Effects of T-Helper 2-Type Cytokines, Interleukin-3 (IL-3), IL-4, IL-5, and IL-6 on the Survival of Cultured Human Mast Cells

By Makoto Yanagida, Hiromi Fukamachi, Kinya Ohgami, Tomoaki Kuwaki, Hiromi Ishii, Hiroya Uzemaki, Kenji Amano, Tonomobu Tokiwa, Hideki Mitsui, Hirohisa Saito, Yoji Ikura, Teruko Ishizaka, and Tatsutoshi Nakahata

Although stem cell factor (SCF) has been identified as a critical cytokine for the development of human mast cells from their progenitors, the effects of other cytokines on human mast cells are less well understood. We examined the effects of several cytokines on the survival of human mast cells of 100% purity generated in suspension cultures of umbilical cord blood mononuclear cells in the presence of 100 ng/mL recombinant human (rh) SCF and interleukin-6 (IL-6). Mast cells suspended in conventional serum-containing medium died over a period of 2 to 6 days after the withdrawal of SCF and IL-6. The cells became pyknotic and underwent DNA fragmentation characteristic of apoptosis. The addition of SCF, IL-3, IL-4, IL-5, or IL-6 to the cultures in both serum-containing and serum-free medium prolonged their survival in a dose-dependent manner. Some other cytokines, such as IL-2, IL-9, IL-10, IL-11, tumor necrosis factor-α, transforming growth factor-β1, and nerve growth factor, had no survival-promoting effect at 100 ng/mL. Preincubation of mast cells with SCF, IL-4, IL-5, or IL-6 for 24 hours during sensitization with IgE enhanced IgE/anti-IgE antibody-induced histamine release from mast cells, whereas IL-3 showed a negligible effect. Polymerase chain reaction amplification of α-chains of IL-3 receptor (R), IL-4 R, IL-5 R, and IL-6 R yielded products of the correct size predicted from the sequence of each receptor. The binding assay using 125I-labeled IL-3 indicated that these mast cells bear receptors for IL-3. These findings suggest that IL-3, IL-4, IL-5, and IL-6, which are mainly produced by T-helper 2 lymphocytes, might regulate the functions of human mast cells in vivo via specific receptors in allergic reactions.

© 1995 by The American Society of Hematology.

M AST CELLS ARE UNIQUE immune cells that release a variety of chemical mediators, such as histamine, leukotrienes,1 and cytokines,2-4 induced by the reaction of allergen with cell-bound IgE antibodies. Progenitors of human mast cells have been shown to be derived from CD34+ cells in bone marrow.5 Stem cell factor (SCF) is known to act as a major growth and differentiation factor for human mast cells from progenitors in cord blood mononuclear cell,6 bone marrow,7,8 and fetal liver,9 whereas several murine cytokines, such as SCF, interleukin-3 (IL-3), IL-4, IL-9, and IL-10, promote differentiation and proliferation of mouse mast cells.10-14 In human mast cells, it is of interest to determine whether cytokines other than SCF are involved in the development of mast cells and affect their function. Kirshenbaum et al15,16 have reported that IL-3 alone and in combination with SCF promoted the growth and survival of human mast cells from bone marrow. However, there is little information about the effects of cytokines other than SCF on human mast cells, partly because sufficient amounts of pure human mast cells have yet to be obtained from lungs, skin, or tonsils or developed in vitro from their progenitors.

Furitsu et al17 first succeeded in developing human mast cells in vitro by coculture of mononuclear cord blood cells and Swiss albino/3T3 fibroblasts. Subsequently, we established a method for long-term suspension culture of human mast cells that were developed from mononuclear cord blood cells in the presence of recombinant human (rh) SCF.6 We recently succeeded in developing pure human mast cells efficiently from CD34+ or mononuclear cells16,17 of cord blood in the presence of SCF and IL-6. IL-6 might act synergistically to expand the number of human mast cells progenitors and to promote their differentiation. All these mast cells were immunohistologically positive for tryptase, whereas only 20% to 30% of the cells were immunoreactive for chymase.16,17 Human mast cells developed from cord blood cells by SCF and IL-6 could be sensitized with IgE and released mediators upon challenge with anti-IgE antibody.16,17 They are functionally active and more like tryptase-positive mast cells (MC-1)8 seen in human lung and gastrointestinal mu cosa. The development of this culture method enabled us to study the biology of human mast cells more precisely.

