0026-895X/93/030365-07$03.00/0

MOLECULAR PHARMACOLOGY, 43:365-371

Histamine-Induced Production of Interleukin-1α from Murine Bone Marrow Stromal Cells and Its Inhibition by H_2 Blockers

KENJI TASAKA, MITSUNOBU MIO, MASAMITSU SHIMAZAWA, and NAOKI NAKAYA

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Okayama University, Okayama, Japan 700

Received October 13, 1992; Accepted December 11, 1992

SUMMARY

In this study, the role of histamine in interleukin-1 (IL-1) formation in murine bone marrow stromal cells was investigated *in vitro*. It was found that histamine and 4-methylhistamine increased the number of granulocyte colony-forming units in murine bone marrow cells. A similar effect was elicited by dibutyryl-cAMP and theophylline. When histamine and H_2 agonists, such as 4-methylhistamine and dimaprit, were added to the culture medium containing murine bone marrow stromal cells, thymocyte comitogenic activity detected in the medium increased significantly. However, no such effect was observed in the case of 2-methylhistamine, an H_1 agonist. Histamine-induced production of thymocyte comitogenic activity in bone marrow stromal cells was inhibited by some H_2 antagonists, such as cimetidine, ranitidine, and famotidine, but not by the H_1 antagonist pyrilamine. Histamine was also effective in inducing the colony-promoting activity in murine bone marrow stromal cells. This was also inhibited by H_2 antagonists such as cimetidine, ranitidine, and famotidine. Histamine elicited an increase in cAMP content in bone marrow stromal cells. From gel filtration analysis, the molecular mass of the active substance produced by bone marrow stromal cells in response to histamine was in the range of 15 to 20 kDa. By means of Western blotting analysis, it was found that production of pro-IL-1α in the bone marrow stromal cells was induced by histamine. The production of pro-IL-1α in bone marrow stromal cells stimulated by histamine was inhibited not only by H_2 antagonists, such as cimetidine, ranitidine, and famotidine, but also by the protein kinase A antagonist KT-5720. These results indicate that histamine stimulates the production of IL-1α in bone marrow stromal cells and that this results in the proliferation and differentiation of neutrophil progenitor cells.

It has been indicated that various H_2 antagonists, such as cimetidine and ranitidine, often cause leukocytopenia (a decrease in peripheral leukocyte counts) or agranulocytosis (1, 2). In a few cases, incidences of thrombocytopenia and pancytopenia have also been reported (3, 4). On the other hand, it has been reported that histamine at submicromolar concentrations induces differentiation of neutrophil progenitor cells via H_2 receptor stimulation in both murine bone marrow cells and HL-60 cells (5, 6). From these findings, it is possible to assume that histamine at physiological concentrations may induce the differentiation of neutrophil progenitors. Furthermore, various cytokines are known to stimulate the proliferation and the differentiation of bone marrow stem cells. As one of such cytokines, IL-1 is known to exert a stimulative effect on the hematopoietic systems through an increase in the release of CSFs from stromal cells (7). It has also been reported that IL-1 is capable of stimulating the differentiation of pluripotent stem cells in cooperation with CSFs (8). Using a long term culture of murine bone marrow cells, Dexter and Testa (9) observed that the bone marrow stromal cells play some important roles in the proliferation and differentiation of hematopoietic cells in culture. It has also been suggested that the proliferation and differentiation of the progenitor cells are regulated not only by the active substances released from the stromal cells but also by the cell-cell interactions between stromal cells and progenitor cells (9). It has been reported that IL-1 synthesized in the bone marrow stromal cells may stimulate the bone marrow stromal cells to produce CSFs (7). However, little is known about the effect of histamine on the bone marrow stromal cells and the production of IL-1. In this study, the effects of histamine and its antagonists on the bone marrow stromal cells were investigated.

Materials and Methods

Long term culture of bone marrow stromal cells. Bone marrow stromal cells were cultured according to the method described by Dexter and Testa (9). In brief, male BALB/c mice (4–6 weeks of age) were sacrificed by cervical dislocation and the bone marrow cells were flushed out of their femurs and tibias. The cells (1×10^6 cells/ml) were suspended in RPMI 1640 medium supplemented with 20% horse serum and were cultured for 3 weeks at 37° in a 5% CO_2 incubator with

ABBREVIATIONS: IL-1, interleukin-1; CFU-c, colony-forming unit in culture; db-cAMP, dibutyryl-cAMP; CSF, colony-stimulating factor; FCS, fetal calf serum; PHM, phytohemagglutinin-M; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; BSA, bovine serum albumin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; G-CFU-c, granulocyte colony-forming unit in culture.

humidified air, by using 24-well culture plates (1 ml/well) or 35-mm-diameter culture dishes (3 ml/dish); half of the medium in each well was replaced with newly prepared medium once each week. Proliferating adherent cells were washed twice with RPMI 1640 medium to remove nonadherent cells, and the remaining cells were used as bone marrow stromal cells. Usually, 2×10^5 stromal cells were obtained from 10^6 bone marrow cells. Before any of the following experiments were started, the cells were washed twice with RPMI 1640 and nonadherent cells were removed. Thereafter, the cells were preincubated for 30 min at 37°, in RPMI 1640 medium containing 0.05% BSA, before the effect of the test compounds was studied.

