BALB/3T3 Fibroblast-Conditioned Medium Attracts Cultured Mast Cells Derived From W/W But Not From mi/mi Mutant Mice, Both of Which Are Deficient in Mast Cells

By Tomoko Jippo-Kanemoto, Shido Adachi, Yoshitaka Ebi, Hiroshi Matsuda, Tsutomu Kasugai, Shin-ichi Nishikawa, and Yukihiko Kitamura

Proliferation of murine mast cells is induced by both T-cell-derived and fibroblast-derived growth factors. Because the most potent T-cell-derived mast cell growth factor, interleukin-3 (IL-3), promotes the migration of mast cells, we investigated whether fibroblast-derived growth factors had the chemotactic activity as well. Conditioned medium (CM) of BALB/3T3 fibroblasts induced the migration of cultured mast cells (CMC) derived from normal (+/+ mice). BALB/3T3-CM contained the mast cell growth factor (MGF)/stem cell factor (SCF)/kit ligand (KL), which is the ligand for the receptor encoded by the W (c-kit) gene. CMC derived from the spleen of W/W mice lack the extracellular domain of the W (c-kit) receptor, and W/W CMC did not proliferate in response to BALB/3T3-CM. However, W/W CMC did migrate normally toward BALB/3T3-CM and, moreover, the antibody to the extracellular domain of the W (c-kit) receptor did not inhibit the chemotactic activity of +/+ CM toward BALB/3T3-CM. These results indicated that MGF/SCF/KL itself did not represent the major chemotactic activity. On the other hand, BALB/3T3-CM induced neither proliferation nor migration of CMC derived from mi/mi mice. Both W/W and mi/mi mice are deficient in mast cells, but the present results suggest that the mechanism of the abnormality is different between W/W and mi/mi mice.

In addition to the potential of IL-3 to induce the proliferation of CMC, Matsuura and Zetter showed that IL-3 induced the migration of murine peritoneal mast cells. There is a possibility that MGF/SCF/KL may also induce the migration of mast cells. In the present study, we investigated whether conditioned medium (CM) of various fibroblast cell lines had the activity to induce the migration of CMC. CM of BALB/3T3 fibroblasts contains MGF/SCF/KL and induces the proliferation of +/+ CM, but not the proliferation of W/W CMC. BALB/3T3-CM induced the migration of +/+ CM, but it also induced the migration of W/W CMC, suggesting that the major chemotactic activity contained in BALB/3T3-CM is different from MGF/SCF/KL. On the other hand, BALB/3T3-CM induced neither proliferation nor migration of CMC derived from mi/mi mice, suggesting that the mechanism of mast cell deficiency is different between W/W and mi/mi mutant mice.

MATERIALS AND METHODS

Mice, cells, and antibodies. Mast cell-deficient (WB × C57BL/6)F1-W/W (hereafter called W/W'), WB-W/W (W/W'), C57BL/6-

From the Department of Pathology, Osaka University Medical School, Suita, Osaka; the Department of Veterinary Surgery, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka; and the Department of Pathology, the Institute of Medical Immunology, Kumamoto University Medical School, Kumamoto, Japan.

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Address reprint requests to Yukihiko Kitamura, MD, Department of Pathology, Osaka University Medical School, Yamada-oka 2-2, Suita, Osaka 565, Japan.

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mi/mi (mi/ni) mice, and their normal (+/+ ) littermates were raised in our laboratory.

The origin of NIH/3T3 cells has been described.27 The BALB/3T3 and Swiss-Albino/3T3 fibroblast cell lines were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). The WCB6F1+/-+/3T3-1 fibroblast cell line was established in our laboratory.28 Fibroblast cell lines were maintained in a-minimal essential medium (a-MEM; Flow Laboratories, Irvine, UK) containing 10% fetal calf serum (FCS; Hyclone, Logan, UT), 100 U/mL penicillin, and 100 µg/mL streptomycin (Sigma Chemical Co, St Louis, MO).

The preparation and specificity of the ACK2 rat monoclonal antibody (MoAb) against the extracellular domain of the W (c-kit) receptor have been described in detail.2930 Murine MoAb to 2.5 S NGF was a generous gift from Dr E.M. Shooter3' (Stanford University, Stanford, CA).