In the present study, we examined the in vitro survival-promoting activity of several cytokines on human mast cells of 100% purity. Our data showed that IL-3, IL-4, IL-5, and IL-6 as well as SCF promote the in vitro survival of human mast cells developed from cord blood mononuclear cells in the presence of SCF and IL-6. Using reverse transcriptase-polymerase chain reaction (RT-PCR) and binding assays, we showed that cultured human mast cells express mRNAs of IL-3 receptor (R), IL-4 R, IL-5 R, and IL-6 R α-chains and have specific binding sites for at least IL-3. In this report, we show the new aspects of the human cytokine network in allergic reactions through the interactions between human mast cells and T-helper 2 (Th2)-type cytokines.

MATERIALS AND METHODS

Cell culture. The culture methods of human mast cells that had been established previously by the several investigators18,19,20 were modified. Mononuclear cells were obtained from heparinized umbilical cord blood and suspended in α-minimum essential medium (α-

From the Pharmaceutical Development Laboratory and Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., Gunma, Japan; The Second Department of Internal Medicine, Osaka University Medical School, Osaka, Japan; the Division of Allergy, National Children’s Medical Research Center, Tokyo, Japan; the La Jolla Institute for Allergy and Immunology, La Jolla, CA; and the Department of Clinical Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Submitted March 20, 1995; accepted July 11, 1995.

Address reprint requests to Makoto Yanagida, Pharmaceutical Development Laboratory, Kirin Brewery Co., Ltd, 2-2 Souja-machi, 1-chome, Maebashi-shi, Gunma 371, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1995 by The American Society of Hematology.

0006-4971/95/8610-0021$3.00/0
MEM; Gibco-BRL Laboratories, Grand Island, NY) supplemented with 20% fetal bovine serum (FCS; HyClone Laboratories, Logan, UT); 1% bovine serum albumin (BSA; Sigma Chemicals, St Louis, MO); 10 μg/mL deoxyadenosine, deoxyguanosine, deoxythymidine, adenine, guanine, cytidine, thymidine, and uridine (Sigma); 100 U/mL penicillin (Sigma); and 50 μg/mL streptomycin (Sigma). The cell suspensions were cultured in the presence of 100 ng/mL rhSCF and rhIL-6 (Kirin Brewery, Tokyo, Japan). Nonadherent cells were harvested weekly after gently pipetting the culture media over the bottom of a flask and resuspending the cells in culture media, half of which was replaced. Total cell numbers were counted using hemacytometers (Kayagaki Clinical and Scientific Equipment, Tokyo, Japan), and the purity of mast cells was determined by staining with May-Grünwald and Giemsa reagents every 2 weeks. The immunoperoxidase staining for tryptase and chymase \(^{[7]}\) was performed every 4 weeks.

**Survival assay.** Cultured human mast cells were washed twice in 20% FCS-containing α-MEM and then replated in 96-well culture dishes (Becton Dickinson, Lincoln Park, NJ) at a density of 2 × 10⁷ cells in 200 μL on day 0. These cells were maintained at 37°C in a humidified atmosphere of 95% air:5% CO₂ in each medium supplemented with various cytokines for an additional 4 to 6 days at concentrations that varied with the particular experiment. Cell viability was assessed at various intervals thereafter according to the trypan blue exclusion test. In the survival-free assays, mast cells were washed in FCS-free α-MEM and BSA-coated 96-well culture dishes prepared by incubation of dishes at 37°C for 2 hours after 200 μL of 5% BSA was added into each well.

**Cytokines.** The sources of cytokines were as follows: rhIL-2, rhIL-3, rhIL-4, rhIL-5, and rhIL-6 from R&D Systems (Minneapolis, MN); rh nerve growth factor (NGF) from Austral Biologicals (Placid, NY); rh tumor necrosis factor-α (TNF-α) from Upstate Biotechnology (Lake Placid, NY); rh nerve growth factor (NGF) from Austral Biologicals (San Ramon, CA); and rh transforming growth factor-β1 (TGF-β1) from Kirin Brewery.

