The Effect of Three Human Recombinant Hematopoietic Growth Factors (Granulocyte-Macrophage Colony-Stimulating Factor, Granulocyte Colony-Stimulating Factor, and Interleukin-3) on Phagocyte Oxidative Activity

By Gail W. Sullivan, Holliday T. Carper, and Gerald L. Mandell

Hematopoietic growth factors not only modulate blood progenitor cell activity but also alter the function of mature phagocytes. Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF; 1 ng/mL for 60 min) did not stimulate luminal-enhanced chemiluminescence of polymorphonuclear leukocytes (PMNs) in suspension but primed PMN for as much as a 15-fold increase in chemiluminescence in response to f-met-leu-phe (fMLP). Mixed mononuclear leukocytes (monocytes (~20%) and lymphocytes (~80%); MNL) chemiluminescence was very low even after rhGM-CSF priming, but MNLs added to the PMNs (PMN-MNL) resulted in near doubling of rhGM-CSF-primed PMN fMLP-stimulated chemiluminescence. The enhancing factor(s) from MNLs were inherent rather than induced by the GM-CSF, and primed lymphocytes increased GM-CSF-primed PMN chemiluminescence equal to mixed MNLs. We could not detect cell-free "enhancing factor(s)" but cell-to-cell contact further enhanced rhGM-CSF-primed fMLP-stimulated PMN-MNL oxidative activity by 40%. Polyclonal rabbit anti-tumor necrosis factor (TNF) (but not preimmune serum) decreased both fMLP-stimulated rhGM-CSF-primed PMNs and PMN-MNL chemiluminescence, suggesting that TNF on the PMN surface is enhancing GM-CSF-primed chemiluminescence. GM-CSF priming markedly increased PMN superoxide release (sevenfold), but PMN superoxide release was not further enhanced by the presence of MNLs. Recombinant human granulocyte colony-stimulating factor (rhG-CSF) and interleukin-3 (rhIL-3) displayed much smaller effects on pure PMNs and mixed PMN-MNL chemiluminescence and superoxide release than rhGM-CSF. rhG-CSF primes PMNs for increased oxidative activity more than rhG-CSF and rhIL-3. Maximal oxidative activity was observed when mixed PMN-MNL were primed with GM-CSF in a cell pellet–promoting cell-to-cell contact. This enhanced activity can be attributed, in part, to both inherent enhancing factor(s) on lymphocytes and PMN-associated TNF induced by GM-CSF.

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Materials and Methods

Materials

Luminol, fMLP, trypan blue, catalase, O-dianisidine, human leukocyte myeloperoxidase (MPO), and ferricytochrome c were from Sigma Chemical Co (St Louis, MO). Ficoll-Hypaque was purchased from Flow Laboratories (McLean, VA) and Los Alamos Diagnostics (Los Alamos, NM). Hanks' balanced salt solution (HBSS) and the limulus amebocyte lysate assay kit were from Whittaker Bioproducts (Walkersville, MD). Human serum albumin (HSA) was from Cutter Biological (Elkhart, IN).

Recombinant human granulocyte-macrophage colony-stimulating factor expressed in yeast (rhGM-CSFy; 5 × 10^10 colony forming units [CFUs]/mg), recombinant human granulocyte colony-stimulating factor (G-CSF), and interleukin-3 (IL-3) are three major growth factors that have been sequenced and cloned. There is a growing list of proposed applications for these growth factors, including treatment of cytopenias and infectious diseases, amelioration of myelosuppression induced by chemotherapy and radiotherapy and facilitation of bone marrow engraftment following transplantation. In addition to their proliferating and differentiating effects on blood progenitor cells, growth factors can also alter the activity of mature leukocytes. The oxidative burst is triggered by ingestion of particles or microbes, by contact with certain biological surfaces, and by certain soluble molecules such as f-met-leu-phe (fMLP) and endotoxin. Products of the oxidative burst serve important microbicidal and tumoricidal functions but can damage host tissue. Thus, knowledge of the influence of growth factors on the polymorphonuclear leukocyte (PMN) oxidative burst is important for evaluation of these factors for various clinical uses. The present study examines and compares the effects of human recombinant GM-CSF, G-CSF, and IL-3 on in vitro human PMN oxidative activity and explores the role of leukocyte interaction in growth factor stimulation of PMN oxidative activity.

Materials and Methods

Materials

Luminol, fMLP, trypan blue, catalase, O-dianisidine, human leukocyte myeloperoxidase (MPO), and ferricytochrome c were from Sigma Chemical Co (St Louis, MO). Ficoll-Hypaque was purchased from Flow Laboratories (McLean, VA) and Los Alamos Diagnostics (Los Alamos, NM). Hanks' balanced salt solution (HBSS) and the limulus amebocyte lysate assay kit were from Whittaker Bioproducts (Walkersville, MD). Human serum albumin (HSA) was from Cutter Biological (Elkhart, IN).

Recombinant human granulocyte-macrophage colony-stimulating factor expressed in yeast (rhGM-CSFy; 5 × 10^10 colony forming units [CFUs]/mg), recombinant human granulocyte colony-stimulating factor expressed in yeast (rhG-CSF; 11.1 ± 9.2 × 10^10 CFU/mg), and recombinant human interleukin-3 expressed in yeast (IL-3; 0.9 to 6.7 × 10^6 IU/mg) were supplied by Immunex Corp (Seattle, WA). rhGM-CSF expressed in Escherichia coli (5 × 10^5 IU/mL) was supplied by Behringwerke AG, Marburg, Germany. The endotoxin content of these growth factor preparations was <1 ng/mg protein by limulus amebocyte lysate assay (Hoechst-Roussel Pharmaceuticals, Somerville, NJ).