Establishment of CMC. Pokeweed mitogen-stimulated spleen cell conditioned medium (PWM-SCM) was prepared as described by Nakahata et al.22 Spleen cells (2 × 107/mL) were incubated for 5 days in a-MEM containing a 1:300 dilution of PWM (GIBCO, Grand Island, NY), 10% FCS, and 10-4 mol/L 2-mercaptoethanol (2-ME; Sigma). The culture supernatant was centrifuged, filtered through a 0.22-µm filter (Millipore Corp, Bedford, MA), and stored at -80°C. PWM-SCM contained IL-3 and IL-427 and, probably, IL-9.'

Culture flasks (Nunc, Roskilde, Denmark) containing 2 × 107 spleen or bone marrow cells and 5 mL a-MEM supplemented with 10-4 mol/L 2-ME, 10% FCS, and 10% PWM-SCM were incubated at 37°C in a humidified atmosphere of 5% CO2 in air. Half of the medium was replaced every 7 days, and more than 95% of cells were CMC at 4 weeks after the initiation of culture.35

Preparation of fibroblast CM. Fibroblast CM was prepared by culturing BALB/3T3, NIH/3T3, Swiss-Albino/3T3, or WCB6F1+/-+/3T3-1 fibroblasts in Cosmedium (Cosmo Bio Co, Ltd, Tokyo, Japan), a serum-free culture medium. After 5 days of culture, conditioned supernatant was harvested, centrifuged at 600g, and stored at -80°C. In one experiment, BALB/3T3-CM was concentrated by using a vacuum concentrator CC-180 (Tomy Co, Tokyo, Japan).

Reagents. Recombinant murine IL-6 was a gift from Dr T. Hirano (Osaka University, Osaka, Japan). Recombinant human colony-stimulating factor-1 (CSF-1) was a gift from Morinaga Milk Industry Company (Tokyo, Japan). Recombinant human differentiation-inducing factor (D-factor), which is also designated as leukemia inhibitory factor (LIF), human interleukin for DA cells (HILDA), and differentiation inhibitory activity (DIA), a gift from Dr M. Hozumi (Saitama Cancer Center Research Institute, Saitama, Japan). Nerve growth factor (NGF) purified from murine submaxillary glands was a gift from Drs A.M. Stanisz and J. Bienenstock (McMaster University, Hamilton, Ontario, Canada).34

Chemotaxis assay. The migration of CMC was measured with blind well chambers (Nuclepore Corp, Pleasanton, CA).35 The lower well containing chemoattractive substances (220 µL) was covered with a sheet of polycarbonate filter (pore size, 8 µm; Nuclepore). In some experiments, filters were coated with laminin (100 µg/15 mL; Sigma) for 30 minutes at room temperature. CMC (5 × 104) suspended in 850 µL of Cosmedium containing 0.1% bovine serum albumin (BSA; Sigma) were placed in the upper well. Blind well chambers were incubated at 37°C in a humidified atmosphere of 5% CO2 in air. The filters were then removed, fixed, and stained with Diff-Quick solution (American Scientific Products, McGaw Park, IL). The area occupied by the cells, which migrated to the lower surface of the filter, was quantitated by an image analyser (Nexus 6400; Nexus Inc, Tokyo, Japan). The migration index (MI) was calculated by the following formula:

\[
MI = \frac{\text{Area Occupied by Migrating Cells}}{\text{Total Area}} \times 100\% 
\]

At least five microscopic fields were quantitated per filter. In each experiment, assays were performed in triplicate.

Characterization of chemoattractive activity. Heat treatment was performed at 56°C or 70°C for 1 hour. To test the sensitivity to procase, BALB/3T3-CM was incubated with 6 U of insoluble trypsin that was bound to beaded agarose (Sigma). After incubation at 37°C for 1 hour, the beads were removed by centrifugation for 10 minutes at 80g. Conventional dialysis was performed with a Spectrapore tube (Sanko Junyaku Co, Ltd, Tokyo, Japan; cutoff, molecular weight, 12 Kd) for 24 hours at 4°C against two changes of Cosmedium.

[3H]Thymidine ([3H]Tdr) incorporation. CMC were washed and resuspended at 2 × 104 cells/mL in a-medium containing 5% FCS. Cells (2 × 104) in a total volume of 100 µL were plated in each well of 96-well microtiter plates, in which 25% BALB/3T3-CM had been applied at a volume of 100 µL. The microtiter plates were incubated at 37°C in humidified atmosphere of 5% CO2 for 24 hours. Wells were pulsed with 1 µCi [3H]Tdr (Amersham International, Amersham, UK; specific activity, 2 Ci/mmol) dissolved in 20 µL of a-medium, and the cells were harvested 18 hours later by an automatic cell harvester (Labo Mash LM 101; Labo Science Co, Tokyo, Japan).

