 3*</td>
</tr>
<tr>
<td>Jejunal RMCP II (μg/g wet wt)</td>
<td>ND</td>
<td>ND</td>
<td>1,752 ± 106</td>
<td>1,186 ± 111†</td>
</tr>
<tr>
<td>Serum RMCP II (ng/mL)</td>
<td>95 ± 4</td>
<td>360 ± 21</td>
<td>452 ± 62</td>
<td>386 ± 121</td>
</tr>
</tbody>
</table>

Each of the groups contained five rats that were killed 24 hours after the last injection of anti-SCF or normal sheep IgG. Thus, n = 10 for day 0 and day 10 values (after blood was drawn on day 10, 5 of the 10 rats received anti-SCF treatment, and the other 5 received normal sheep IgG treatment), whereas n = 5 for the day 14 values.

* P < .03
† P < .01 by two-tailed Student's t-test

and vehicle-treated (control) rats and diminished until no eggs were detected by day 11 (Fig 6A). While values for fecal egg output were greater in the rrSCF144-treated as opposed to the control rats at many intervals of the infection, these differences did not achieve statistical significance.

In the experiments in which rats were treated with anti-SCF or normal sheep IgG, the worm egg output was monitored over the latter part of the infection to determine whether depressing the intestinal mucosal mast cell response would also lead to an alteration of the normal worm expulsion kinetics. Interestingly, we found that the animals that were treated with anti-SCF (and had reduced densities of jejunal MMCs) had significantly lower fecal egg-counts on both days 8 and 9 than did the normal IgG-treated controls (P < .03 at both intervals, Fig 6B). Over the entire time course of the response shown in Fig 6B, values for anti-SCF treated rats were significantly lower than those in control rats by ANOVA (P = .001). However, egg output had virtually ceased by day 11 in both groups.

DISCUSSION

Our findings provide additional evidence that SCF importantly contributes to the MMC hyperplasia that occurs during nematode infection in rats, show that this response can be markedly suppressed by administration of an anti-SCF antibody, and raise interesting questions about the role of SCF and mast cells in the regulation of parasite fecundity during N brasiliensis infection.

In confirmation of our previous study,14 we found that treatment of uninfected rats with rrSCF at 25 μg/kg/d, intravenous (IV) for 14 days produced a significant (85%) increase in jejunal MMC numbers. However, in contrast to our previous study,14 the elevation in jejunal RMCP II content in rrSCF144-treated versus vehicle-treated rats did not achieve statistical significance. On the other hand, rrSCF144

Table 5. Hematologic Parameters in Rats Treated With Anti-SCF or Normal Sheep IgG (Control) From Day 10 to 13 of N brasiliensis Infection

<table>
<thead>
<tr>
<th></th>
<th>WBC (X 10⁹/L)</th>
<th>RBC (X 10⁹/L)</th>
<th>Hgb (g/dL)</th>
<th>Hct %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>10</td>
<td>1.4 ± 0.05</td>
<td>6.7 ± 0.1</td>
<td>15.7 ± 0.2</td>
</tr>
<tr>
<td>Day 10</td>
<td>10</td>
<td>1.1 ± 0.11</td>
<td>7.1 ± 0.2</td>
<td>16.1 ± 0.3</td>
</tr>
<tr>
<td>Day 14 control</td>
<td>5</td>
<td>1.2 ± 0.05</td>
<td>6.7 ± 0.1</td>
<td>15.1 ± 0.3</td>
</tr>
<tr>
<td>Day 14 anti-SCF</td>
<td>5</td>
<td>1.1 ± 0.12</td>
<td>6.4 ± 0.1</td>
<td>14.5 ± 1.6</td>
</tr>
</tbody>
</table>

Rats were killed 24 hours after the last injection of anti-SCF or normal sheep IgG. Day 0 and day 10 values are from all 10 rats (the 5 that later received anti-SCF treatment and the 5 that later received normal sheep IgG treatment; the treatments began after blood was drawn on day 10).
treatment had only modest effects on the changes in jejunal MMC numbers, or on the jejunal or serum levels of RMCP II, that were associated with N brasiliensis infection. Compared with vehicle-treated rats, rrSCF-treated rats had higher levels of jejunal MMCs and significantly higher levels of jejunal RMCP II at day 8 of infection. Also, the peak mean levels of serum RMCP II occurred earlier (day 8 v. day 10) and reached a roughly twofold higher maximum in rrSCF-versus vehicle-treated rats. These findings indicate that administration of exogenous SCF can increase the kinetics and magnitude of jejunal MMC development and RMCP II production during N brasiliensis infection. However, later during the infection, numbers of jejunal MMCs and serum levels of RMCP II were quite similar in rrSCF- and vehicle-treated rats, and, due to the broad range of individual values, the higher mean levels of RMCP II in the jejunal mucosa of rrSCF-treated rats were not significantly different than those in the vehicle-treated controls. One possible explanation for these findings, which we favor, is that endogenous levels of SCF in normal rats are sufficient to permit near maximal MMC hyperplasia (presumably, in response to T-cell-associated cytokines) in the setting of N brasiliensis infection.