**Electron microscopy (EM):** Cells maintained in 20% FCS-containing medium supplemented with or without 100 ng/mL rhSCF were collected on day 2, fixed in 2% glutaraldehyde for 1.5 hours, and washed in 0.1 mol/L sodium phosphate buffer. The cells were then postfixed in 1% osmic acid in 0.1 mol/L sodium phosphate buffer for 1.5 hours and embedded in epoxy resin. These blocks were processed for EM studies as described previously. \(^{[30]}\)

**In situ detection of DNA fragmentation.** Some samples of the cells collected for EM were subjected to cytocentrifugation (Shandon, Pittsburgh, PA) on glass slides, dried, and fixed promptly in Carnoy’s fixative overnight. After washing with water, slides were incubated at 37°C in 2.5 μg/mL proteinase K (Sigma) for 15 minutes, washed with phosphate-buffered saline (PBS), and dehydrated by passage through a series consisting of 50%, 75%, 95%, and 100% ethanol. In situ detection of DNA fragmentation was performed using an immunoperoxidase staining kit (ApopTag; Oncor, Gaithersburg, MD). \(^{[21]}\) Briefly, slides were quenched in 2% hydrogen peroxide to block nonspecific staining and then reacted with a mixture of terminal deoxynucleotidyl transferase (TdT), digoxigenin-UTP, and dATP to label the 3'-OH ends of DNA. Digoxigenin incorporated into the tails of DNA molecules was identified by immunohistochemical procedures. The nuclei of multiple DNA fragment-containing cells were stained reddish brown. Cell nuclei were counterstained with methyl green.

**Histamine release.** BSA-coated 24-well culture dishes were prepared by incubation of dishes (Becton Dickinson) at 37°C for 2 hours after 1 mL of 5% BSA was added to each well. Cultured cells were washed twice in FCS-free α-MEM and incubated with 10 μg/mL human IgE (a generous gift from Dr Kishigake Ishizaka, La Jolla Institute for Allergy and Immunology, La Jolla, CA) for 24 hours at 37°C under FCS-free conditions with or without rhSCF, rhIL-3, rhIL-4, rhIL-5, or rhIL-6 (1, 10, and 100 ng/mL) in BSA-coated 24-well culture dishes. After washing, the cells were incubated at 37°C for 30 minutes with 4 μg/mL anti-IgE antibody (Chemicon, Temecula, CA). Histamine content in supernatants and that remaining in cell pellets was determined by the automated technique of Siraganian. \(^{[2]}\) The percentage of enhancement of histamine release was calculated using the following formula: (% stimulated release – % control release)/% control release) × 100.

**RNA preparation.** Cellular mRNA was isolated from cultured

---

**Table 1. Development of Mast Cells From Cord Blood Cells by SCF and IL-6**

<table>
<thead>
<tr>
<th>Culture Period (wk)</th>
<th>Total Cells per Flask ((×10^5))</th>
<th>Mast Cells (%)</th>
<th>Tryptase-Positive Cells (%)</th>
<th>Chymase-Positive Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>5.9 ± 1.6</td>
<td>41.5 ± 12.5</td>
<td>41.3 ± 11.1</td>
<td>24.2 ± 2.2</td>
</tr>
<tr>
<td>8</td>
<td>2.4 ± 0.9</td>
<td>99.6 ± 0.6</td>
<td>99.8 ± 0.5</td>
<td>24.8 ± 17.5</td>
</tr>
<tr>
<td>12</td>
<td>2.4 ± 0.6</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>27.8 ± 19.9</td>
</tr>
<tr>
<td>16</td>
<td>2.2 ± 0.4</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>37.5 ± 36</td>
</tr>
</tbody>
</table>

Mononuclear cells \((5 × 10^5)\) were cultured per flask. The numbers of cells represent the means ± SD of four separate cultures. Mast cells were identified by May-Grünwald-Giemsa staining.

Abbreviation: ND, not determined.
human mast cells and the U-937 cell line by using a QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech, Piscataway, NJ). Briefly, extraction buffer containing a high concentration of guanidinium isothiocyanate was added to the cell pellet. Elution buffer was then added. After centrifugation, supernatants were transferred to microcentrifuge tubes containing oligo(dT)-cellulose. The polyadenylated RNA bound to the oligo(dT)-cellulose pellet was then washed sequentially with high-salt buffer and eluted with low-salt buffer. The mRNA pellet was then precipitated in ethanol, and the purified mRNA was resuspended in water.