RESULTS

Matsuura and Zetter26 showed a considerable chemoattractive activity of IL-3 by using laminin-coated filters, whereas only weak chemoattractive activity of IL-3 was found by Thompson et al.,36 who used uncoated filters. We examined whether the chemoattractive activity of PWM-SCM was influenced by coating filters with laminin. When the lower wells were covered with filters that had not been coated with laminin, the chemoattractive activity of PWM-SCM was hardly demonstrable (Table 1). In contrast, a significant chemoattractive activity of PWM-SCM was detected when filters had been coated with laminin as described by Matsuura and Zetter.36 We also evaluated the

<table>
<thead>
<tr>
<th>Substances in Wells</th>
<th>MI (%)</th>
<th>Uncoated Filters</th>
<th>Laminin-Coated Filters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosmedium Cosmedium</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Cosmedium 100% PWM-SCM</td>
<td>0.7 ± 0.5</td>
<td>5.3 ± 1.3t</td>
<td></td>
</tr>
<tr>
<td>Cosmedium BALB/3T3-CM</td>
<td>18.0 ± 2.5t</td>
<td>17.4 ± 2.1t</td>
<td></td>
</tr>
<tr>
<td>25% PWM-SCM BALB/3T3-CM</td>
<td>15.7 ± 1.2t</td>
<td>14.2 ± 1.3t</td>
<td></td>
</tr>
<tr>
<td>50% PWM-SCM BALB/3T3-CM</td>
<td>12.1 ± 0.9t</td>
<td>15.3 ± 0.9t</td>
<td></td>
</tr>
<tr>
<td>75% PWM-SCM BALB/3T3-CM</td>
<td>11.4 ± 1.0t</td>
<td>14.2 ± 1.2t</td>
<td></td>
</tr>
<tr>
<td>100% PWM-SCM BALB/3T3-CM</td>
<td>12.3 ± 0.5t</td>
<td>12.6 ± 0.6t</td>
<td></td>
</tr>
</tbody>
</table>

* CMC derived from the bone marrow of C57BL/6+/-+/+ mice were used as a target.

 tp < .01 when compared with the value of the case, in which Cosmedium was applied in both upper and lower wells, by t-test.

 tp < .01 when compared with the value of the case, in which the uncoated filters were used, by t-test.

 tp < .01 when compared with the value of the case, in which Cosmedium was applied in the upper well and BALB/3T3-CM in the lower well, by t-test.
To examine whether the chemoattractive activity of BALB/3T3-CM. The chemoattractive activity of BALB/3T3-CM was significantly greater than that of PWM-SCM. Moreover, the pretreatment of filters with laminin was not necessary for demonstration of the chemoattractive activity of BALB/3T3-CM (Table 1). To examine whether the chemoattractive activity of BALB/3T3-CM competed with that of PWM-SCM containing IL-3, chemotaxis assays were performed by applying various concentrations of PWM-SCM in the upper wells and BALB/3T3-CM in the lower wells. The chemoattractive activity of BALB/3T3-CM was significantly decreased when PWM-SCM was placed in the upper wells (Table 1). Because coating of laminin did not enhance the migration of CMC induced by BALB/3T3-CM, we hereafter used coated filters. In one experiment, BALB/3T3-CM was concentrated 10-fold and then diluted to various concentrations. The concentrated BALB/3T3-CM had not the greater chemoattractive activity, and the MI of approximately 20% appeared to be the peak value in the present experimental condition (Fig 1).

Chemoattractive activity of CM from various fibroblast cell lines was compared by using CMC derived from the bone marrow of C57BL/6-+/+ mice as a target. CM obtained from fibroblast cell lines other than the BALB/3T3 cell line did not show significant chemoattractive potential (Table 2). Because the activity of an equal mixture of BALB/3T3-CM and NIH/3T3-CM was comparable to that of 50% BALB/3T3-CM, NIH/3T3-CM did not appear to contain inhibitory factor(s). Stimulated cell migration may be either directional (chemotaxis) or random (chemokinesis). These can be distinguished by varying the concentrations of chemoattractant on each side of the filter in the assay chamber, as originally described by Zigmond and Hirsch.37 The result of this type of “checkerboard” analysis showed that BALB/3T3-CM primarily stimulated chemotaxis of CMC with a minor chemokinetic component (Table 3).