To assess the importance of endogenous SCF in the intestinal MMC response to nematode infection in normal rats, we administered a polyclonal sheep antirat SCF antibody to rats infected with N brasiliensis or T spiralis, as well as to normal rats. Treatment of normal rats with anti-SCF for 4 days totally ablated the intestinal MMC population and virtually eliminated circulating RMCP II; it also significantly reduced numbers of peritoneal mast cells and diminished numbers of all circulating white blood cells, probably because of an effect on hematopoietic progenitor cells. Treatment with anti-SCF beginning at the time of infection profoundly interfered with mast cell hyperplasia in the jejunum and significantly diminished the systemic release of RMCP II after infection with either N brasiliensis or T spiralis.

These effects were most marked at the earlier intervals of infection, probably because of the later development of antibodies to sheep IgGs in the anti-SCF-treated rats. When anti-SCF was administered to rats that already had undergone expansion of jejunal MMCs in response to N brasiliensis infection, anti-SCF treatment resulted in a significant decrease in both intestinal RMCP II concentrations and mast cell numbers, but produced neither a significant depression of RMCP II concentrations in the serum nor a significant change in numbers of peripheral blood white cells.

One interpretation of our findings with anti-SCF is that endogenous SCF is necessary for the survival of mast cell populations, including both baseline populations of MMCs in uninfected rats and the markedly expanded MMC population in nematode-infected rats. rrSCF can maintain mouse mast cell survival in vitro and in vivo by suppressing apoptosis, and the abrogation of this effect by anti-SCF could account for our findings. Of course, rrSCF can promote the recruitment of mast cell precursors in vivo and can favor their local development into mature mast cells. It also can synergize with IL-3 to promote rat mast cell proliferation. These additional effects of SCF also may have been antagonized by anti-SCF treatment, particularly when treatment was begun at the time of infection.

However, the total ablation of mature MMCs from the intestinal mucosa of normal rats by anti-SCF cannot easily be explained by a suppression of recruitment of mast cell precursors. The half-life of mucosal mast cells in rat intestine has been estimated at around 40 days, and blocking recruitment for only 4 days would be expected to have a negligible effect on mast cell numbers. Nor can an effect solely on recruitment of precursors readily explain the depletion of MMCs from the intestinal mucosa that was observed when anti-SCF treatment was started on day 10 of infection, when T-cell–driven mastocytosis was already well established. These findings can, perhaps, best be explained by effects of anti-SCF treatment on the SCF-dependent suppression of mast cell apoptosis.

In T spiralis infection in mice, treatment with an anti-c-kit receptor antibody not only diminished intestinal mast cell hyperplasia, but also reduced spontaneous parasite expulsion. This finding supports the widely held view that mast cells can represent an important component of host immunity to parasite infection. Yet we found that treatment with rrSCF which accelerated the development of the MMC response, did not diminish and actually somewhat increased parasite egg production during N brasiliensis infection. This effect was particularly evident at day 8 of infection, the day on which rrSCF-treated rats had significantly more jejunal MMCs than did control rats (Table 1). Moreover, treatment with anti-SCF, which markedly suppressed the MMC hyperplasia associated with N brasiliensis infection, substantially (by >50%) and significantly (P = .001) diminished N brasiliensis egg production during the later stages of the infection.

In considering these findings, it should be noted that genetically mast cell-deficient (W/W, SISP) mice or (Ws/Ws) rats exhibit little or no impairment in their ability to expel a primary infection with N brasiliensis (reviewed in), findings which suggest that mast cells are not an essential component of the host immune response to this nematode. Indeed, Arizono et al found that, at the peak of egg output on day 8 of N brasiliensis infection, parasite egg production was ~3.5-fold greater in the congenic +/- normal rats than in mast cell-deficient Ws/Ws rats (133,300 v. 38,100 eggs/g of feces, P < .01); egg production in both the Ws/Ws and +/- rats declined rapidly thereafter, so that there was no significant difference in the low levels of egg output observed on day 11 of infection.

Both the findings in Ws/Ws and congenic +/- rats, and our findings in normal rats raise the interesting possibility that mast cells (and/or other SCF-responsive cells) may actually have some effects that favor parasite fecundity during N brasiliensis infection. For example, perhaps the activation of increased numbers of intestinal MMCs for mediator release, and the resulting increased permeability of gut blood vessels and intestinal mucosa, promotes the nutrition of the parasites. Whatever mechanism(s) might account for the apparent increased fecundity of N brasiliensis in rats treated with anti-SCF, it has been shown recently that suppression of IgE levels can decrease both the worm burden and the
parasite egg production associated with primary infection with Schistosoma mansoni in mice. Taken together with our results and those of Arizono et al in rats infected with N. brasiliensis, these findings suggest that, in certain parasite infections, aspects of the mast cell- and/or IgE-dependent immune responses that are elicited by the organisms may have effects which are more advantageous to the parasite than to the host.

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