**Oligonucleotide primers.** Primers, the sources of which are not described below, were prepared in the oligonucleotide synthesis core at Kirin Brewery. The primers used were as follows: IL-3 R, (A1; nucleotides 611 to 631, sense) AAGGGATGCGGAGCAACGG and (A2; nucleotides 1144 to 1164, sense) GAGGGATGCGGAGAAAGTGC; IL-4 R, (B1; nucleotides 605 to 625, sense) AGCGGGTGATATCGCCGCG and (B2; nucleotides 1099 to 1119, sense) TCCAGAAAAACACGAAAGAC; and (B3; nucleotides 1339 to 1359, sense) AAGGTATCCCTGCTGCTCTCA; IL-5 R, (C1; nucleotides 607 to 627, sense) CCAAGAATACAGCAAAGAC and (B3; nucleotides 1339 to 1359, sense) AAGGTATCCCTGCTGCTCTCA; IL-6 R, (D1; nucleotides 1143 to 1163, sense; Clontech) ATGGATGATGATATCGCCGCG and (D2; nucleotides 1393 to 1414, sense; Clontech) ATGGATGATGATATCGCCGCG; human granulocyte colony-stimulating factor receptor (G-CSF R, E1; nucleotides 1790 to 1810, sense) AACTGAGCAGTGTGGAGGAGG and (E2; nucleotides 2179 to 2199, sense) CAGCGATGGTGTAGCTGGTGC; human monocyte colony-stimulating factor receptor (fc), (F1; nucleotides 654 to 674, sense) CGAGGACCAACGACGACTACT and (F2; nucleotides 1003 to 1023, sense) GTGTTGTGTGTTGGAAAGAC; and human β-actin, (G1; nucleotides 1 to 21, sense; Clontech) ATGGATGATGATATCGCCGCG and (G2; nucleotides 1908 to 1940, sense; Clontech) CTAGAGCCATTTCCGGTTCGAGG.

**RT-PCR.** First-strand cDNAs were synthesized from the purified mRNAs using a first-strand cDNA synthesis kit (Pharmacia). Briefly, 1.0 μg of RNA in 11 μL of reaction buffer (muriate reverse transcriptase, 500 μg/mL yeast RNA guard, BSA, dATP, dCTP, dGTP, and dITP), 1 μL of 200 mmol/L dithiothreitol (DTT), and 0.2 μg of random primer were mixed in a final volume of 33 μL and incubated at 37°C for 1 hour. One microliter of the double-stranded products from human mast cells and the U-937 cell line was added to the reaction mixture and incubated at 15°C in a water bath. Medium and serum were then removed by aspiration and the radioactivity of the cell sediment was counted in a gamma counter. Nonspecific binding of radiolabeled rHL-3 was quantified in the presence of an at least 200-fold excess of unlabeled rHL-3.

**RESULTS**

Development of human mast cells by SCF and IL-6. The growth pattern of cord blood mononuclear cells in suspension cultures in the presence of SCF and IL-6 is shown in Table 1. The proportion of tryptase-positive cells in the cultures was in agreement with the proportion of mast cells identified by May-Grunwald-Giemsa staining. During the first 4 weeks, large numbers of differentiated cells, including neutrophils, macrophages, monocytes, and basophils, as well as mast cells, were generated. Beyond 8 weeks, the purity of mast cells reached almost 100%, and then the viability was constantly more than 95%. Apoptotic cells were seldom or never observed in the cultures under phase-contrast microscopy. The proportion of chymase-positive cells recovered from the cultures varied depending on the cord blood specimens used (15% to 85% in the period of 16 weeks).

**Apoposis of cultured human mast cells after withdrawal of SCF and IL-6.** We initially determined the time course of viability of cultured human mast cells after withdrawal of rhSCF and rHL-3.  Human mast cells cultured for more than 15 weeks were used for experiments. In the absence of SCF and IL-6, the viability of the cells maintained in serum-containing medium declined rapidly between day 2 and day 6 (Fig 1). In contrast, the viability was perfectly maintained for 6 days in the presence of 100 ng/mL SCF or both 100 ng/mL SCF and IL-6. The viability of the cells in the presence of 100 ng/mL IL-6 alone gradually declined but was higher than that cultured in the absence of any cytokine.