The BALB/3T3 fibroblast cell line was known to secrete MGF/SCF/KL.9,12,19 but NIH/3T3, Swiss albino/3T3, and +/+ 3T3 fibroblast cell lines did not appear to secrete detectable levels of MGF/SCF/KL.20 There is a possibility that the result shown in Table 2 may reflect the secretion of MGF/SCF/KL by BALB/3T3 fibroblasts. Because CMC derived from WBB6F1-W/Wv mice did not proliferate in the presence of W/W CMC.38-40 To exclude the involvement of extracellular domain of the c-kit protein in the chemotaxis, we hereafter used the transmembrane portion of the c-kit protein.39-40 To exclude the involvement of extracellular domain of the c-kit protein in the chemotaxis, the ACK2 antibody was not stimulated by BALB/3T3-CM. Chemotaxis of W/W CMC was comparable to that of +/+ CMC (Table 4). Because the Wv allele is a point mutation at the intracellular tyrosine kinase domain of the c-kit protein, normal extracellular domain of the c-kit protein is expressed on the surface of W/W CMC.38-40 To exclude the involvement of extracellular domain of the c-kit protein in the chemotaxis, W/W CMC were used. The transmembrane portion of the c-kit protein is not produced by W/W CMC, and the truncated c-kit protein is not expressed on the surface.39,40 As shown in Table 4, BALB/3T3-CM did not stimulate proliferation of W/W CMC, but did induce their migration. Another experiment was performed to exclude the involvement of the c-kit protein in the chemotaxis. The chemotaxis of +/+ CMC was evaluated in the presence of the ACK2 anti-c-kit antibody. The concentration of the ACK2 antibody was 5 μg/mL that inhibited attachment of +/+ CMC to +/+ fibroblasts.41 The chemotaxis of +/+ CMC to BALB/3T3-CM was not significantly reduced (Table 5).

We have already shown that the proliferation of CMC derived from another mutant, mi/mi, was not stimulated by the coculture with fibroblasts.21 Proliferation of mi/mi

| Table 2. Chemotaxis of CMC to CM of Various Fibroblast Cell Lines |
|-----------------|-----------------|
| Chemoattractants | MI (%)*         |
| BALB/3T3-CM     | 18.5 ± 2.5      |
| NIH/3T3-CM      | 1.4 ± 0.1       |
| Swiss-Albino/3T3-CM | 1.2 ± 0.1      |
| WCB6F1-+/+/3T3-1-CM | 1.1 ± 0.1     |
| 50% BALB/3T3-CM | 7.8 ± 1.0       |
| 50% BALB/3T3-CM + 50% NIH/3T3-CM | 7.6 ± 1.2     |

Table 2. Chemotaxis of CMC to CM of Various Fibroblast Cell Lines

| Table 3. Checkerboard Analysis of BALB/3T3-CM |
|-----------------|-----------------|
| BALB/3T3-CM in Lower Well (%) | MI/%* |
| 0 | 0 25 100 |
| 0 | 0.2 ± 0.1 1.5 ± 0.7 1.9 ± 0.3 1.0 ± 0.4 |
| 25 | 3.6 ± 1.7 5.7 ± 1.7 1.7 ± 0.6 1.5 ± 0.6 |
| 50 | 7.1 ± 3.8 8.3 ± 0.9 1.8 ± 0.4 0.7 ± 0.3 |
| 100 | 18.0 ± 2.5 10.0 ± 0.6 2.4 ± 0.5 2.2 ± 0.3 |

Table 3. Checkerboard Analysis of BALB/3T3-CM

<graph>Fig 1. Migration of CMC toward BALB/3T3-CM at various concentrations. BALB/3T3-CM was concentrated 10-fold and the chemotaxis of variously diluted samples was tested by using CMC derived from C57BL/6-+/+ mice as a target. CM obtained from fibroblast cell lines other than the BALB/3T3 cell line did not show significant chemoattractive potential (Table 2). Because the activity of an equal mixture of BALB/3T3-CM and NIH/3T3-CM was comparable to that of 50% BALB/3T3-CM, NIH/3T3-CM did not appear to contain inhibitory factor(s). Stimulated cell migration may be either directional (chemotaxis) or random (chemokinesis). These can be distinguished by varying the concentrations of chemoattractant on each side of the filter in the assay chamber, as originally described by Zigmond and Hirsch.37 The result of this type of “checkerboard” analysis showed that BALB/3T3-CM primarily stimulated chemotaxis of CMC with a minor chemokinetic component (Table 3).

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We have already shown that the proliferation of CMC derived from another mutant, mi/mi, was not stimulated by the coculture with fibroblasts.21 Proliferation of mi/mi
fibroblast cell lines are known to produce laminin, D-fac-
tor, CSF-1, IL-6, and NGF, we examined whether NGF showed weak chemoattractive activity, but CSF-1 and
ble 5).

than that of PWM-SCM. Although laminin-coated filters
activity in BALB/3T3-CM was protein in nature. Because
IL-6 did not (Table 7). The chemoattractive activity of the
mixture (laminin 25 µg/mL + D-factor 250 U/mL + CSF-
1 500 U/mL + IL-6 500 U/mL) was significantly lower than
that of BALB/3T3-CM. Similar results were obtained by
using laminin-coated filters (data not shown). Moreover,
the addition of anti-NGF antibodies (20 µg/mL) did not
inhibit the chemoattractive activity of BALB/3T3-CM (Ta-
ble 5).

We examined the physicochemical characteristics of the
chemoattractive activity in BALB/3T3-CM. The chemo-
attractive activity was destroyed by heating BALB/3T3-CM
at 57°C for 1 hour (Table 6). The activity was not lost after
dialysis. These data suggested that the chemoattractive
activity in BALB/3T3-CM was protein in nature. Because
fibroblast cell lines are known to produce laminin, D-factor,
CSF-1, IL-6, and NGF, we examined whether +/+ CMC showed chemotaxis toward these substances. In the
present experimental condition, laminin, D-factor, and
NGF showed weak chemoattractive activity, but CSF-1 and
IL-6 did not (Table 7). The chemoattractive activity of the
mixture (laminin 25 µg/mL + D-factor 250 U/mL + CSF-
1 500 U/mL + IL-6 500 U/mL) was significantly lower than
that of BALB/3T3-CM. Similar results were obtained by
using laminin-coated filters (data not shown). Moreover,
the addition of anti-NGF antibodies (20 µg/mL) did not
inhibit the chemoattractive activity of BALB/3T3-CM (Ta-
ble 5).

DISCUSSION

Chemoattractive activity of BALB/3T3-CM was greater
than that of PWM-SCM. Although laminin-coated filters

Table 4. Comparison Between BALB/3T3-CM-Induced Proliferation
and Migration in CMC of Various Genotypes

<table>
<thead>
<tr>
<th>Genotype of CMC</th>
<th>[3H] TdR Uptake (cpm)*</th>
<th>MI (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+ †</td>
<td>12,732 ± 1,942</td>
<td>18.3 ± 2.2</td>
</tr>
<tr>
<td>W/W †</td>
<td>151 ± 68#</td>
<td>19.3 ± 1.1</td>
</tr>
<tr>
<td>W/W</td>
<td>114 ± 50†</td>
<td>19.2 ± 3.2</td>
</tr>
<tr>
<td>mi/mi</td>
<td>83 ± 29‡</td>
<td>4.9 ± 2.4‡</td>
</tr>
</tbody>
</table>

*Mean ± SE of four experiments.
†CMC derived from the bone marrow of C57BL/6-/+ mice. CMC
derived from the spleen of C57BL/6/+/+ mice gave a comparable
result.
‡P < .01 when compared with the value of +/+ CMC by t-test.

Table 5. Effects of Antibodies on the Migration of CMC to the
BALB/3T3-CM

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>MI (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>18.6 ± 1.8</td>
</tr>
<tr>
<td>Anti-c-kit (5 µg/mL)†</td>
<td>17.5 ± 2.2</td>
</tr>
<tr>
<td>Anti-NGF (20 µg/mL)†</td>
<td>18.2 ± 1.1</td>
</tr>
</tbody>
</table>

CMC derived from the bone marrow of C57BL/6-/+/+ mice were used as a target.
†The concentrations of the antibody were determined according to
the results published by Adachi et al4 and Matsuda et al5, respectively.
There is another explanation that MGF/SCF/KL may be used as a target.

Ando Y, Yagi K, Tadokoro K, Akabane T: Extensive proliferation of W/W mice in the presence of IL-3. In this coculture, W/W+ and mi/mi CMC were maintained by the added IL-3; +/+ and W/W+ CMC acquired the CTMC-like phenotype, but mi/mi CMC did not. Because Matsuda et al showed that NGF played an important role for the phenotypic change, there is a possibility that mi/mi CMC do not respond to NGF. Taken together, mi/mi CMC cannot respond normally to all the above-mentioned inductive stimuli presented by fibroblasts.