CFU-c assay. CUF-c assay was carried out according to the method of Worton *et al.* (10) with slight modifications. Murine bone marrow cells (1.6×10^5 cells/ml/well of a 24-well culture plate), obtained as described above, were incubated for 24 hr with various concentrations of test compounds dissolved in RPMI 1640 medium supplemented with 10% FCS, at 37° in a 5% CO_2 incubator with humidified air. Thereafter, nonadherent cells (0.6×10^5 cells/ml) were suspended in 1 ml of CFU-c assay medium (RPMI 1640 medium supplemented with 0.6% methylcellulose, 20% horse serum, and 5 ng/ml recombinant human granulocyte CSF) and cultured for 7 days at 37° in a 5% CO_2 incubator with humidifed air. After that, colonies consisting of >50 cells each were counted microscopically. The activity stimulating colony formation was regarded as CFU-c stimulation activity. The activity was expressed as percentage of control, taking the nonstimulated control as 100%.

Thymocyte comitogenic activity. Thymocyte comitogenic activity, which is known as the most representative IL-1 activity, was determined by the method of Rosenwasser and Dinarello (11). Thymocytes obtained aseptically from C3H/HeJ mice (4 weeks of age) were suspended at a concentration of 1×10^7 cells/ml in RPMI 1640 medium supplemented with 0.5% FCS. The cell suspension (100 μl), 50 μl of 50 μg/ml PHM, and 50 μl of the samples were added to each well of a 96-well microculture plate and cultured at 37° for 48 hr. In this experiment, the supernatant obtained from the culture media of stromal cells was used as the sample; stromal cells (6×10^5 cells/ml) were incubated in the presence of test compounds at 37° for 24 hr and then the low molecular substances in the supernatant, including histamine, were removed by 6-hr dialysis using Spectrapore membranes (molecular weight cut-off, 10,000; Spectrum Medical Industries), and the remaining dialysate was used as the sample. Subsequently, 10 μl of [*methyl*-^{3}H]thymidine (20 μCi/ml) were added to each well and further incubation was carried out at 37° for 24 hr. Thereafter, 20 μl of 50 mM thymidine were added to terminate the reaction. The cells were filtered through a Whatman GF/C filter and washed twice with 5 ml of 7% trichloroacetic acid. The radioactivity that remained on the filter was measured by using a liquid scintillation counter. The net radioactivity corresponding to the thymocyte comitogenic activity of the sample was determined as the difference between the total radioactivity (PHM plus sample) and the radioactivity derived from PHM alone. One unit was defined as an activity showing half-maximal incorporation of [^{3}H] thymidine elicited by recombinant murine IL-1α.

Colony-promoting activity assay. Murine bone marrow stromal cells (6×10^5 cells/ml/well of a 24-well culture plate) were incubated at 37° for 24 hr in the presence and in the absence of test compounds, and 100 μl of the supernatant of the culture medium were added to 0.9 ml of colony-promoting activity assay medium (RPMI 1640 medium supplemented with 0.6% methylcellulose and 20% horse serum). Nonadherent murine bone marrow cells (0.8×10^5 cells/ml/well of a 24-well culture plate) were suspended in this medium and cultured for 7 days at 37° in a 5% CO_2 incubator with humidifed air. After that, colonies consisting of >50 cells each were counted microscopically. The activity that increased colony formation was regarded as colony-promoting activity. When the supernatant of nontreated culture medium was used, the activity was taken as the control. The colony-promoting activity was expressed as percentage of the control, with the control value taken as 100%.

Determination of cAMP content in murine bone marrow stromal cells. Murine bone marrow stromal cells (1.8×10^6 cells/3 ml/35-mm-diameter culture dish) were incubated with 1 μM histamine for various incubation periods at 37°. Thereafter, the medium was discarded and the cells were disrupted by an addition of 2 ml of 5% trichloroacetic acid (containing 1 mM theophylline). After centrifugation at $2000 \times g$ for 20 min at 4°, the supernatant was extracted four times with water-saturated ether. After that, the water layer was collected, dried *in vacuo*, and stored at −20°. The sample was dissolved in 100 μl of distilled water and cAMP content was determined by means of a cAMP radioimmunoassay kit (Yamasa, Tokyo, Japan).

Gel filtration. After the incubation of stromal cells (6×10^5 cells/ml/well of a 24-well plastic plate) with test compounds at 37° for 24 hr, 200 μl of the supernatant were applied onto a column (0.6 × 20 mm) packed with Sephacryl S-200, which had been equilibrated with 0.15 M NaCl, 5 mM HEPES, pH 7.4. Proteins were eluted with the same solution at a flow rate of 0.05 ml/min and fractionated; 200 μl of each fraction were collected. Ferritin (M_r 460,000), aldolase (M_r 150,000–160,000), BSA (M_r 65,000), and cytochrome *c* (M_r 12,400) were used as molecular weight markers. After that, colony-promoting activity in each fraction was determined according to the method described above.