A large number of mast cells cultured in serum-containing medium in the absence of any cytokine after day 4 were smaller than normal and had a wrinkled appearance under phase-contrast microscopy. To determine whether such cell death was caused by apoptosis, we observed the process of cell death morphologically by light and electron microscopy and examined in situ DNA fragmentation of the cells. As shown in Fig 2A through D, light microscopical and ultrastructural analysis showed that a typical dying mast cell became pyknotic with a wrinkled membrane and condensation and clumping of chromatin after 2 days. Furthermore, only the nuclei of dying mast cells in the absence of SCF and IL-6 were stained reddish brown (Fig 2E and F), with only the nuclei of dying mast cells in the absence of SCF and IL-6, the viability of the cells maintained in serum-containing medium declined rapidly between day 2 and day 6 (Fig 1). In contrast, the viability was perfectly maintained for 6 days in the presence of 100 ng/mL SCF or both 100 ng/mL SCF and IL-6. The viability of the cells in the presence of 100 ng/mL IL-6 alone gradually declined but was higher than that cultured in the absence of any cytokine.

Survival-promoting activities of SCF, IL-3, IL-4, IL-5, and IL-6 on cultured human mast cells.** We examined the effects of the 12 cytokines SCF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-11, TNF-α, TGF-β1, and NGF on the survival of cultured human mast cells in serum-containing medium. Almost 100% of the cells remained viable in the presence of 190 ng/mL SCF, and the addition of 100 ng/mL...
Fig 2. Morphologic changes and in situ detection of DNA fragments of cultured mast cells in serum-containing suspension culture after withdrawal of SCF and IL-6. Photomicrographs of May-Grünwald/Giemsa-stained mast cells were taken after 48 hours in serum-containing suspension culture in the presence of 100 ng/mL of both SCF and IL-6 (A) or in the absence of cytokines (arrows indicate 2 representative cells of apoptosis). (B) Typical ultrastructure and a photomicrograph of in situ detection of DNA fragments corresponding to (A) are shown in (C) and (E) and those corresponding to (B) are shown in (D) and (F). (A, B, E, and F) original magnification × 100; (C and D) bar = 1 μm.
IL-3, IL-4, IL-5, or IL-6 to mast cell cultures resulted in a doubling of viability on day 4 in comparison with medium alone, whereas 100 ng/mL IL-2, IL-9, IL-10, IL-11, TNF-α, TGF-β1, and NGF showed no significant effect (Fig 3). During the experiment, total cell numbers (viable and dead cells) did not increase and the cells were shown not to proliferate by oxidation-reduction indicator assay (AlamarBlue Assay; BioSource International, Camarillo, CA; data not shown). Remaining viable cells were well-rounded, exhibited a bright appearance under phase-contrast microscopy, and were all stained immunohistologically for tryptase (data not shown). Viability of the cells in the presence of 100 ng/mL IL-3, IL-4, IL-5, or IL-6 gradually declined between day 4 and day 6 and did not improve even when the cytokines were added back to the cultures at 100 ng/mL on day 4 (data not shown).

To assess the effect of SCF, IL-3, IL-4, IL-5, and IL-6 on survival of mast cells in detail, we examined the dose-response of the activity in the presence or absence of serum. BSA-coated 96-well plates were used in this experiment because mast cells were too adherent to conventional plastic plates to be collected easily in the absence of serum. As shown in Fig 4A and B, the survival-promoting activity of IL-3, IL-4, IL-5, and IL-6 was less strong than that of SCF at 100 ng/mL, but IL-3, IL-4, and IL-6 remained effective at lower concentrations than SCF. The dose-reaction curve of IL-5 was sharp and similar to that of SCF. These cytokines showed survival-promoting activity even under serum-free conditions. Twenty percent FCS was shown to have a slight favorable effect on the survival of mast cells by itself and also showed synergistic activity with some other cytokines.

