Down Regulation of Myelopoiesis by Mediators Inhibiting the Production of Macrophage-Derived Granulomonopoietic Enhancing Activity (GM-EA)

By Sheng-Yuan Wang, Chi-Kuan Ho, Ling-Yang Chen, Rueen-Chiou Wang, Min-Hsiung Huang, Hugo Castro-Malaspina, and Malcolm A.S. Moore

Monocyte-derived lipid-containing macrophages (MDLMs) constitutively synthesize a granulomonopoietic enhancing activity (GM-EA) that potentiates the function of granulocyte-macrophage colony-stimulating activity (GM-CSA). In the study reported, we show that GM-EA is distinct from interleukin-1 (IL-1) in biochemical and functional properties and that its production is negatively regulated by several mediators. Thus, MDLM cultures pretreated with interferon-$\gamma$ (IFN-$\gamma$, 3 to 900 U/mL), prostaglandin E$_2$ (PGE$_2$, $10^{-13}$ to $10^{-8}$ mol/L), or lactoferrin (LF, $10^{-13}$ to $10^{-8}$ mol/L) invariably produced less GM-EA than untreated controls. The relative potency of inhibition was in the order of IFN-$\gamma$ > PGE$_2$ > LF. The extent of the inhibitory effects was proportional to dosage and the duration of treatment.

MONOCYTID CELLS can constitutively synthesize granulocyte-macrophage colony-stimulating activity (GM-CSA), including a mixture of granulocyte colony-stimulating factor (G-CSF) and macrophage CSF (M-CSF), that stimulate the clonal growth and differentiation of committed granulocyte-macrophage progenitor cells. On the other hand, they elaborate prostaglandin E (PGE) that inhibits myelopoietic colony formation. Furthermore, other monokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) have also been shown to be potent in regulation of hematopoiesis. The former can stimulate endothelial cells and/or fibroblasts to produce GM-CSA as well as growth factors of erythroid burst-forming units (BFU-E) and pluripotent progenitor cells (CFU-GEMM) while the latter acts on directly inhibiting the colony growth of both CFU-GM and BFU-E.

Recently, we have shown that well-differentiated macrophages known as monocyte-derived lipid-containing macrophages (MDLMs) produce a previously unreported mediator designated granulomonopoietic enhancing activity (GM-EA) that has no direct stimulatory effect on the colony formation of CFU-GM but can enhance the function of GM-CSA.

In this report we provide evidence that GM-EA is not related antigenically or functionally to IL-1, and we also extend our previous findings to demonstrate that the production of GM-EA is negatively regulated by interferon-$\gamma$ (IFN-$\gamma$), PGE$_2$, and LF, which can suppress the production of GM-EA at relatively low doses. Furthermore, this inhibitory effect is reversible and can be overridden by the stimulatory effect of zymosan. Our results substantiate the existence of a novel growth-promoting factor of myelopoiesis and provide some important insights into the negative feedback mechanisms of myelopoiesis.

MATERIALS AND METHODS

Reagents

Four reagents known to affect macrophage functions were used in the present study. PGE$_2$ and zymosan A (from Saccharomyces cerevisiae) were purchased from the Sigma Chemical Co (St. Louis) and could be observed following only a brief exposure (two hours) of the MDLMs to physiologic doses of the mediators. Under optimal conditions, IFN-$\gamma$ (300 U/mL for 24 to 48 hours) and PGE$_2$ ($10^{-8}$ mol/L for 24 to 48 hours) could totally abrogate the ability of the MDLMs to produce GM-EA. However, the drug-inhibited MDLMs could be reactivated to produce GM-EA by treatment with zymosan (80 $\mu$g/mL). These results demonstrate that a mechanism for the control of myelopoiesis by mediators such as IFN-$\gamma$, PGE$_2$, and LF may involve the inhibition of GM-EA production. Furthermore, this negative feedback control is reversible and can be overridden when a proper stimulatory signal is given.

Preparation of MDLMs

MDLMs were prepared as described in detail previously. The low-density mononuclear cells separated from normal human peripheral blood were seeded at a concentration of 1.6 x 10$^6$ cells/mL into T-25 culture flasks or 35-mm petri dishes and incubated at 37°C for two hours. After that time, nonadherent cells were removed by repeated washings and adherent cells were further cultured in α-MEM containing 20% horse serum (Gibco Laboratories, St. Lawrence, MA) at 37°C in a humidified atmosphere of 5% CO$_2$ in air. After 3 weeks of cultivation with medium change every three to four days, the monocyteid cells became progressively

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enlarged up to above eight times their original size and could be morphologically identified as well-developed MDLMs.