SDS-PAGE and Western blotting analysis. Murine bone marrow stromal cells (1.8×10^6 cells/3 ml/dish) were incubated with test compounds at 37° for 24 hr, and the cells were washed twice with ice-cold PBS (in mM: NaCl, 137; KCl, 2.68; KH_2PO_4, 1.47; Na_2HPO_4, 9.27; pH 7.2). The cells were suspended in SDS-PAGE sample solution (1% SDS, 20% glycerol, 1% 2-mercaptoethanol, 0.002% bromphenol blue, 10 mM Tris·HCl, pH 6.8), and the suspension was placed in boiling water for 5 min to accomplish protein denaturation. SDS-PAGE was carried out according to the method of Laemmli (12), using a slab gel (12 × 10 cm, 1-mm thickness) containing 10–20% polyacrylamide. In each run, the proteins obtained from 10^6 stromal cells were applied. After electrophoresis, the gel was washed for 30 min with a transfer solution containing 20 mM Tris, 50 mM glycine, and 20% methanol. The proteins in the gel were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Richmond, CA) by using an electroblotting apparatus (Sartorius) at a constant current of 250 mA for 90 min. Thereafter, the membrane was washed overnight at 4° with blocking solution (PBS containing 3% BSA and 3% skim milk) and then incubated at 37° for 2 hr with rabbit anti-mouse IL-1α serum diluted with blocking solution (1/200). Thereafter, the membrane was washed with PBS supplemented with 0.1% Tween 20 (PBS-Tween) and was incubated for 3 hr at room temperature with sheep anti-rabbit IgG conjugated to biotin (1/400). The membrane was washed with PBS-Tween, and biotinylated antibody was further labeled with avidin and biotinylated peroxidase, by using a VECSTAIN Elite ABC kit (Vector Laboratories Inc., Burlingame, CA), for 30 min at room temperature. After that, the membrane was washed with PBS-Tween and incubated in a substrate solution (PBS containing 0.013% diaminobenzidine, 0.004% H_2O_2, and 0.037% $NiCl_2$) for visualization of the labeled protein bands.

Chemicals. The compounds used were as follows (sources are indicated in parentheses): RPMI 1640 medium (Nissui, Tokyo, Japan), horse serum (Hazelton, Lenaxa, KS), BSA (Sigma Chemical Co., St. Louis, MO), methylcellulose (Sigma), recombinant human granulocyte CSF (kindly provided by Kyowa Hakko, Tokyo, Japan), histamine dihydrochloride (Wako Pure Chemicals, Osaka, Japan), 4-methylhistamine (SmithKline & French, Welwyn, UK), cimetidine (SmithKline & French), ranitidine (Glaxo, Greenford, UK), famotidine (Yamanouchi, Tokyo, Japan), pyrilamine (Rhône-Poulenc, Paris, France), FCS (Hazelton), PHM (Difco Laboratories, Detroit, MI), [*methyl*-^{3}H]thymidine (2.0 Ci/mmol; Du Pont NEN, Wilmington, DE), cAMP radioimmunoassay kit (Yamasa), db-cAMP (Sigma), Sephacryl S-200 (Pharmacia LKB Biotechnology, Uppsala, Sweden), polyvinylidene difluoride membrane (Bio-Rad), recombinant murine IL-1α (kindly provided by Dr. P. T. Lomedico, Hoffmann-La Roche, Inc., Nutley, NJ), anti-

murine IL-1α rabbit serum (Genzyme, Cambridge, MA), biotinylated anti-rabbit IgG goat serum (Jackson Immunoresearch Laboratories Inc., West Grove, PA), VECSTAIN Elite ABC kit (Vector), KT-5720 (Kyowa Hakko), and HA1004 (Seikagaku Corp.). All other chemicals used were reagent grade and were purchased from commercial sources.

Statistical analysis. A one-way analysis of variance with Dunnett's test was used to determine the statistical significance.

Results

CFU-c counts in murine bone marrow cells. After murine bone marrow cells had been incubated for 24 hr at 37° in RPMI 1640 supplemented with 10% FCS containing various concentrations of histamine, the CFU-c assay was carried out. As indicated in Fig. 1, histamine increased the number of G-CFU-c in a dose-dependent fashion at concentrations equal to or higher than 10^{-7} M, without affecting the total CFU-c count. A significant effect was observed at concentrations equal to or higher than 10^{-6} M. 4-Methylhistamine, an H_2 agonist, was also effective in increasing the G-CFU-c count (Fig. 1). On the other hand, 2-methylhistamine, an H_1 agonist, did not affect either G-CFU-c or the total colony counts (data not shown). However, when either histamine or 4-methylhistamine was added directly, without pretreatment with the bone marrow cells, to the CFU-c assay medium containing nonadherent cells, the G-CFU-c count did not increase at all. These findings seem to indicate that activation of H_2 receptors of adherent stromal cells may play a crucial role in the histamine-induced G-CFU-c increase but nonadherent cells may not provide such activity.

Because it is known that H_2 receptor stimulation brings about an increase in intracellular cAMP levels, the effects of theophylline and db-cAMP on the G-CFU-c counts in murine bone marrow cells were tested. After incubation of murine bone marrow cells with either theophylline or db-cAMP for 24 hr at 37°, the G-CFU-c assay was carried out using nonadherent cells as before. As indicated in Fig. 2, each compound significantly increased the G-CFU-c counts in a concentration-dependent manner, indicating that an increase in intracellular cAMP levels may be related in some way to an increase of G-CFU-c count.

Production of thymocyte comitogenic activity in murine bone marrow stromal cells induced by histamine. Because it was thought that adherent stromal cells may be involved in increasing the G-CFU-c counts in association with histamine pretreatment, murine stromal cells were cultured at 37° for 24 hr in the presence of histamine and related compounds, and thymocyte comitogenic activity in the supernatant of the culture medium was determined. As indicated in Table 1, the thymocyte comitogenic activity in the culture medium of murine stromal cells significantly increased after treatment with histamine at concentrations equal to or higher than 0.01 μM, in a concentration-dependent manner. Although the H_2 agonists 4-methylhistamine and dimaprit also increased the thymocyte comitogenic activity in the culture medium of stromal cells, the H_1 agonist 2-methylhistamine did not induce such activity.