The effects of cell-density on survival-promoting activity of 100 ng/mL SCF, IL-3, IL-4, IL-5, and IL-6 were examined by preparing three cultures of different densities, ie, $1 \times 10^4$, $2 \times 10^4$ and $4 \times 10^4$ cells/200 μL/well. After washing with FCS-containing medium, mast cells were maintained at dif-
Effects of 12 human cytokines on the survival of mast cells in serum-containing suspension cultures on day 4. After washing with serum-containing medium containing no cytokine, $2 \times 10^5$ mast cells were maintained in medium containing 20% FCS but no cytokines (1), 100 ng/mL SCF (2), 100 ng/mL IL-2 (3), 100 ng/mL IL-3 (4), 100 ng/mL IL-4 (5), 100 ng/mL IL-5 (6), 100 ng/mL IL-6 (7), 100 ng/mL IL-9 (8), 100 ng/mL IL-10 (9), 100 ng/mL IL-11 (10), 100 ng/mL TNF-α (11), 100 ng/mL TGF-β1 (12), or 100 ng/mL NGF (13) for up to 4 days. Viability of the cells was determined on day 4. The data are the means ± SD of three separate cultures (experiments of each separate culture were performed in triplicate). Data were analyzed by the two-tailed Student's t-test. ***P < .001 compared with control cultures containing no cytokines.

Effects of IL-3, IL-4, IL-5, and IL-6 in regulation of IgE-mediated histamine release from cultured human mast cells. To test the possibility that IL-3, IL-4, IL-5, and IL-6 as well as SCF act also to enhance IgE-mediated histamine release, mast cells were sensitized for 24 hours with human IgE under FCS-free conditions with or without SCF, IL-3, IL-4, IL-5, or IL-6 at 100 ng/mL, and the sensitized cells were challenged with anti-IgE antibody. As shown in Fig 5A, SCF, IL-4, IL-5, and IL-6 enhanced IgE-mediated histamine release significantly at 100 ng/mL, whereas IL-3 showed no significant effect in comparison with cytokine-untreated control (n = 4). As shown in Fig 5B, the effects of the cytokines on histamine release were dose-dependent, with maximum effects observed with 100 ng/mL of the cytokines. The control cells spontaneously released less than 5% histamine without challenge, and any cytokine did not induce histamine release by itself (data not shown).

Expression of IL-3 R, IL-4 R, IL-5 R, and IL-6 R in cultured human mast cells. To support the hypothesis that cultured human mast cells express IL-3 R, IL-4 R, IL-5 R, and IL-6 R as well as c-kit, mRNA was prepared from whole mast cells cultured in the presence of SCF and IL-6, and segments of α-chains of IL-3 R, IL-4 R, IL-5 R, and IL-6 R were amplified by PCR. Message of the expected size was detected for IL-3 R, IL-4 R, IL-5 R, and IL-6 R, whereas those for G-CSF R and c-fms were not detected (Fig 6).

We examined the expression of IL-3 R on cultured mast cells by binding assay using $^{125}$I-radiolabeled IL-3, because different densities in FCS-containing medium in the presence or absence of the cytokines. The ability of the cytokines to promote the survival of the cells over 4 days did not depend on the density of the cells in the cultures (data not shown).

Fig 3. Effects of 12 human cytokines on the survival of mast cells in serum-containing suspension cultures on day 4. After washing with serum-containing medium containing no cytokine, $2 \times 10^5$ mast cells were replated into BSA-coated 96-well plates and maintained in serum-containing (A) or serum-free (B) medium containing SCF (○), IL-3 (●), IL-4 (□), IL-5 (■), or IL-6 (△) of several concentrations for up to 4 days. Viability of the cells was determined on day 4. The data are the means ± SD of triplicate cultures. The results presented are representative of three independent experiments.

Fig 4. Dose-response of the survival-promoting activity of SCF, IL-3, IL-4, IL-5, and IL-6. After washing with serum-free medium containing no cytokines, $2 \times 10^5$ mast cells were replated into BSA-coated 96-well plates and maintained in serum-containing (A) or serum-free (B) medium containing SCF (○), IL-3 (●), IL-4 (□), IL-5 (■), or IL-6 (△) of several concentrations for up to 4 days. Viability of the cells was determined on day 4. The data are the means ± SD of triplicate cultures. The results presented are representative of three independent experiments.
IL-3, -4, -5, AND -6 PROMOTE MAST CELL SURVIVAL