Production of GM-EA and GM-CSA

GM-EA and GM-CSA were prepared according to the procedures described previously. MDLM cultures at various times of cultivation were washed twice with medium and further incubated for three days in fresh medium at 37°C in 5% CO₂. Then the conditioned media were harvested, centrifuged at 1,500 rpm for ten minutes to remove possible debris, filtered, and stored at −70°C until assay for GM-EA and GM-CSA as described below.

Assay for CFU-GM

A slightly modified version of the method described by Metcalf was used to assay for CFU-GM. Briefly, 50,000 nonadherent low-density normal bone marrow cells were plated in 1-mL layer of 0.3% agar (Difco Laboratories Inc, Detroit) in McCoy's 5A medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), essential and nonessential amino acids, vitamins, and pyruvate. Colony formation was induced by the inclusion of 10% GCT-CM (Gibco) as a source of GM-CSA. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air and scored for colonies after seven days of incubation.

Assay for GM-EA

Media conditioned by normal or various drug-treated MDLMs were assessed for GM-EA using the technique of CFU-GM assay as described above. Various preparations of MDLM conditioned media (MDLM-CM) (as sources of GM-EA) were added to the CFU-GM assay cultures at a final concentration of 10% (vol/vol), and 10% GCT-CM was included as the source of GM-CSA as described before. After seven days of incubation at 37°C in 5% CO₂, the number of GM colonies were counted and results were expressed as follows:

\[ \text{GM-EA (% Enhancement)} = \frac{A - B}{B} \times 100 \]

A = No. of colonies induced by GM-CSA plus MDLM-CM
B = No. of colonies induced by GM-CSA alone

The morphologic typing of GM colonies was performed according to the method described elsewhere.

Effect of IL-1 and Anti-IL-1 on Colony Enhancement

Nonadherent low-density bone marrow cells were prepared as described above and seeded in CFU-GM assay dishes at 5 x 10^4 cells/dish in complete medium supplemented with the following: (1) GM-CSA alone (10% vol/vol), (2) GM-CSA plus GM-EA (10% vol/vol), (3) GM-CSA plus different doses of IL-1 (10 to 100 U/mL), and (4) GM-CSA plus GM-EA in the presence of various concentrations of anti-IL-1 (final concentration of 10 to 100 neutralizing U/mL). Before addition to CFU-GM assay cultures, GM-EA and anti-IL-1 antibody were preincubated for 90 minutes at 37°C. The cultures were then incubated at 37°C in 5% CO₂ for seven days and the number of colonies formed were scored. Results were expressed as percentage of colonies with GM-CSA alone.

Effect of IFN-γ, PGE₂, and LF on GM-EA Production

MDLM cultures (21 to 24 days old) were incubated in media with or without various concentrations of IFN-γ (3 to 900 U/mL), PGE₂ (10⁻⁸ to 10⁻¹⁵ mol/L), or LF (10⁻³ to 10⁻¹⁵ mol/L) for 24 hours at 37°C under normal culture conditions. The cells were washed three times to remove the reagents and further incubated for three days in fresh normal medium. All conditioned media were then collected for assay of GM-EA.

In some experiments, MDLMs were treated or untreated with IFN-γ (300 U/mL) and/or an antisera to IFN-γ (300 neutralizing units/mL) for 24 hours before use for the production of GM-EA as described above.

All tests were performed in triplicate and repeated at least three times with similar results.

Kinetics of Inhibition

MDLMs (21 days old) were cultured in the presence or absence of IFN-γ (300 U/mL), PGE₂ (10⁻⁹ mol/L), or LF (10⁻⁴ mol/L) for various times (two to 48 hours). Following treatment, the cells were washed three times and subsequently cultured for three days in normal medium. Supernatants were then harvested for the determination of GM-EA.

Assay for IFN-γ and PGE₂

MDLM-CM treated or untreated with the various agents for 24 to 48 hours were assayed for the presence of IFN-γ and PGE₂. IFN-γ activity was assayed by a conventional bioassay based on 50% inhibition of the cytopathic effect (CPE) of sindbis virus on the amniotic cell line WISH. PGE₂ was determined by radioimmunoassay using a commercially available assay kit (NEN Products, N Billerica, MA).