Dubreuil et al introduced the c-fms proto-oncogene, which encodes the receptor for CSF-1, to +/+ W/W+ and mi/mi CMC. After the introduction, CMC of +/+ W/W+ genotypes proliferated in response to CSF-1, but mi/mi CMC did not. Because both c-kit and c-fms proteins are receptor tyrosine kinases, Dubreuil et al speculated that the c-kit and c-fms proteins shared common substrates for phosphorylation. There is a possibility that the abnormality caused by the mi mutation may result in the lack of response to various factors other than MGF/SCF/KL and CSF-1. The impaired migration of mi/mi CMC to BALB/3T3-CM may result from the unresponsiveness of mi/mi CMC to one of such growth factors.

### Table 7. Chemotaxis of CMC to Known Products of Fibroblasts

<table>
<thead>
<tr>
<th>Substances</th>
<th>MI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosmedium alone</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>BALB/3T3-CM</td>
<td>17.7 ± 2.8†</td>
</tr>
<tr>
<td>Laminin (25 µg/mL)</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>Laminin (50 µg/mL)</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Laminin (100 µg/mL)</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Laminin (200 µg/mL)</td>
<td>0.9 ± 1.0</td>
</tr>
<tr>
<td>D-factor (50 U/mL)</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>D-factor (250 U/mL)</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>D-factor (1,000 U/mL)</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>D-factor (2,000 U/mL)</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>CSF-1 (500 U/mL)</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>IL-6 (500 U/mL)</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Laminin + D-factor + CSF-1 + IL-6†</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>NGF (50 ng/mL)</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>NGF (100 ng/mL)</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>NGF (500 ng/mL)</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

CMC derived from the bone marrow of C57BL/6-+/+ mice were used as a target.

*Mean ± SE of four experiments.
†P < .01 when compared with the value of Cosmedium, in which Cosmedium alone was applied, by t-test.
‡Laminin (25 µg/mL), D-factor (250 U/mL), CSF-1 (500 U/mL), and IL-6 (600 U/mL).

The chemoattractive activity of MGF/SCF/KL appeared to depend on the type of cells that expressed the c-kit protein. There is another explanation that MGF/SCF/KL may interact with the W/W CMC through some receptors other than W (c-kit) receptors, particularly in the context of other fibroblast products.

The chemoattractive activity in BALB/3T3-CM was sensitive to heat and trypsin treatment, and was not dialyzable. These results suggested that the chemoattractant is a protein. We examined the chemoattractive activity of some known proteins produced by fibroblasts. However, laminin, D-factor, CSF-1, IL-6, and NGF did not show chemoattractive activity that was comparable to BALB/3T3-CM. Moreover, the anti-NGF antibody did not neutralize the chemoattractive activity of BALB/3T3-CM. Further studies are necessary to identify the substance(s) contained by BALB/3T3-CM, which show the chemoattractive activity to CMC.

Fibroblasts affect mast cells in at least three different fashions: (1) induction of proliferation through MGF/SCF/KL; (2) induction of migration shown in the present study; (3) induction of phenotypic change from CMC resembling mucosal mast cells to connective tissue-type mast cells (CTMC). Proliferation of both W/W and mi/mi CMC was not induced by BALB/3T3-CM containing MGF/SCF/KL. Migration potential of W/W CMC toward BALB/3T3-CM was comparable to that of +/+ CMC, but migration potential of mi/mi CMC was apparently impaired. Recently, we cocultured +/+ W/W+ and mi/mi CMC with fibroblasts in the presence of IL-3. In this coculture, W/W+ and mi/mi CMC were maintained by the added IL-3; +/+ and W/W+ CMC acquired the CTMC-like phenotype, but mi/mi CMC did not. Because Matsuda et al showed that NGF played an important role for the phenotypic change, there is a possibility that mi/mi CMC do not respond to NGF. Taken together, mi/mi CMC cannot respond normally to all the above-mentioned inductive stimuli presented by fibroblasts.

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BALB/3T3 fibroblast-conditioned medium attracts cultured mast cells derived from W/W but not from mi/mi mutant mice, both of which are deficient in mast cells

T Jippo-Kanemoto, S Adachi, Y Ebi, H Matsuda, T Kasugai, S Nishikawa and Y Kitamura