Fig 5. Effects of SCF, IL-3, IL-4, IL-5, and IL-6 on IgE-mediated histamine release from mast cells. (A) After washing, the cells were incubated with 10 ng/mL human IgE overnight at 37°C under serum-free conditions in the presence of no cytokines (a), 100 ng/mL SCF (b), 100 ng/mL IL-3 (c), 100 ng/mL IL-4 (d), 100 ng/mL IL-5 (e), or 100 ng/mL IL-6 (f). The cells were then washed and challenged with 4 µg/mL anti-IgE antibody. The data are the means ± SD of four separate experiments. Data were analyzed by the two-tailed Student’s t-test. **P < .01 and ***P < .001 compared with control cultures containing no cytokines. (B) The cells were similarly sensitized with IgE in the absence or presence of SCF (○), IL-3 (■), IL-4 (□), IL-5 (▲), or IL-6 (△) of several concentrations (1, 10, and 100 ng/mL) and challenged with anti-IgE antibody. The data are the means of duplicate experiments. The results presented are representative of three independent experiments.

Valent et al.27 reported that they detected no IL-3–binding sites on either human lung mast cells or a human mast cell line (HMC-1).28 The optimal concentration of 125I-radiolabeled IL-3 was determined in a preliminary experiment using TF-1 cells (data not shown). When TF-1 cells were incubated with 125I-radiolabeled IL-3 at the optimal concentration, the total and nonspecific binding rates were 9,185 and 3,260 cpm/5 × 10^6 cells, respectively (the data are means of duplicate experiments). Mast cells were incubated with 125I-radiolabeled IL-3, and total and nonspecific binding rates were quantified in the presence or absence of nora radiolabeled competitor. The nonspecific binding rate (3,686 ± 430 cpm/5 × 10^6 cells) was apparently less than the total binding rate (6,582 ± 340 cpm/5 × 10^6 cells), as shown in Fig 7. The results of the binding assay indicated that human mast cells express IL-3 R on their surfaces.

DISCUSSION

SCF is known to be a critical factor for the development of human mast cells and to enhance IgE-mediated degranulation of these cells.7,29,30 In this report, we confirmed that SCF is also a strong survival factor for human mast cells, as predicted. To exclude the effects of other-lineage cells, we used pure cultured human mast cells developed from cord blood mononuclear cells in the presence of SCF and IL-6. After withdrawal of SCF and IL-6, mast cells died rapidly, showing several changes typical of apoptosis. The results of the present study suggest that SCF is an important mediator in the regulation of apoptosis of human mast cells. SCF promoted the survival of mast cells in vitro sharply in a dose-dependent manner, and this may also be representative of the mechanism of regulation of the size of mast cell populations in vivo. Possibly, the long-term survival of human mast cells in vivo may be maintained by cell-bound SCF, which is produced by fibroblasts or stroma cells. As expected from the results of previous reports,9,31 these mast cells were shown to express c-kit on their surfaces by flow cytometric analysis (data not shown).

According to a previous report, no cytokine except SCF showed a remarkable effect on the development of human mast cells from their progenitors.7 IL-4 was shown to down-regulate the expression of c-kit on the HMC-1 cell line and human mast cells from fetal liver22,33 and to inhibit certain aspects of development of human mast cells in long-term culture. However, we found that IL-3, IL-4, IL-5, and IL-6 promoted the survival of human mast cells in vitro in both serum-containing and serum-free medium. FCS showed a slight favorable effect on the survival of human mast cells by itself and acted synergistically with some cytokines. This effect was likely caused by bovine SCF and various nutrients present within serum. The activity of 20% FCS is supposed to be equivalent to that of approximately 3 ng/mL SCF on the basis of the work of Shiohara et al.34 IL-3, IL-4, IL-5, and IL-6 likely promote the survival of mast cells directly without involvement of any autocrine factors, because the survival-promoting activity of these factors did not depend on the density of mast cells. Some cytokines, such as SCF,35,37 IL-3,38 and NGF,39 have been reported to promote the survival of rodent mast cells in vitro by suppressing apoptosis. In the present study, NGF showed no effect on the survival of human mast cells.

Preincubation of mast cells with some of these cytokines, such as SCF, IL-4, IL-5, and IL-6, for 24 hours was shown...
to upregulate IgE-mediated histamine release significantly. Some investigators previously reported that a brief incubation (10 to 45 minutes) of human lung, skin, and cultured mast cells with SCF enhanced IgE-mediated histamine release more than 2 times, whereas that with IL-3, IL-4, or IL-6 showed no significant effect (data not shown). The mechanism of enhancement of IgE-mediated histamine release induced by preincubation with the cytokines for 24 hours remains to be clarified. The cytokines might affect the signal transduction pathway of IgE-mediated degranulation or possibly enhance the expression of Fe ε RI on the cells during sensitization.