Purification of GM-EA

Anion-exchange chromatography. Crude MDLM-CM was prepared in serum-free cultures at days 21 through 24. One-liter aliquots of GM-EA containing CM were concentrated about 100-fold by ammonium sulfate (60%) precipitation and then dialysed extensively against 0.05 mol/L Tris-HCl buffer pH 7.8 (buffer A). The protein solution was then loaded onto a DEAE-TSK column (1 x 10 cm) in buffer A and eluted with a stepwise gradient of Tris-buffered NaCl (0 to 0.5 mol/L NaCl). Five-mL fractions were collected and then assayed for GM-EA. Those with activity were pooled, concentrated by ultrafiltration (Amicon Corp, Danvers, MA; mol wt cutoff 5,000), and subjected to further purification.

Preparative polyacrylamide gel electrophoresis. The semi-purified GM-EA material was analyzed by 7.5% preparative polyacrylamide gel electrophoresis (PAGE). Following electrophoresis at 4°C for 12 hours, one set of the gel was stained with coomassie blue R-250 to reveal the protein profile, and the protein bands of the intact gel were then carefully sliced and each slice was minced in 1 mL of PBS. The protein content released was then assayed for GM-EA and the apparent mol wt(s) of the biologically active protein(s) were determined by 12% sodium dodecyl sulfate-PAGE (SDS-PAGE) according to Laemmli using phosphorylase b (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactalbumin (14,400) as mol wt markers. In all assays, protein content of samples was measured by the Lowry method.

RESULTS

Production of GM-CSA and GM-EA. As indicated in Table 1, the level of GM-CSA production was optimal in one- to three-day-old cultures, but with the prolongation of the culture period, production diminished to an undetectable level after 2 weeks. Conversely, GM-EA production was...
initially low but increased progressively with time and peaked at 20 to 30 days postincubation. Thereafter, the capacity of cells to produce GM-EA decreased and fell to about one third of the peak value in about 50 days. After 20 to 30 days of cultivation, cells could be identified as typical MDLMs and the maximal GM-enhancing activity produced by these cells usually ranged from 50% to 100% enhancement with a mean value of 81 ± 6% enhancement.

**Effect of IL-1 and anti-IL-1 on GM-CSA and GM-EA activities.** As illustrated in Table 2, rIL-1 (10 to 100 U/mL) had no apparent enhancement or suppression on the formation of GM colonies induced by GM-CSA. Also demonstrated in Table 2 is that the presence of anti-IL-1 antibody (10 to 100 neutralizing units/mL) had no inhibitory effect on the potency of GM-CSA by GM-EA (78.4% ± 67.5% to 75.7% enhancement). These results suggest that the function of GM-EA cannot be replaced by IL-1 and that GM-EA is not antigenically related to IL-1.

**Effect of IFN-γ, PGE₃₃, and LF on GM-EA production.** As shown in Figure 1, a dose-dependent inhibition of GM-EA production was observed in cultures pretreated with IFN-γ, PGE₂, and LF. The level of suppression was in the order of IFN-γ > PGE₂ > LF. In the presence of IFN-γ (900 U/mL) or PGE₂ (10⁻⁹ mol/L) for 24 hours, the production of GM-EA by MDLMs was negligible. However, LF was unable to achieve total suppression, and about 36% of GM-EA activity remained in cultures treated with LF (10⁻⁸ mol/L) for 24 hours (Fig 1). Since both IFN-γ and PGE₂ could directly inhibit the colony formation of CFU-GM, we examined whether these two mediators were present in the different preparations of MDLM-CM (sources of GM-CSA), and our results indicate that neither IFN-γ nor PGE₂ was detectable in any of the samples tested (results not shown). Furthermore, the colony type (G, M, GM) distribution induced by GM-CSA was unaffected by the addition of GM-EA prepared from IFN-γ, PGE₂, and LF-treated or untreated MDLMs (Table 3).

Also shown in Fig 1 is that when MDLMs were treated simultaneously with IFN-γ and anti-IFN-γ, suppression of GM-EA production was not observed, indicating that the

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**Table 1. Production of GM-EA and GM-CSA by Cultured Human Macrophages at Different Times Postestablishment**

<table>
<thead>
<tr>
<th>Time of CM Preparation (d)</th>
<th>GM-EA (No. of Colonies)</th>
<th>GM-CSA* (No. of Colonies)</th>
<th>Percentage of GM-CSA alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>0</td>
<td>58 ± 4</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>7-9</td>
<td>19 ± 2</td>
<td>8 ± 1</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>14-16</td>
<td>50 ± 4</td>
<td>1</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>21-23</td>
<td>81 ± 6</td>
<td>0</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>28-31</td>
<td>80 ± 7</td>
<td>0</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>35-37</td>
<td>66 ± 6</td>
<td>0</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>42-44</td>
<td>55 ± 5</td>
<td>0</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>49-51</td>
<td>31 ± 3</td>
<td>0</td>
<td>100 ± 1</td>
</tr>
</tbody>
</table>

Three-day-old conditioned media (CM) from human monocyte/macrophage cultures at various times postincubation were collected and assayed for GM-EA and GM-CSA as described in Materials and Methods. Results are given as mean ± SEM.

*The activity of GM-CSA is expressed as the number of GM colonies formed per 5 x 10⁶ bone marrow mononuclear cells.

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**Table 2. Effect of rIL-1 and Anti-IL-1 Antibody on the Enhancement of GM-CSA-Induced Colony Formation by GM-EA**

<table>
<thead>
<tr>
<th>Material Assayed</th>
<th>No. of CFU-GM</th>
<th>Δ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSA (GCT-CM)</td>
<td>74 ± 4</td>
<td>100</td>
</tr>
<tr>
<td>GM-CSA + GM-EA (MDLM-CM)</td>
<td>132 ± 7</td>
<td>178.4</td>
</tr>
<tr>
<td>GM-CSA + rIL-1</td>
<td>10 U/mL</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>GM-CSA + Anti-IL-1</td>
<td>100 U/mL</td>
<td>93.2</td>
</tr>
<tr>
<td>GM-CSA + GM-EA + Anti-IL-1</td>
<td>124 ± 8</td>
<td>167.5</td>
</tr>
</tbody>
</table>

Recombinant human IL-1 (rIL-1) or anti-human IL-1 antibody (anti-IL-1) was added at 10% (vol/vol) to the assayed cultures containing an optimal concentration of GM-CSA (10% GCT-CM) with or without 10% MDLM-CM (GM-EA). Results are expressed as number of GM colonies (mean ± SEM) per 5 x 10⁶ nonadherent low-density bone marrow cells.

Abbreviation: NU, neutralizing unit.

*Percentage of GM-CSA alone.

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**Fig 1. Dose effect of IFN-γ, PGE₂, and LF on GM-EA production.** Well-developed MDLMs at 21 to 24 days of cultivation were treated or untreated with various concentrations of IFN-γ, PGE₂, or LF for 24 hours at 37°C. After removal of the reagents, the cells were further cultured for three days, and MDLM-CM were then collected for assay of GM-EA. Results are expressed as percent GM-EA activity of the untreated controls. (A) PGE₂ (●) and LF (○). (B) IFN-γ (□) and IFN-γ (300 U/mL) + anti-IFN-γ (300 neutralizing units/mL) (△).
Well-developed MDIMs were treated with 300 U/mL IFN-γ for two hours. Cultures pretreated with Zym for 48 hours exhibited the lowest GM-EA activity (35 ± 5% of untreated control). Treatment with IFN-γ and PGE2 or LF for 24 hours alone had a more pronounced effect on GM-EA production compared to the combined treatments (Fig 2). Zym alone was less effective than IFN-γ or PGE2/LF, indicating that synergy between these mediators is crucial for optimal suppression of GM-EA production.

Effect of zymosan on inhibition. Results in Table 4 show that GM-EA containing zymosan (Zym) similarly produced higher levels of GM-EA than the untreated control regardless of whether the inhibitors are present or not. Furthermore, similar results were obtained when cells were pretreated with the inhibitors followed by Zym treatment or when cells were treated simultaneously with both the inhibitors and the stimulator (Table 4). These results suggest that Zym can override the inhibitory signals produced by the inhibitors.

Purification of GM-EA. Crude GM-EA was concentrated about 100-fold by ammonium sulfate precipitation and then loaded onto a DEAE-TSK column and eluted with 0.04 mol/L NaCl. Fractions with GM-EA activity were pooled, concentrated, and then analyzed by SDS-PAGE. As shown in Fig 3, GM-EA corresponded to two pairs of closely linked bands (lanes C). When the column-purified GM-EA preparation was analyzed in 7.5% preparative PAGE, only two bands were found to be biologically active, suggesting that GM-EA may exist in two different forms (data not shown). However, when these two protein bands were analyzed on 12% SDS-PAGE, the proteins were found to have similar apparent mol wts (Fig 3, lanes D and E) of about 73 to 74 Kd and both of them were glycosylated (data not shown). Preliminary amino acid sequencing data of these two proteins revealed that the two forms of GM-EA are similar but different. Details of the results concerning the purification and biochemical characterization of GM-EA will be published elsewhere (Wang et al, manuscript in preparation).

Table 3. Morphologic Analysis of GM Colonies Induced by GM-CSA With or Without Various Preparations of GM-EA

<table>
<thead>
<tr>
<th>Material Assayed</th>
<th>Colony Types (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
</tr>
<tr>
<td>GM-CSA (GCT-CM)</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>GM-CSA + GM-EA</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>GM-CSA + GM-EA (t)*</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>PGE2</td>
<td>59 ± 3</td>
</tr>
</tbody>
</table>

GM-EA containing supernatant prepared from drug-treated or untreated MDLMs was added at a final concentration of 10% (vol/vol) to CFU-GM assay cultures. Morphologic analysis of day-7 GM colonies was performed after agar gel fixation and staining.

Abbreviations: G, granulocytes; M, monocytes/macrophages; GM, granulocytes and monocytes/macrophages.

*MDLMs were pretreated with IFN-γ (30 to 100 U/mL), PGE2 (10^-10 to 10^-11 mol/L), or LF (10^-8 to 10^-9 mol/L) for 24 hours at 37°C before preparation of GM-EA.

Inhibition was specific for IFN-γ and was not mediated by contaminants in the drug preparation.

To determine whether the mediators are cytotoxic to MDLMs, we treated cells with IFN-γ (300 U/mL), PGE2 (10^-9 mol/L) or LF (10^-8 mol/L) for 24 to 48 hours, and then assessed their viability and phagocytic capacity. Our results indicated that the percent viability (96%) and the phagocytic capacity were proportional to the duration of treatment. The effect of IFN-γ appeared to be most efficient so that exposure of cells to IFN-γ for as little as two hours was sufficient to reduce the production of GM-EA by 66% and total suppression was observed when target cells were treated for 48 hours. The action of PGE2 was similar but less obvious and required a longer incubation period to manifest its function. Finally, at all the time points tested, LF appeared to be less potent than the other two reagents (Fig 2).

Effect of zymosan on inhibition. Results in Table 4 show that the cultures containing zymosan (Zym) invariably produce higher levels of GM-EA than the untreated control regardless of whether the inhibitors are present or not. Furthermore, similar results were obtained when cells are pretreated with the inhibitors followed by Zym treatment or when cells are treated simultaneously with both the inhibitors and the stimulator (Table 4). These results suggest that Zym can override the inhibitory signals of all three types of inhibitors.

Purification of GM-EA. Crude GM-EA was concentrated about 100-fold by ammonium sulfate precipitation and then loaded onto a DEAE-TSK column and eluted with 0.04 mol/L NaCl. Fractions with GM-EA activity were pooled, concentrated, and then analyzed by SDS-PAGE. As shown in Fig 3, GM-EA corresponded to two pairs of closely linked bands (lane C). When the column-purified GM-EA preparation was analyzed in 7.5% preparative PAGE, only two bands were found to be biologically active, suggesting that GM-EA may exist in two different forms (data not shown). However, when these two protein bands were analyzed on 12% SDS-PAGE, the proteins were found to have similar apparent mol wts (Fig 3, lanes D and E) of about 73 to 74 Kd and both of them were glycosylated (data not shown). Preliminary amino acid sequencing data of these two proteins reveal that the two forms of GM-EA are similar but different. Details of the results concerning the purification and biochemical characterization of GM-EA will be published elsewhere (Wang et al, manuscript in preparation).

Table 4. Effect of Zymosan on GM-EA Production by Normal and Drug-Inhibited MDLMs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GM-EA (%)</th>
<th>Δ %†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>82 ± 6</td>
<td>—</td>
</tr>
<tr>
<td>Zym (60 µg/mL) alone</td>
<td>160 ± 9</td>
<td>195</td>
</tr>
<tr>
<td>LF (10^-8 mol/L) alone</td>
<td>30 ± 3</td>
<td>36</td>
</tr>
<tr>
<td>LF (10^-8 mol/L) + Zym (60 µg/mL)</td>
<td>140 ± 8</td>
<td>170</td>
</tr>
<tr>
<td>LF (10^-8 mol/L)→Zym (60 µg/mL)*</td>
<td>138 ± 8</td>
<td>168</td>
</tr>
<tr>
<td>PGE2 (10^-9 mol/L) alone</td>
<td>3 ± 4</td>
<td></td>
</tr>
<tr>
<td>PGE2 (10^-9 mol/L) + Zym (60 µg/mL)</td>
<td>124 ± 7</td>
<td>151</td>
</tr>
<tr>
<td>PGE2 (10^-9 mol/L)→Zym (60 µg/mL)*</td>
<td>119 ± 9</td>
<td>145</td>
</tr>
<tr>
<td>IFN-γ (300 U/mL) alone</td>
<td>4 ± 5</td>
<td></td>
</tr>
<tr>
<td>IFN-γ (300 U/mL) + Zym (60 µg/mL)</td>
<td>118 ± 7</td>
<td>144</td>
</tr>
<tr>
<td>IFN-γ (300 U/mL)→Zym (60 µg/mL)*</td>
<td>116 ± 6</td>
<td>141</td>
</tr>
</tbody>
</table>

Well-developed MDLMs at 21 to 24 days of cultivation were pretreated with or without different reagents at 37°C for 24 hours and then cultured for three days in normal medium to prepare MDLM-CM for assay of GM-EA. Results are given as mean ± SEM.

†Percent activity of the untreated control.
GRANULOMONOPOIETIC ENHANCING ACTIVITY

DISCUSSION

We have previously demonstrated that well-differentiated macrophages (MDLMs) produce a GM-EA that functions in conjunction with GM-CSA in the promotion of myelopoiesis. Correspondingly, a similar phenomenon has been observed in long-term bone marrow cultures of a colony promoting activity (CPA) that may be the counterpart of the GM-EA in our system.

In this study, we demonstrate that GM-EA production is inhibited by IFN-γ, PGE₂, and LF, all of which are well-established modulators of macrophage functions. The observed inhibition is dose dependent (Fig 1) and can be manifested following only a brief exposure (two hours or more) of MDLMs to either IFN-γ or PGE₂ (Fig 2), suggesting that either one of them can constitute an efficient negative control on GM-EA production. It is noteworthy that the suppressive effect of all these inhibitors is reversible and can be overridden by the addition of Zym, a macrophage stimulator capable of promoting the production of both GM-CSA and GM-EA. This happens regardless of whether Zym is added in parallel with the inhibitors or 24 hours following treatment (Table 4). Thus, it appears that the interaction between Zym and the inhibitors does not involve competition at the cell surface receptor level.

Despite the fact that GM-EA shares certain functional properties with IL-1 and hemopoietin-1 (H-1) in the regulation of hemopoiesis, it is a distinct mediator. Our previous observation as well as those reported by others have shown that IL-1 is a product of the younger macrophages, while GM-EA is synthesized predominantly by the fully mature macrophages (MDLMs). Moreover, IL-1 does not mimic the activity of GM-EA in the potentiation of GM-CSA, and anti-IL-1 antibody does not abrogate the enhancing effect of GM-EA (Table 2). Similar result on the lack of promoting effect of IL-1 on GM-CSA was observed by Segal et al. In addition, highly purified GM-EA has an apparent mol wt of 73 to 74 Kd (Fig 3), which is quite different from those reported for IL-1. Finally, GM-EA differs from H-1 in biochemical properties and in the types of target cells it acts on. Thus, while GM-EA potentiates only the colony formation of granulomonopoietic progenitor cells, H-1 targets on primitive, multipotent stem cells. Therefore, it appears that GM-EA is a new type of positive myelopoietic regulator.

At an earlier stage of differentiation, monocytoid cells can directly or indirectly promote myelopoiesis through production of GM-CSA and GM-EA, and correspondingly they secrete PGE and/or TNF, which mediate the negative feedback control. At the well-differentiated stage, they can exert an auxiliary amplifying signal (GM-EA) to myelopoiesis, but its effect is in turn controlled at the production level by mediators (PGE₂ and IFN-γ) of their less differentiated counterpart and T lymphocytes. Our results together with those of other investigators show that myelopoiesis is controlled by complex interplay of positive and negative feedback mechanisms mediated by monocytoid cells at different stages of differentiation. In addition, the nature and many of the functional characteristics of GM-EA remain to be determined.

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Down regulation of myelopoiesis by mediators inhibiting the production of macrophage-derived granulomonopoietic enhancing activity (GM-EA)

SY Wang, CK Ho, LY Chen, RC Wang, MH Huang, H Castro-Malaspina and MA Moore