Table 2 shows the effect of histamine antagonists on the histamine-induced production of thymocyte comitogenic activity in murine stromal cells. In this case, histamine antagonists were added simultaneously with histamine to the culture medium of stromal cells. As shown in Table 2, histamine (1 μM)-induced production of thymocyte comitogenic activity was significantly inhibited by H_2 antagonists, such as famotidine, cimetidine, and ranitidine, at concentrations equal to or higher than 0.1 μM, 1 μM, and 1 μM, respectively. However, pyrilamine, an H_1 antagonist, did not affect the histamine-induced production of thymocyte comitogenic activity even at a concentration of 10 μM.

From these observations, it was suggested that histamine may enhance the production of a substance possessing thymocyte comitogenic activity from the bone marrow stromal cells though H_2 receptor stimulation.

Histamine-induced production of colony-promoting activity. To confirm that the substance induced by histamine in the culture medium of stromal cells actually increases the colony formation in bone marrow cells, the colony-promoting activity in the culture medium of stromal cells treated with histamine was determined. As indicated in Table 3, histamine at 1 μM significantly increased colony-promoting activity in the culture medium of stromal cells. Histamine-induced production of colony-promoting activity was completely inhibited by simultaneous addition of the H_2 antagonists cimetidine, ranitidine, and famotidine (10 μM each) (Table 3).

Role of cAMP in the histamine-induced production of thymocyte comitogenic activity in murine bone marrow stromal cells. As indicated in Fig. 3, when bone marrow stromal cells were stimulated by histamine (1 μM) cAMP contents increased rapidly, reaching a maximum (almost twice the control) 10 min after stimulation, and thereafter decreased gradually. Moreover, when stromal cells were stimulated by db-cAMP at 37° for 24 hr, thymocyte comitogenic activity in the

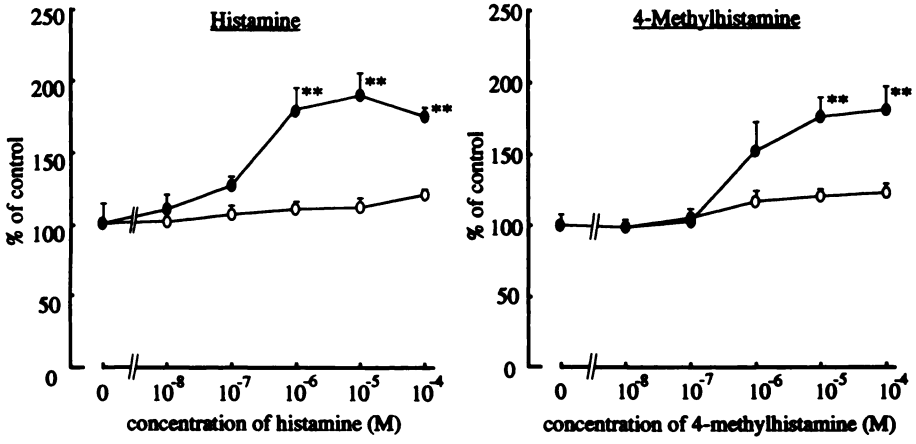

Fig. 1. Effects of histamine and 4-methylhistamine on the G-CFU-c counts in murine bone marrow cells. After treatment of murine bone marrow cells with test compounds at 37° for 24 hr, the CFU-c assay was carried out. ○, Total CFU-c count; ●, G-CFU-c count. Each *point* represents the mean ± standard error of the data obtained from five separate experiments. **, Statistical significance, in comparison with control, at $p < 0.01$.

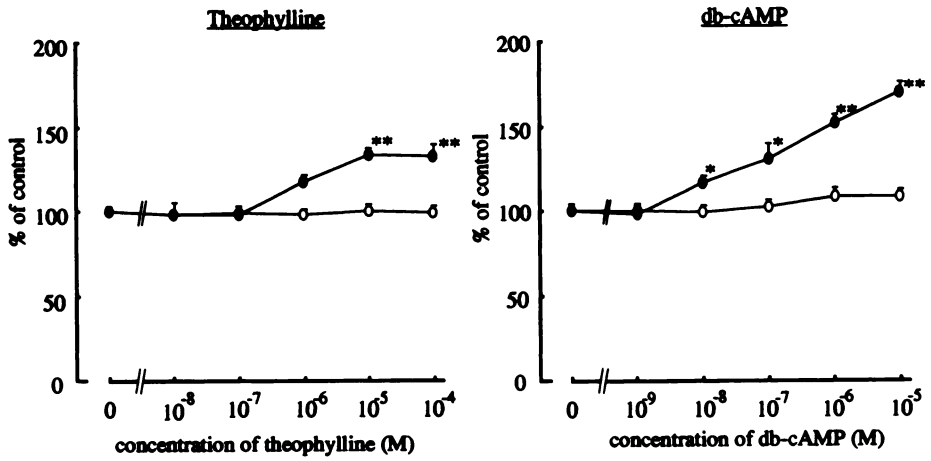

Fig. 2. Effects of theophylline and db-cAMP on the G-CFU-c counts in murine bone marrow cells. After murine bone marrow cells had been treated with test compounds at 37° for 24 hr, the CFU-c assay was carried out. ○, Total CFU-c count; ●, G-CFU-c count. Each *point* represents the mean ± standard error of the data obtained from five separate experiments. * and **, Statistical significance, in comparison with control, at $p < 0.05$ and $p < 0.01$, respectively.

TABLE 1

Effects of histamine agonists on the production of thymocyte comitogenic activity in the supernatant of the culture medium of murine bone marrow stromal cells

Murine bone marrow stromal cells were cultured in the presence of test compounds at 37° for 24 hr. After that, thymocyte comitogenic activity of the supernatant was determined. Half-maximal activity of murine IL-1α was taken as 1 unit. Each value represents the mean ± standard error of data obtained from five separate experiments.

Compounds	Concentration	Thymocyte comitogenic activity
	μM	units/ml
Control		0.390 ± 0.046
Histamine	0.01	0.767 ± 0.055[a]
	0.1	0.806 ± 0.030[b]
	1	0.884 ± 0.068[b]
	10	1.302 ± 0.195[b]
4-Methylhistamine	0.01	0.429 ± 0.039
	0.1	0.715 ± 0.065[a]
	1	0.926 ± 0.051[b]
	10	1.157 ± 0.046[b]
Dimaprit	0.1	0.507 ± 0.065
	1	0.572 ± 0.104
	10	1.482 ± 0.098[b]
2-Methylhistamine	1	0.325 ± 0.065
	10	0.312 ± 0.081

[a] Statistical significance, in comparison with control, at $p < 0.05$.
[b] $p < 0.01$.

culture medium increased significantly and dose-dependently at concentrations equal to or higher than 50 μM (Table 4). In accordance with these observations, the histamine-induced production of thymocyte comitogenic activity was significantly inhibited in the presence of KT-5720, an inhibitor of protein kinase A (13), as indicated in Table 5.

These observations seem to indicate that the effect of histamine on bone marrow stromal cells may be exerted through H_2 receptor stimulation and that the resulting increment of intracellular cAMP levels and the subsequent activation of protein kinase A are essential for the production of thymocyte comitogenic activity.

Gel filtration analysis. After incubation of the bone marrow stromal cells with histamine both in the presence and in the absence of H_2 antagonists, the culture medium was fractionated by means of gel filtration and colony-promoting activity in each fraction was determined. Fig. 4 presents the gel filtration profiles of colony-promoting activity produced by murine stromal cells after histamine treatment. As indicated in this figure, the colony-promoting activity determined in the

TABLE 2

Effects of histamine antagonists on the histamine-induced production of thymocyte comitogenic activity in the culture medium of murine bone marrow stromal cells

Murine bone marrow stomal cells were cultured in the presence of test compounds at 37° for 24 hr. After that, thymocyte comitogenic activity of the supernatant was determined. Half-maximal activity of murine IL-1α was taken as 1 unit. Each value represents the mean ± standard error of data obtained from five separate experiments.

Drugs	Concentration	Thymocyte comitogenic activity	
		Drug alone	+ Histamine (1 μM)
	μM	units/ml	
Control		0.438 ± 0.021	0.863 ± 0.087[a]
Cimetidine	0.1	0.427 ± 0.032	0.707 ± 0.076
	1	0.435 ± 0.015	0.551 ± 0.051[b]
	10	0.434 ± 0.022	0.470 ± 0.061[c]
Ranitidine	0.1	0.441 ± 0.034	0.769 ± 0.073
	1	0.429 ± 0.019	0.530 ± 0.126[b]
	10	0.431 ± 0.042	0.432 ± 0.088[c]
Famotidine	0.01	0.440 ± 0.038	0.697 ± 0.055
	0.1	0.436 ± 0.027	0.383 ± 0.073[c]
Pyrilamine	10	0.433 ± 0.045	0.860 ± 0.082

[a] Statistical significance, in comparison with blank, at $p < 0.01$.
[b, c] Statistical significance in comparison with histamine (1 μM)-treated group at $p < 0.05$ and $p < 0.01$, respectively.

TABLE 3

Histamine-induced production of colony-promoting activity in murine bone marrow stromal cells and its inhibition by H_2 antagonists

Murine bone marrow stromal cells were incubated with 1 μM histamine both in the presence and in the absence of H_2 antagonists. The supernatant was added to the CFU-c assay medium and the CFU-c assay was carried out. Each value represents the mean ± standard error of data obtained from five separate experiments.

Drugs	Concentration	Colony-promoting activity	
		Drug alone	+ Histamine (1 μM)
	μM	% of control	
Control		100.0 ± 14.0	193.6 ± 24.4[a]
Cimetidine	10	89.8 ± 12.3	84.6 ± 5.9[c]
Ranitidine	10	97.9 ± 8.6	101.3 ± 14.1[b]
Famotidine	10	90.7 ± 10.6	87.2 ± 5.2[c]

[a] Statistical significance, in comparison with blank, at $p < 0.01$.
[b, c] Statistical significance, in comparison with histamine (1 μM)-treated group, at $p < 0.05$ and $p < 0.01$, respectively.

fractions corresponding to about 15–20 kDa significantly increased after treatment with 1 μM histamine. In addition, the histamine-induced increase of colony-promoting activity in these fractions was completely suppressed by the H_2 antagonists ranitidine and famotidine (10 μM each). This result may

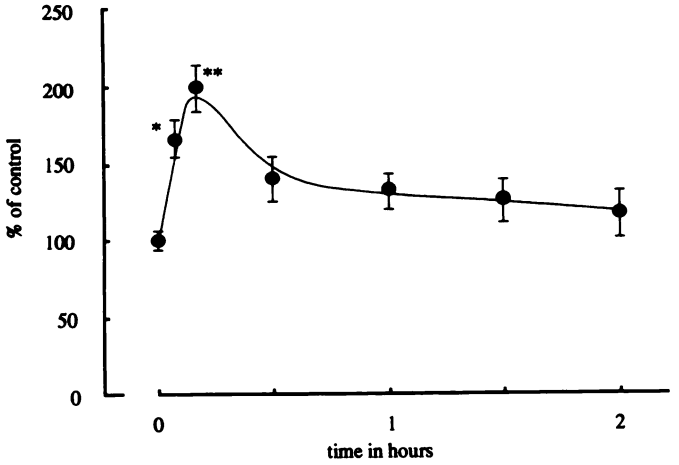

Fig. 3. Effect of histamine on the cAMP level of murine bone marrow stromal cells. Histamine (1 μM) was added to murine bone marrow stromal cells and the changes in cAMP content were determined by means of radioimmunoassay. Each *point* represents the mean ± standard error of the data obtained from four separate experiments. The level of cAMP in control cells was 6.324 ± 0.435 pmol/10^6 cells (four experiments). * and **, Statistical significance, in comparison with control, at $p < 0.05$ and $p < 0.01$, respectively.

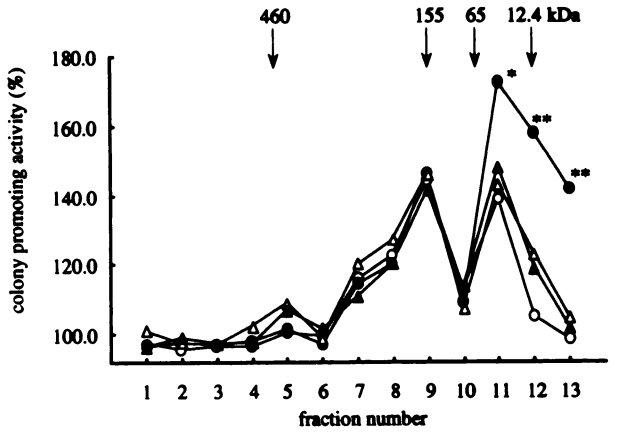

Fig. 4. Gel filtration profiles of colony-promoting activity produced by murine bone marrow stromal cells. Murine bone marrow stromal cells were incubated both in the presence and in the absence of histamine and/or H_2 antagonists at 37° for 24 hr. The supernatant was applied to Sephacryl S-200 and eluted with saline. The colony-promoting activity of each fraction was determined using murine bone marrow cells. The spontaneous increase in CFU-c counts in murine bone marrow cells was taken as 100%. ○, Control; ●, histamine (1 μM); △, histamine (1 μM) plus ranitidine (10 μM); ▲, histamine (1 μM) plus famotidine (10 μM). Each *point* represents the mean value obtained from four separate experiments. Error bars were omitted for clarity. * and **, Statistical significance, in comparison with control, at $p < 0.05$ and $p < 0.01$, respectively.

TABLE 4

Effect of db-cAMP on the production of thymocyte comitogenic activity in the culture medium of murine bone marrow stromal cells

Murine bone marrow stromal cells were cultured with db-cAMP at 37° for 24 hr. After that, thymocyte comitogenic activity of the supernatant was determined. Half-maximal activity of murine IL-1α was taken as 1 unit. Each value represents the mean ± standard error of data obtained from six separate experiments.

Concentration of db-cAMP	Thymocyte comitogenic activity
μM	units/ml
0	0.082 ± 0.041
10	0.071 ± 0.046
50	0.354 ± 0.100[a]
100	1.822 ± 0.271[b]
500	2.927 ± 0.189[b]

[a] Statistical significance, in comparison with control, at $p < 0.05$.
[b] $p < 0.01$.

TABLE 5

Effects of KT-5720 on the histamine-induced production of thymocyte comitogenic activity in the culture medium of murine bone marrow stromal cells

Murine bone marrow stromal cells were cultured in the presence of test compounds at 37° for 24 hr. After that, thymocyte comitogenic activity of the supernatant was determined. Half-maximal activity of murine IL-1α was taken as 1 unit. Each value represents the mean ± standard error of data obtained from five separate experiments.

Concentration of KT-5720	Thymocyte comitogenic activity	
	Drug alone	+Histamine (1 μM)
μM	units/ml	
0	0.419 ± 0.022	0.859 ± 0.066[a]
0.03	0.425 ± 0.034	0.824 ± 0.059
0.1	0.413 ± 0.031	0.595 ± 0.123
0.3	0.372 ± 0.075	0.356 ± 0.066[b]
1.0	0.328 ± 0.084	0.251 ± 0.024[b]

[a] Statistical significance, in comparison with blank, at $p < 0.01$.
[b] Statistical significance, in comparison with histamine (1 μM)-treated group, at $p < 0.01$.

indicate that the molecular mass of the active substance produced by histamine stimulation is 15–20 kDa.

Western blot analysis of IL-1α production in murine bone marrow cells. From the findings indicated above, the active substance produced by bone marrow stromal cells after stimulation by histamine possesses both thymocyte comitogenic activity and colony-promoting activity and its molecular mass is 15–20 kDa. Because such properties are very similar to those of IL-1, Western blot analysis was carried out using anti-IL-1α antibody. When murine bone marrow stromal cells were stimulated by histamine at concentrations equal to or higher than 10^{-8} M, production of a 32-kDa protein in a concentration-dependent manner was detected with anti-IL-1α, as shown in Fig. 5. The molecular mass of this protein seems to correspond to that of pro-IL-1α (14). This may suggest that the production of pro-IL-1α was increased by histamine stimulation. IL-1α released in the culture medium was not detected because the concentration was much lower than that detectable by means of IL-1α antibody.

Effects of H_2 antagonists and protein kinase inhibitors on the histamine-induced production of pro-IL-1α. The effect of H_2 antagonists on the histamine-induced production of pro-IL-1α in murine bone marrow stromal cells was investigated. As shown in Fig. 6, H_2 antagonists such as cimetidine, ranitidine, and famotidine inhibited the production of pro-IL-1α almost to the control level, indicating that the effect of histamine on the production of pro-IL-1α may be exerted via H_2 receptor stimulation. However, these drugs did not affect the spontaneous production of pro-IL-1α at the concentration used.

In Fig. 7, the effect of several protein kinase inhibitors on the histamine-induced production of pro-IL-1α is indicated. As shown in this figure, inhibitors of protein kinase A, such as KT-5720 and HA1004 (15), inhibited the histamine-induced production of pro-IL-1α to the control level, suggesting that protein kinase A may also play some important role in the

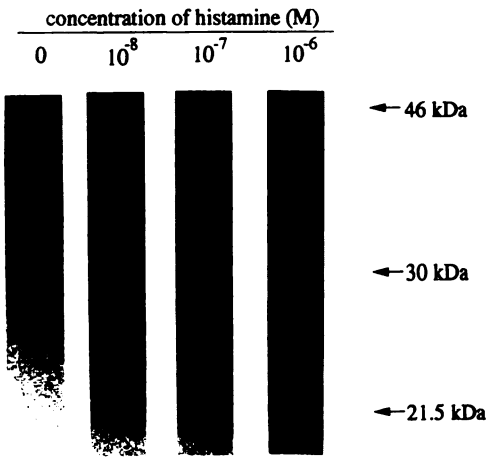

Fig. 5. Western blot analysis of intracellular IL-1α production in murine bone marrow cells cultured in the presence of histamine. Murine bone marrow stromal cells were cultured with various concentrations of histamine at 37° for 24 hr. Thereafter, the cells were harvested and intracellular IL-1α was determined by Western blot analysis using anti-IL-1α antibody. The molecular mass of the detected band corresponded to that of pro-IL-1α (32 kDa).

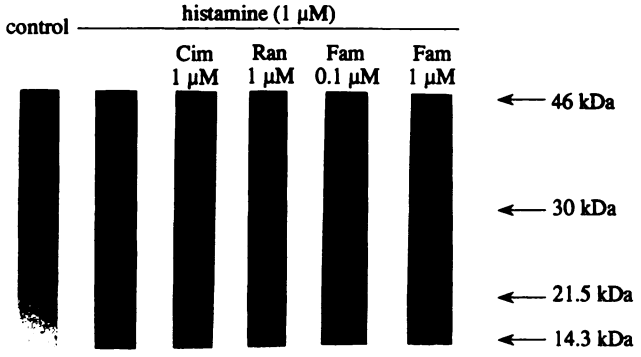

Fig. 6. Effects of H_2 antagonists on intracellular IL-1α production in murine bone marrow stromal cells cultured in the presence of histamine (1 μM). Murine bone marrow stromal cells were cultured with histamine (1 μM), both in the presence and in the absence of H_2 antagonists, at 37° for 24 hr. Thereafter, the cells were harvested and intracellular IL-1α was determined by Western blot analysis using anti-IL-1α antibody. The molecular mass of the detected band corresponded to that of pro-IL-1α (32 kDa). *Cim*, cimetidine; *Ran*, ranitidine; *Fam*, famotidine.

histamine-induced production of pro-IL-1α. These compounds did not affect the spontaneous production of pro-IL-1α at the concentrations used.

Discussion

In clinical observations, it has often been reported that one of the severe adverse effects of H_2 antagonists is a decrease in neutrophil counts in the peripheral blood (16). On the other hand, it has been indicated that histamine, in the physiological concentration range, induces differentiation of neutrophil progenitor cells, such as myeloblasts and promyelocytes, via H_2 receptors *in vitro* (5). From these observations, it has been postulated that histamine is one of the important physiological stimulants for neutrophil differentiation (16). However, it has not been clear whether histamine interacts with immature cells at the stages before CFU-c. Moreover, the effect of histamine on bone marrow stromal cells has not been reported, although it has been shown that bone marrow stromal cells produce various hematopoietic substances (7).

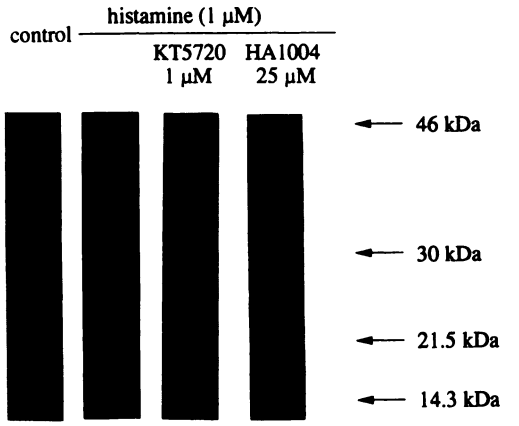

Fig. 7. Effects of protein kinase inhibitors on intracellular IL-1α production in murine bone marrow stromal cells stimulated by histamine (1 μM). Murine bone marrow stromal cells were cultured with histamine (1 μM), both in the presence and in the absence of protein kinase inhibitors, at 37° for 24 hr. Thereafter, the cells were harvested and intracellular IL-1α was determined by Western blot analysis using anti-IL-1α antibody. The molecular mass of the detected band corresponded to that of pro-IL-1α (32 kDa).

As shown in Fig. 1, both histamine and 4-methylhistamine were effective in increasing G-CFU-c counts, suggesting that histamine exerted G-CFU-c stimulation in murine bone marrow cells. However, when nonadherent cells alone were exposed to either histamine or 4-methylhistamine no such increase in G-CFU-c was detected. This seems to indicate that histamine stimulates H_2 receptors of bone marrow stromal cells so as to induce production of hematopoietic substances having G-CFU-c-stimulating activity. A similar effect was observed when theophylline and db-cAMP were used in place of histamine, indicating that an increase in cAMP level in bone marrow stromal cells may be involved in the process that triggers the production of such hematopoietic substances. To clarify the effect of histamine on the bone marrow stromal cells, murine bone marrow stromal cells were isolated and cultured according to the method reported by Dexter and Testa (9). It has been reported that the stromal cells are composed of mononuclear phagocytic cells, epithelial cells, giant fat cells, endothelial cells, reticular cells, fibroblasts, and adipocytes (17). The same types of cells were observed in the present study. When bone marrow stromal cells were stimulated by histamine, both thymocyte comitogenic activity and colony-promoting activity in the culture medium increased significantly. Such effects of histamine were mimicked by H_2 agonists and inhibited by H_2 antagonists, but an H_1 agonist and an H_1 antagonist had no effect. In addition, histamine induced a rapid increase in cAMP levels in bone marrow stromal cells and db-cAMP was also effective in increasing the thymocyte comitogenic activity in the culture medium of bone marrow stromal cells. These observations indicate that H_2 receptor stimulation and the resulting increase in cAMP levels may be inevitably involved in the production of hematopoietic substance from bone marrow stromal cells. The activity and the molecular mass of the substance induced by histamine in bone marrow stromal cells corresponded to those of IL-1. By means of Western blotting analysis, it was indicated that the production of pro-IL-1α was increased by histamine stimulation. As reported previously (18), murine bone marrow stromal cells produced mainly IL-1α, whereas the production of IL-1β was negligible (data not shown). Hista-

mine-induced pro-IL-1α production was inhibited not only by H_2 antagonists but also by inhibitors of protein kinase A, indicating a participation of H_2 receptors and resulting activation of protein kinase A.

It is known that macrophages, endothelial cells, epithelial cells, and fibroblasts are capable of producing IL-1 (14). So far, no report has indicated whether histamine interacts with these cells to produce IL-1 via H_2 receptor stimulation. However, it has been reported that histamine facilitates the release of free fatty acids from fat cells, and this effect seems to be mediated by H_2 receptors (19). It is known that adipocytes are important to maintain the stem cells in the bone marrow (20). Therefore, giant fat cells and adipocytes in the bone marrow may be useful for IL-1α release in response to histamine.

It has been reported that pro-IL-1 is translated as a 31-kDa protein from mRNA and the precursor protein is processed by serine proteases, such as elastase and plasmin, into 17.5-, 11-, and 4-kDa proteins (14). It has also been reported that phosphorylation of the pro-IL-1 molecule by protein kinase A markedly increases the susceptibility to protease (21). From these observations, it was assumed that an increase in cAMP levels via H_2 receptor stimulation may be intimately related to the digestion of pro-IL-1α and the resulting release of IL-1α. In addition, the present findings indicate that an activation of the cAMP-protein kinase system as a result of H_2 receptor stimulation is also related to the production of pro-IL-1α in bone marrow stromal cells.

From the present observations, it was concluded that histamine induces production of IL-1α in murine bone marrow stromal cells through activation of H_2 receptors in association with activation of the cAMP-protein kinase A system. As reported previously, IL-1 is effective in producing various CSFs from bone marrow stromal cells, fibroblasts, and endothelial cells (7, 22, 23). It has also been reported that IL-1 is capable of activating pluripotent stem cells so as to induce proliferation and differentiation (8). Therefore, it is possible to assume that the G-CUF-c stimulation exerted by histamine may be derived from the production of IL-1α and, consequently, that G-CSF production from bone marrow stromal cells may be induced by released IL-1α. Furthermore, it has been reported that IL-1 is capable of inducing IL-1 production by a positive feedback mechanism (24). It is well known that both histamine and IL-1 are released in the case of infections, injuries, and allergic reactions. Moreover, the production of IL-1 markedly decreased in patients with aplastic anemia, and it has been suggested that IL-1 plays physiologically important roles as a hematopoietic substance (25). In the bone marrow, stromal cells have important roles in proliferation and differentiation of stem cells. Therefore, it is possible to assume that IL-1α released from stromal cells in the bone marrow as a result of histamine may stimulate hematopoietic progenitor cells not only by the production of CSFs but also by direct action on pluripotent stem cells. In addition, histamine also acts directly on the myeloblasts and promyelocytes to induce differentiation through the H_2 receptors (5).

From the present experiments, it has become clear that histamine acts upon bone marrow stromal cells through H_2 receptors and, consequently, IL-1α production and an increase of colony-promoting activity are elicited. cAMP plays an essential role in these events. The IL-1-induced IL-1 release from the stromal cells may enhance the effect of histamine on neutrophil differentiation.

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Send reprint requests to: Prof. Kenji Tasaka, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Okayama University, Okayama, Japan 700.