It is of great interest that IL-3, IL-4, IL-5, and IL-6 are all produced by Th2 lymphocytes (IL-3 is produced by both Th1 and Th2 lymphocytes), which play an important role in allergic inflammatory responses by enhancing the production of IgE antibodies and promoting the development of eosinophils and basophils. The effects of IL-3, IL-4, IL-5, and IL-6 on human mast cells described above agree well with the proposal that Th2-type cytokines promote allergic reactions by regulating various immune cells. At local sites in allergic inflammation, IL-3, IL-4, IL-5, and IL-6 are likely present at high concentrations, and so these cytokines might also influence the survival and function of human mast cells in vivo.

In addition, these cultured human mast cells were shown to express mRNAs of IL-3 R, IL-4 R, IL-5 R, and IL-6 R α-chains and to have specific binding sites for IL-3. That α-chains of IL-3 R, IL-4 R, IL-5 R, and IL-6 R mRNAs were detected in the mast cells by PCR supports the hypothesis that these mast cells have specific binding sites for these cytokines. Transcripts of the expected sizes for either G-CSF R or c-fms, which are known to be expressed in granulocytes and macrophages, respectively, were not detected. This indicates that the cultured mast cells used for this experiment were pure and that the mRNAs of IL-3 R, IL-4 R, IL-5 R, and IL-6 R detected by PCR were derived from mast cells.

Our results in the binding assay of IL-3 R were contrary to those reported by Valent et al. The reason for the discrepancy is not clear, but there are two possible explanations. The first possibility is that there are subtypes of human mast cells and that these cultured mast cells express IL-3 binding sites, whereas there are no IL-3 binding sites on either natural human lung mast cells or the HMC-1 cell line. The second possibility is that human mast cells express extremely low numbers of IL-3 binding sites that are not easily detectable by binding assay. Indeed, we used a larger number of cells and higher radioactivity of labeled IL-3 than did Valent et al. Kirshenbaum et al. previously showed that IL-3 alone and in combination with SCF promoted the development of human mast cells from CD34+ bone marrow cells and that the mast cells developed in the presence of IL-3 alone were immature. Durand et al. also reported recently that human mast cells were generated in serum-free cultures of CD34+...
cord blood cells in the presence of SCF and IL-3. These mast cells remained immature in morphology and died in the absence of either SCF or IL-3. It is possible that IL-3 R might be highly expressed on immature human mast cells and their progenitors and that the expression of IL-3 R might decrease during the maturation of human mast cells.

In conclusion, we investigated the effects of IL-3, IL-4, IL-5, and IL-6 as well as SCF in vitro on the survival of human mast cells developed from cord blood mononuclear cells in the presence of SCF and IL-6. These cytokines, with the exception of IL-3, enhanced IgE-mediated histamine release. In addition, the findings of RT-PCR and binding assays suggested that these human mast cells express specific receptors for each cytokine. These cytokines, which are mainly produced by Th2 lymphocytes, might regulate the functions of human mast cells in allergic reactions.

ACKNOWLEDGMENT

The authors are grateful to Dr Kimishige Ishizaka for support in commencement of this study of human mast cells, Dr Akimitsu Imai and his coworkers for supplying cord blood, and Keiko Hakoda, Mie Toriyama, Toshiko Aoki, Mayumi Kawagishi, and Keiko Kobayashi for excellent technical assistance.

REFERENCES

1. Ishizaka T, Ishizaka K: Activation of mast cells for mediator release through IgE receptors. Prog Allergy 34:188, 1984


18. Irani AA, Schecter NM, Craig SS, DeBlois G, Schwartz LB:
Two types of human mast cells that have distinct neutral protease compositions. Proc Natl Acad Sci USA 83:4464, 1986


Effects of T-helper 2-type cytokines, interleukin-3 (IL-3), IL-4, IL-5, and IL-6 on the survival of cultured human mast cells

M Yanagida, H Fukamachi, K Ohgami, T Kuwaki, H Ishii, H Uzumaki, K Amano, T Tokiwa, H Mitsui and H Saito

Updated information and services can be found at:
http://www.bloodjournal.org/content/86/10/3705.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml