MYELOPOIETIC ENHANCING EFFECTS OF MURINE MACROPHAGE INFLAMMATORY PROTEINS 1 AND 2 ON COLONY FORMATION IN VITRO BY MURINE AND HUMAN BONE MARROW GRANULOCYTE/ MACROPHAGE PROGENITOR CELLS

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Myeloid blood cell production from hematopoietic stem and progenitor cells (1, 2) is regulated by a network of interacting accessory cell populations (1). Accessory cells release biomolecules or cytokines, which in turn can act either directly on hematopoietic stem and progenitor cells (3–6), or indirectly on other accessory cells (5, 7–10). Cytokines implicated in the modulation of blood cell production include, but are not limited to, the hematopoietic colony-stimulating factors: granulocyte/macrophage CSF (GM-CSF),[1] M-CSF, G-CSF, multi-CSF (also termed IL-3) and erythropoietin, as well as IL-1 through IL-6, cachectin/TNF-α, lymphotoxin/TNF-β, IFN-α, -β, and -γ, transforming growth factor β, prostaglandins E_1, and E_2, lactoferrin, acidic isoferritin, activin, and inhibin (1–10). These well-characterized mediators have been shown to act on more than one cell type and to exert more than one effect (1–10). New cytokines that act to regulate myelopoiesis are being described as well. Herein we report a new and unique myelopoietic enhancing activity in vitro for the recently descibed heparin-binding macrophage inflammatory proteins, MIP-1 and MIP-2 (11–15) on granulocyte/macrophage progenitor cells (CFU-GM) from bone marrows of normal mice and humans.

Materials and Methods

Cells and Cell Separation Procedures

Femoral bone marrow cells were obtained from 4–6-wk-old (C57Bl/6 × DBA/2)F_1 (BDF_1) female mice purchased from Cumberland View Farms (Clinton, TN). Cells were used as

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[1] *Abbreviations used in this paper:* BFU-E, erythroid burst-forming unit; CFU-GM, granulocyte/macrophage progenitor cell; G, granulocyte; hu, human; MIP, macrophage inflammatory protein; mu, murine; n, natural.

isolated or following purification as described elsewhere with minor modification (16). In short, purified CFU-GM were obtained as follows: mice were injected intraperitoneally with 200 mg/kg cyclophosphamide, marrow cells were removed 3 d later, and the low density cells (<1.077 g/cm^3) were retrieved after density cut separation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ). The low density cells were then purified further by centrifugal elutriation in a Sanderson Chamber at 4°C rather than 10^{8}C. In the present studies, the peak CFU-GM-containing fractions elutriated at a slower sedimentation rate (16–20 ml/min) than previously reported (24–28 ml/min), but the yields and purity of CFU-GM were similar.

Human bone marrow cells were obtained by aspiration from the posterior iliac crest of healthy volunteers who had given informed consent according to the guidelines established by the Human Investigation Committee of the Indiana University School of Medicine. Low density marrow cells were purified on Ficoll-Hypaque and cultured.

Biomolecules and Antibodies

Natural murine (mu) MIP 1 (comprised of α and β peptides) and MIP 2 were isolated from the supernatant fluid of LPS-stimulated RAW 264.7 cells and purified as described previously (11–14). Recombinant preparations of mu and human (hu) GM-CSF and muIL-4 (sp act, $\sim 10^8$ U/mg each) (5) and huIL-6 (sp act, 5×10^6 U/mg) were kind gifts from Dr. David Urdal and Dr. Steven Gillis, Immunex Corp., Seattle, WA. Natural muCSF-1 (sp act, 2.3×10^7 U/mg) (5) was a kind gift from Dr. Richard K. Shadduck, University of Pittsburgh School of Medicine, Pittsburgh, PA. Recombinant huG-CSF (95% pure, sp act, $>5 \times 10^7$ U/mg) (5) was a kind gift from Dr. Peter Ralph and Dr. Robert Drummond, Cetus Corp., Emeryville, CA. Recombinant huIL-1-α (sp act, 10^9 U/mg using the D10 cell assay) (6) was a kind gift from Dr. Peter T. Lomedico, Hoffman-La Roche, Nutley, NJ. The purified Ig fractions of anti-MIP-1 and anti-MIP-2 (17) were prepared from serum of rabbits injected respectively with purified preparations of MIP-1 and MIP-2. *Escherichia coli* LPS was purchased from Sigma Chemical Co., St. Louis, MO.

Colony Assays

CFU-GM. Unseparated mouse bone marrow cells (0.5, 0.75, and 1.0×10^5 cells/ml) and low density human bone marrow cells (1.0×10^5 cells/ml) were plated in standard 35-mm standard tissue culture dishes in 1 ml of 0.3% agar (Difco Laboratories, Inc., Detroit, MI) culture medium containing McCoy's 5A medium supplemented with additional essential and nonessential amino acids, glutamine, serine, asparagine, sodium pyruvate (Gibco Laboratories, Grand Island, NY) and 10% inactivated (56°C for 0.5 h) FCS (HyClone Laboratories, Logan, UT) with or without purified growth factors (5). Purified murine CFU-GM were plated at 200 cells/ml in 0.4% agarose (16). Serum-free culture conditions were as described elsewhere (18). Culture dishes were incubated at 37°C in a humidified atmosphere flushed with 5% CO_2 at lowered (5%) O_2 tension and scored after 7 d for colonies (>50 cells/aggregate) and clusters (5–50 cells/aggregate) for human and mouse cells, and also after 14 d for human cells. Day 7 and day 14 colonies appear to derive from different human CFU-GM progenitors (19, 20) and colonies plus clusters provide a more accurate estimate of the actual number of progenitor cells stimulated than sampling of colonies only (19). Colony and cluster morphology were assessed in whole plates stained with α-naphthyl acetate esterase and luxol fast blue and counter-stained with hematoxylin (21). CFU-GM in DNA synthesis (S-phase) were killed by pulse exposure to 50 μCi/ml high specific activity [^{3}H]TdR (sp act, 20 Ci/mmol; New England Nuclear, Boston, MA) as described (22, 23).

BFU-E. Mouse bone marrow cells (2×10^5 cells/ml) were plated in standard 35-mm tissue culture dishes containing a 1-ml mixture of Iscove's modified Dulbecco's medium, 1.3% methylcellulose, 30% FCS, 5×10^{-5} M 2-ME, 0.1 mM hemin (Eastman Kodak Co., Rochester, NY), and 2 U (r)erythropoietin (Amgen Corp., Thousand Oaks, CA) (22). Cultures were incubated as above for CFU-GM and scored after 7 d of incubation.

Statistics

The results are expressed as the mean ± 1 SEM of three plates per point for the CFU-GM assay and four plates per point for the BFU-E assay. Levels of significance for comparisions between samples were determined using student's t distribution.

Results

Myelopoietic Enhancing Activities In Vitro of MIP-1 and MIP-2. MIP-1 and MIP-2 were assessed alone and in combination, at various concentrations, for their influence on colony and cluster formation by mouse bone marrow CFU-GM stimulated with suboptimal concentrations of nmuCSF-1 or rmuGM-CSF. Representative results of one of two identical sets of experiments are shown in Table I. MIP-1 and MIP-2 each significantly enhanced colony, and colony plus cluster, formation stimulated by 10 U/ml of either nmuCSF-1 or rmuGM-CSF. Maximal levels of enhancement were noted at 100–200 ng/ml MIP-1 or MIP-2 (Table I) and concentrations of up to 1,000 ng/ml of MIP-1 or MIP-2 did not further enhance colony or cluster formation (data not shown). No synergy was observed when MIP-1 and MIP-2 were assayed in combination (Table I). Neither MIP-1 nor MIP-2, at concentrations ranging from 100 to 300 ng/ml, stimulated colony or cluster formation of mouse marrow CFU-GM in the absence of an added source of CSF (Table II). Both MIP-1 and MIP-2 enhanced colony and cluster formation of mouse marrow CFU-GM maximally stimulated by nmuCSF-1 or rmuCSF-1 (100 U/ml or either), but the percent enhancement noted was not as much as that seen when colonies and clusters were stimulated with suboptimal concentrations of either CSF (10 U/ml of each) (Table II). The enhancing effects of MIP-1 or MIP-2 were similar whether colonies or colonies plus clusters were evaluated except when 10 U/ml of nmuCSF-1 was used to

TABLE I

Influence of Various Concentrations of MIP-1 and MIP-2, Alone and In Combination, on Colony and Cluster Formation by Mouse Bone Marrow CFU-GM Stimulated In Vitro with CSF-1 or GM-CSF

		CFU-GM per 7.5×10^4 BDF_1 cells stimulated with:			
		nmuCSF-1 (10 U)		rmuGM-CSF (10 U)	
Additions to culture	Amount	Colonies	Colonies and clusters	Colonies	Colonies and clusters
	ng				
Control medium		17 ± 2	64 ± 6	49 ± 2	61 ± 3
MIP-1	200	40 ± 4*	104 ± 7*	79 ± 5*	101 ± 6*
MIP-1	100	35 ± 2*	100 ± 2*	79 ± 1*	102 ± 4*
MIP-1	50	22 ± 2	57 ± 6	64 ± 3*	84 ± 1*
MIP-1	25	16 ± 1	51 ± 1	59 ± 2*	76 ± 2*
MIP-1	12.5	17 ± 1	64 ± 6	47 ± 3	61 ± 3
MIP-1	6.25	17 ± 1	61 ± 4	48 ± 2	60 ± 3
MIP-2	200	30 ± 2*	109 ± 6*	72 ± 2*	89 ± 2*
MIP-2	100	30 ± 5*	103 ± 5*	75 ± 6*	97 ± 3*
MIP-2	50	18 ± 2	68 ± 7	57 ± 6*	71 ± 6
MIP-2	25	16 ± 1	59 ± 3	47 ± 2	60 ± 3
MIP-2	12.5	18 ± 3	60 ± 2	45 ± 3	57 ± 4
MIP-2	6.25	16 ± 2	61 ± 1	46 ± 3	59 ± 4
MIP-1 + MIP-2	6.25 + 6.25	15 ± 1	58 ± 1	47 ± 2	61 ± 3
MIP-1 + MIP-2	100 + 100	32 ± 2*	92 ± 9*	81 ± 5*	94 ± 6*

* $p < 0.05$ compared with control medium.

stimulate the cells; in this case enhancement of colonies by MIP-1 or MIP-2 was greater than the enhancement for colonies plus clusters (Table II). The enhancing effects of MIP-1 and MIP-2 were noted whether the cells were plated in the absence or presence of 10^{-6} M indomethacin (Table II, legend). The enhancing activity of MIP-1 or MIP-2 was also apparent in the absence of serum in the culture system, with 200 ng of each significantly enhancing colony and colony plus cluster formation by 67–89% in the presence of 100 U nmCSF-1 and by 77–122% in the presence of 100 U rmuGM-CSF. When the types of colonies and clusters enhanced by MIP-1 and MIP-2 were evaluated, it was evident that both those containing only macrophages, or containing both macrophages and neutrophils, were significantly ($p < 0.001$) enhanced by MIP-1 and MIP-2 (data for five separate experiments not shown).

Specificity of MIP-1 and MIP-2 Myelopoietic Enhancing Activities. To substantiate that the enhancing effects noted were due to MIP-1 and MIP-2 themselves, the preparations of MIP-1 and MIP-2 were each preincubated with purified Ig fractions of rabbit anti-murine MIP-1 or rabbit anti-murine MIP-2 before their addition to cultures containing 10 U/ml of either nmuCSF-1 or rmuGM-CSF. The representative results of one or two similar experiments are shown in Fig. 1. The antibodies by themselves had no effect on CSF-stimulated colony or cluster formation ($p > 0.05$). Anti-MIP-1 neutralized the myelopoietic enhancing activity of MIP-1, but not of MIP-2, and anti-MIP-2 neutralized the myelopoietic enhancing activity of MIP-2, but not of MIP-1, suggesting that the enhancing effects of MIP-1 and MIP-2 were independent and not due to contaminating molecules.

TABLE II

Influence of MIP-1 and MIP-2 on Colony and Cluster Formation by Mouse Bone Marrow CFU-GM Co-stimulated In Vitro with CSF-1, GM-CSF, or G-CSF

		Percent increase with MIP on numbers of CFU-GM stimulated with CSF*			
		Colonies		Colonies and clusters	
CSF	Amount	MIP-1	MIP-2	MIP-1	MIP-2
	U				
None		0 (4)	0 (4)	3 ± 4 (4)	6 ± 6 (4)
nmuCSF-1	10	108 ± 20 (14)‡	146 ± 46 (9)‡	59 ± 9 (14)‡	63 ± 7 (9)‡
nmuCSF-1	100	33 ± 5 (10)§	44 ± 7 (5)§	39 ± 3 (10)§	43 ± 13 (5)§
rmuGM-CSF	10	55 ± 7 (12)‡	68 ± 8 (8)‡	57 ± 6 (12)‡	58 ± 4 (8)‡
rmuGM-CSF	100	36 ± 6 (7)§	40 ± 4 (5)§	46 ± 8 (7)§	41 ± 7 (5)§
rhuG-CSF	100	7 ± 7 (3)	4 ± 4 (3)	11 ± 6 (3)	9 ± 4 (3)

* Results are expressed as the mean ± 1 SEM, with numbers of experiments done shown in parentheses, for BDF_1 mouse bone marrow cells plated at 5.0×10^4, 7.5×10^4, or 1.0×10^5 cells/ml in the absence and presence of added CSF and in the absence or presence of 100–300 ng MIP-1 or MIP-2. Results were similar regardless of the cell concentration plated or if cells were plated in the absence or presence of 10^{-6} M indomethacin and were therefore pooled. The percent increases are based on control CFU-GM numbers of: 0 colonies and 0 to 8 ± 1 colonies and clusters without CSF, 7 ± 1 to 39 ± 1 colonies and 30 ± 1 to 120 ± 10 colonies plus clusters with 10 U CSF-1, 62 ± 1 to 151 ± 5 colonies and 109 ± 6 to 313 ± 13 colonies plus clusters with 100 U CSF-1, 19 ± 1 to 49 ± 2 colonies and 28 ± 1 to 103 ± 5 colonies plus clusters with 10 U GM-CSF, 31 ± 1 to 67 ± 2 colonies and 45 ± 3 to 137 ± 4 colonies plus clusters with 100 units GM-CSF, 8 ± 1 to 15 ± 3 colonies and 19 ± 2 to 38 ± 5 colonies plus clusters with 100 U G-CSF.

‡ Increases for each experiment within these groups were statistically significant to $p < 0.01$.

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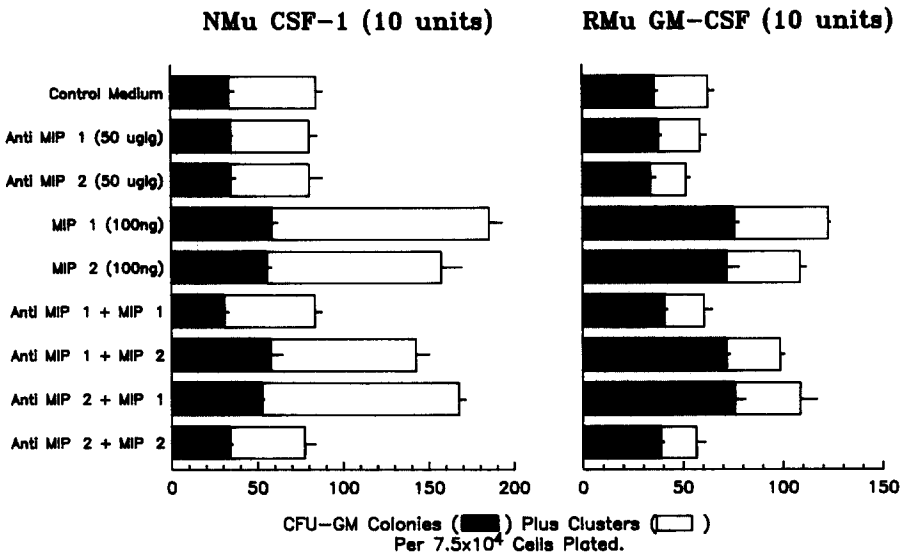

FIGURE 1. Influence of anti-MIP-1 and anti-MIP-2 antibodies on the myeloid enhancing activity of MIP-1 and MIP-2. Preparations of MIP were preincubated with control medium or anti MIP for 1.5 h at room temperature before addition to culture dishes with 5×10^4 BDF_1 bone marrow cells/milliliter in the presence of nmuCSF-1 or rmuGM-CSF. Significant increases in colony and colony plus cluster formation ($p < 0.001$) were noted with MIP-1, MIP-2, anti-MIP-1 plus MIP-2, and anti-MIP-2 plus MIP-1. The other values were not significantly different from control ($p > 0.05$).

While MIP-1 and MIP-2 enhanced CFU-GM colony and cluster formation stimulated by nmuCSF-1 and rmuGM-CSF as noted above (Tables I and II), both MIP-1 and MIP-2 were without effect on colonies or clusters stimulated by rhuG-CSF (Table II). Colonies and clusters stimulated by rhuG-CSF were >95% composed of only neutrophilic granulocytes. We also tested MIP-1 and MIP-2 for enhancement of erythroid progenitor cell proliferation by mouse bone marrow erythroid burst-forming units (BFU-E). In the absence of Epo, no BFU-E colonies formed in the absence or presence of MIP-1 or MIP-2. Neither MIP-1 nor MIP-2, at concentrations ranging from 1 to 1,000 ng/ml, enhanced BFU-E colony numbers or size in the presence of 0.25–2.0 U of Epo ($p > 0.05$). Control BFU-E numbers formed in the absence of MIP-1 and MIP-2 but in the presence of 2.0 U of Epo were, respectively, 25 ± 1 and 25 ± 2 per 2×10^5 BDF_1 mouse bone marrow cells plated in two separate experiments.

The myelopoietic enhancing effects of MIP-1 and MIP-2 on mouse bone marrow cells were not mimicked by rhuIL-1α, rmuIL-4, rhuIL-6, or *E. coli* LPS (data not shown). rhuIL-1α and rhuIL-6, both of which are titered for activity on mouse cells, were each tested at 1, 5, and 10 ng/ml and were not found to significantly influence colony or cluster formation stimulated with 10 U/ml of nmuCSF-1 or rmuGM-CSF, or 100 U/ml rhuG-CSF. Moreover, neither 10 ng/ml of rhuIL-1α nor of rhuIL-6 significantly influenced colonies or clusters formed in the presence of 100 ng/ml MIP-1 or MIP-2 with 10 U/ml of nmuCSF-1 or rmuGM-CSF. We have previously shown that rmuIL-4 only enhances mouse bone marrow neutrophil colony and cluster for-

mation in the presence of rhuG-CSF; it does not enhance neutrophil, neutrophil-macrophage, or macrophage colony or cluster formation stimulated with nmuCSF-1 or rmuGM-CSF (5). *E. coli* LPS tested at 0.01–100 μg/ml (in 10-fold increments) in the absence or presence of 10^{-6} M indomethacin did not enhance colony or cluster formation stimulated by 10 U/ml nmuCSF-1 or rmuGM-CSF. LPS did suppress CSF-stimulated colonies and clusters in a dose-dependent fashion with up to 93% inhibition apparent with 100 μg LPS/ml. The LPS-induced suppression was still apparent and only minimally counteracted when cells were plated with 10^{-6} M indomethacin.

Influence of MIP-1 and MIP-2 on Purified Populations of Mouse Marrow CFU-GM. Since CFU-GM make up <0.5% of the population of unseparated marrow cells (as is apparent from the <0.5% colony and cluster cloning efficiency of cells plated in the presence of 100 Units/ml nmCSF-1 or rmGM-CSF in Table II) it was not possible to determine from the above studies whether MIP-1 and MIP-2 were acting directly on the CFU-GM, or indirectly through an action on accessory cells. To determine whether MIP-1 and MIP-2 had direct acting effects on CFU-GM, mouse bone marrow cells were purified (16) and the preparations of these two mediators were evaluated for their influence on colony and cluster formation by 200 purified cells plated/milliliter and stimulated with 50 U/ml of either nmuCSF-1 or rmuGM-CSF. The results of one representative of two similar experiments are shown in Fig. 2. The colony plus cluster cloning efficiencies of the various fractions ranged from 15 to 44% when cells were stimulated with nmuCSF-1 and from 17 to 39% when cells were stimulated with rmuGM-CSF. These concentrations of CSF (50 U/ml) result in maximal or near maximal stimulation of colony and cluster formation by purified CFU-GM when only one type of CSF is used, although combinations of CSFs can result in higher cloning efficiencies (3). MIP-1 and MIP-2 (100 ng/ml) each significantly enhanced (p at least <0.01 when compared with cells in that particular fraction cultured with control medium) CSF-stimulated colony and cluster formation by purified CFU-GM in the various fractions (Fig. 2). Cloning efficiencies of up to 82 and 65% were respectively noted for cells plated in the presence of MIP plus either nmuCSF-1 or rmuGM-CSF. These results suggest that MIP-1 and MIP-2 probably exert direct acting effects on mouse marrow CFU-GM *in vitro*.

Cell Cycle–related Myelopoietic Enhancing Activities of MIP-1 and MIP-2. To evaluate whether MIP-1 and MIP-2 had preferential effects on CFU-GM in S-phase or during non–S-phase portions of the cell cycle, mouse bone marrow cells were pulse treated with nonradioactive (cold) thymidine (shown to have no effect on subsequent colony or cluster formation by the cells [22, 23]) or high specific activity tritiated thymidine before washing and plating in the presence of 10 or 100 U/ml of nmuCSF-1 or rmuGM-CSF and in the absence or presence of 100 ng/ml MIP-1 or MIP-2. Table III shows the data from one of two experiments with similar results. Both MIP-1 and MIP-2 significantly enhanced colony and cluster formation by cells first pulsed with cold thymidine. In contrast, CFU-GM in DNA synthesis (S-phase) at the time of pulse exposure with high specific activity [^{3}H]TdR are reproductively killed and only CFU-GM not in S-phase of the cell cycle at that time go on to proliferate in response to CSF to form a colony or cluster. MIP-1 and MIP-2 had no myelopoietic enhancing activity on cells that were first pulse treated with high specific activity [^{3}H]TdR to

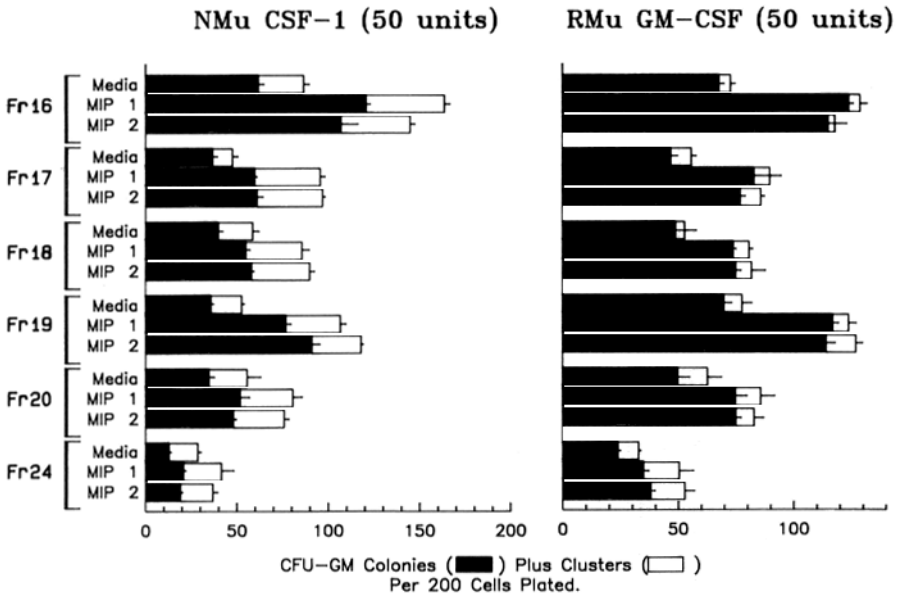

FIGURE 2. Influence of MIP-1 and MIP-2 on colony and cluster formation of purified mouse CFU-GM stimulated by nmuCSF-1 or rmuGM-CSF. Mouse CFU-GM were purified as described elsewhere (16).

TABLE III

Influence of MIP-1 and MIP-2 on Untreated Mouse Bone Marrow CFU-GM versus Bone Marrow Depleted of CFU-GM in S-Phase

	Colonies		Colonies & clusters	
Cells plated with	Cold TdR	[^{3}H]TdR	Cold TdR	[^{3}H]TdR
A) nmuCSF-1 (10 U)				
Control medium	18 ± 2	7 ± 1	60 ± 2	36 ± 3
MIP-1 (200 U)	32 ± 2*	6 ± 1	105 ± 5*	32 ± 2
MIP-2 (200 U)	30 ± 2*	8 ± 1	100 ± 4*	32 ± 3
B) nmuCSF-1 (100 U)				
Control medium	125 ± 4	49 ± 3	313 ± 13	180 ± 17
MIP-1 (200 U)	167 ± 9*	45 ± 2	414 ± 7*	153 ± 10
MIP-2 (200 U)	181 ± 12*	52 ± 2	410 ± 15*	196 ± 9
C) rmuGM-CSF (10 U)				
Control medium	41 ± 1	14 ± 2	69 ± 3	30 ± 3
MIP-1 (200 U)	66 ± 3*	13 ± 2	107 ± 7*	30 ± 4
MIP-2 (200 U)	72 ± 4*	13 ± 1	104 ± 3*	32 ± 2
D) rmuGM-CSF (100 U)				
Control medium	58 ± 4	26 ± 2	70 ± 2	39 ± 3
MIP-1 (200 U)	89 ± 3*	25 ± 2	106 ± 3*	40 ± 4
MIP-2 (200 U)	84 ± 3*	24 ± 1	99 ± 1*	35 ± 1

Cells were pulse treated with cold thymidine or high specific activity [^{3}H]TdR as described in Materials and Methods, washed, and plated at 5×10^4 cells/ml in the presence of CSF and in the absence and presence of MIP-1 or MIP-2.

* Significant increase from control medium, $p < 0.01$; other numbers are not significant, $p > 0.05$.

TABLE IV

Influence of MIP-1 and MIP-2 on Colony and Cluster Formation by Normal Human Bone Marrow Cells

Material added to plate	Day 7 CFU-GM				Day 14 CFU-GM	
	Exp. 1		Exp. 2		Exp. 1	Exp. 2
	Colonies	Colonies and clusters	Colonies	Colonies and clusters	Colonies	Colonies
A. No CSF						
Control medium	0	239 ± 22	0	149 ± 9	37 ± 2	0
MIP-1	0	365 ± 16*	0	223 ± 13*	58 ± 2*	0
MIP-2	0	355 ± 8*	0	170 ± 2*	54 ± 6*	0
B. rhuGM-CSF						
Control medium	50 ± 3	304 ± 9	16 ± 2	162 ± 5	68 ± 3	24 ± 4
MIP-1	88 ± 6*	409 ± 11*	24 ± 2*	179 ± 9	125 ± 5*	49 ± 6*
MIP-2	80 ± 7*	398 ± 15*	25 ± 3*	173 ± 8	110 ± 10*	45 ± 3*
C. rhuG-CSF						
Control medium	29 ± 2	183 ± 7	16 ± 1	72 ± 7	40 ± 3	16 ± 4
MIP-1	31 ± 3	179 ± 7	17 ± 1	70 ± 5	39 ± 3	14 ± 3
MIP-2	32 ± 2	191 ± 9	15 ± 1	66 ± 5	38 ± 4	15 ± 2

10^5 Low density cells/ml were plated in the absence and presence of 100 U CSF and 200 ng MIP and scored for colonies and colonies plus clusters after 7 d of incubation and for colonies after 14 d of incubation.

* Significant increase compared with control medium, $p < 0.01$; other values are not significantly different from control, $p > 0.05$.

remove S-phase CFU-GM. These results suggest that the myelopoietic enhancing activities of MIP-1 and MIP-2 are initiated mainly or entirely during the DNA synthetic phase of the CFU-GM cell cycle.

Influence of MIP-1 and MIP-2 on Colony and Cluster Formation by Human Bone Marrow CFU-GM. MIP-1 and MIP-2 (200 ng/ml) were evaluated for their effects on colony and cluster formation by CFU-GM present in the low density fraction of normal human bone marrow (Table IV). Cells were plated at 10^5 cells/ml in the absence or presence of 100 U/ml rhuGM-CSF or rhuG-CSF and scored after 7 and 14 d of incubation. Low density human bone marrow cells can form colonies and clusters in the absence of an exogenously added source of CSF, but the numbers of colonies and clusters formed are related to the number of cells plated and are a result of the endogenous release of CSFs from marrow accessory cells (24). In the two experiments shown, MIP-1 and MIP-2 in the absence of added CSF significantly enhanced colony formation when colonies formed in the absence of MIP, but not when colonies did not form in the absence of MIP. MIP-1 and MIP-2 each enhanced cluster formation in the absence of exogenously added CSF. MIP-1 and MIP-2 significantly enhanced colony and cluster formation by day 7 and day 14 CFU-GM stimulated with rhuGM-CSF, but similar to the results noted for mouse colony and cluster formation stimulated by G-CSF (Table II), neither MIP-1 nor MIP-2 enhanced colony or cluster formation of human bone marrow cells stimulated with rhuG-CSF.

Discussion

The heparin-binding proteins MIP-1 and MIP-2 have been shown to elicit a localized inflammatory response when injected subcutaneously into footpads of C3H/HeJ

mice (11, 13). MIP-1 acts as a prostaglandin-independent endogenous pyrogen when administered to mice (15) and is capable in vitro of inducing chemokinesis of human neutrophils and of triggering adherent neutrophils to release hydrogen peroxide (11). MIP-2 acts as a chemotactic agent for neutrophils and induces neutrophil degranulation of lysozyme, but not of beta-glucuronidase (13).

The results presented here demonstrate a new and as yet unique role for MIP-1 and MIP-2 at the level of the granulocyte/macrophage progenitor cell. While MIP-1 and MIP-2 had no hematopoietic CSF activity when tested alone, they did significantly enhance colony and cluster formation by murine bone marrow CFU-GM costimulated with nmuCSF-1 and rmuGM-CSF and by human bone marrow CFU-GM costimulated with rhuGM-CSF. Studies using purified mouse marrow CFU-GM suggested that the myelopoietic enhancing effects of MIP-1 and MIP-2 were due to a direct effect on the CFU-GM itself. The exact manner and mechanism of action of MIP-1 and MIP-2 on CFU-GM remains to be determined, but the action appears to be mediated, or at least initiated, during S-phase of the cell cycle. The fact that MIP-1 and MIP-2 can act directly on CFU-GM does not however rule out the possibility that MIP-1 and MIP-2 might be able also to modulate myelopoiesis indirectly through an action on accessory cells.

A number of molecules without CSF activity can modulate myelopoiesis in a positive fashion, but the type of enhancing activity noted for MIP-1 and MIP-2 was not duplicated in our hands by IL-1α, IL-6, or bacterial LPS. We had previously shown that IL-4 synergizes with G-CSF to enhance neutrophil colony formation (5), but MIP-1 and MIP-2 did not enhance the activity of rhuG-CSF against mouse or human bone marrow cells, and IL-4 does not enhance the activities of nmuCSF-1 and rmuGM-CSF (5). IL-5 acts as an eosinophil-CSF (25; Lu, L., Z. H. Lin, R. N. Shen, D. J. Warren, T. Leemhuis, and H. E. Broxmeyer, manuscript in preparation) and MIP-1 and MIP-2 did not stimulate or enhance eosinophil colony or cluster formation in our studies. IL-2 has not been shown to directly enhance CFU-GM numbers (1). In the type of assay used in the present studies, cachectin/TNF-α, lymphotoxin/TNF-β, interferons, and acidic isoferritin suppress colony formation (1, 4). Activin enhances and inhibin suppresses BFU-E colony formation by an action mediated through T lymphocytes and monocytes, but has no effect on CFU-GM colony formation (10), and MIP-1 and MIP-2 had no demonstratable effect on BFU-E colony formation. E-type prostaglandins 1 and 2 enhance Epo-dependent BFU-E, but suppress CSF-dependent CFU-GM colony formation (21). The reported effects of transforming growth factor β (26, 27) are also not consistent with the effects noted herein for MIP-1 and MIP-2.

MIP-1 and MIP-2 have predicted amino acid sequence homology with a number of other molecules activated in T lymphocytes, including LD78, JE, and TCA3 (12). It will be of interest to see if these latter molecules have myelopoietic modulatory activities also. To be determined, as well, is whether MIP-1 and MIP-2 can act in vivo to enhance hematopoietic progenitor cell cycling and myelopoiesis.

Summary

Two recently identified and purified murine macrophage inflammatory proteins MIP-1 and MIP-2 were tested in vitro both alone, and in combination with purified recombinant (r) murine (mu) GM-CSF, natural (n)muCSF-1, or rhuman (hu)G-

CSF, for effects on mouse marrow CFU-GM, in combination with erythropoietin for effects on mouse marrow BFU-E, and in combination with rhuGM-CSF or rhuG-CSF for effects on human marrow CFU-GM. MIP-1 and MIP-2 did not stimulate, but did enhance by up to threefold, colony formation of mouse CFU-GM co-stimulated by rmuGM-CSF and nmuCSF-1, but not by rhuG-CSF, in the absence or presence of serum. MIP-1 and MIP-2 were maximally active at concentrations ≥100 ng/ml and the actions appeared to be initiated during the DNA synthetic portion of the cell cycle. Neither MIP-1 nor MIP-2 at up to 1 μg/ml had any effect on mouse BFU-E, in the absence or presence of erythropoietin. Both MIP-1 and MIP-2 had direct acting effects on purified mouse CFU-GM. The cloning efficiency of 200 purified cells plated with 50 U muCSF-1 was 82% with and 43% without MIP; the cloning efficiency with 50 U rmuGM-CSF was 65% with and 35% without MIP. MIP effects were not mimicked by bacterial LPS, rhuIL-1α, rhuIL-6, or rmuIL-4, and were neutralized by their respective specific antibodies. MIP-1 and MIP-2 also enhanced endogenously stimulated and rhuGM-CSF-, but not rhuG-CSF-, stimulated colony formation by human marrow CFU-GM. These results demonstrate a new role for MIP-1 and MIP-2 in vitro as myelopoietic enhancing activities for CFU-GM.

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