Preimmune rabbit serum, rabbit polyclonal anti-human GM-CSF (10,000 neutralizing units [NU]/mL), rabbit polyclonal anti-human IL-18 (10,000 NU/mL), and rabbit polyclonal anti-human TNF (10,000 NU/mL) were purchased from Endogen Inc (Boston, MA).

Leukocyte Preparation

Purified PMNs (~95% PMNs and >95% viable by trypan blue exclusion) containing ~1 platelet/5 PMNs and <50 pg/mL endotoxin (limulus amebocyte lysate assay) were obtained from normal heparinized (10 U/mL) venous blood by a one-step ficoll-Hypaque sep-
uation procedure. Residual red blood cells (RBCs) were lysed by hypotonic lysis with 3 mL of iced 0.22% sodium chloride solution for 45 seconds followed by 0.88 mL 3% sodium chloride solution.

The mononuclear leukocyte fraction (MNLs) (>95% viable by trypan blue exclusion) was retained from the ficoll-Hypaque separation described above. This fraction was composed of monocytes (~15% to 20%) and lymphocytes (~80% to 85%). Mixed leukocyte preparations (PMN-MNL) were made by combining PMNs with MNLs.

Monocyte-depleted preparations of MNLs were prepared by allowing the cells to adhere to a tissue culture-grade plastic Petri plate for 45 minutes at 37°C (5% CO2). The nonadherent cells were retained, centrifuged 150 g × 5 min and resuspended in HBSS. The preparation of nonadherent cells was ~94% lymphocytes as confirmed by negative nonspecific esterase stain.

**PMN-MNL—Conditioned Media**

PMNs (5 × 10^6/mL) plus MNLs (5 × 10^6/mL) HBSS containing 0.1% human serum albumin were incubated for 60 minutes at 37°C with or without rhGM-CSF (1 mg/mL). The samples were then iced and the cells removed by centrifugation, yielding a cell-free PMN-MNL—conditioned medium.

**Leukocyte Oxidative Activity**

**Chemiluminescence**

Chemiluminescence is a measure of PMN oxidative activity. The light is emitted from unstable high-energy oxygen species generated by activated phagocytes. Purified PMNs (5 × 10^6 PMNs) and/or MNLs (5 × 10^6) were incubated in HBSS containing 0.1% human serum albumin (1 mL) with or without growth factors 0 to 120 minutes at 37°C in a shaking water bath. Then luminol-enhanced chemiluminescence was read with a Chronolog Photometer (Chrono-log Corp, Havertown, PA) at 37°C for 12 minutes. Chemiluminescence is reported as relative peak light emitted (height of the curve) in arbitrary units (AU).

**Superoxide Release**

The growth factors were added to PMNs (5 × 10^6/mL), MNLs (5 × 10^6/mL), or PMNs (5 × 10^6/mL) plus MNLs (5 × 10^6/mL), and the cells were pelleted by centrifugation (150g × 5 min) into buttons. The cell buttons were incubated for 60 minutes at 37°C and then resuspended by vortex agitation. Catalase (0.062 mg/mL) was added to prevent H2O2 reoxidation of cytochrome c. Cytochrome c (120 μmol/L) and fMLP (10^-4 mol/L) were added to the cells and the samples incubated for 10 minutes more at 37°C in a shaker bath. Matched samples containing superoxide dismutase (SOD) (200 U/ sample) were also prepared. The samples were iced and centrifuged (2,000g × 10 min). The optical density of the supernatants was read at 550 nm, and the nanomoles of SOD-inhibitable superoxide released in 10 minutes were calculated with the extinction coefficient of 2.11 × 10^5 cm²/mmol.

**Leukocyte Myeloperoxidase Release**

PMNs (5 × 10^6/mL), MNLs (3 × 10^6/mL), or PMNs (5 × 10^6/mL) plus MNLs (3 × 10^6/mL) were primed ± rhGM-CSF (8 ng/mL) for 120 minutes. The cells were then stimulated with fMLP (1 μmol/L × 10 min). The samples were iced and the cells removed by centrifugation (2000g × 10 min). The cell-free supernatants were assayed spectrophotometrically for MPO release by measuring oxidation of O-dianisidine. The amount of MPO released was determined by comparing dilutions of the cell-free supernatant activity to a standard curve made by reacting known concentrations of human leukocyte MPO (0 to 3 μg/mL) with O-dianisidine (2 mg/mL) in the presence of hydrogen peroxide (2.5 mmol/L) for 20 minutes at 23°C, 350 nm. The results are expressed as MPO released (μg).

**Statistical Analyses**

The data were analyzed by Student’s two-tailed t-test.

**RESULTS**

**Leukocyte Oxidative Activity**

**Chemiluminescence**

GM-CSF and G-CSF (but not II-3) prime PMNs and PMN-MNL for increased fMLP-stimulated chemiluminescence. Leukocytes were primed for 120 minutes with or without rhIL-3, rhG-CSF, or rhGM-CSF (8 ng/mL), then stimulated with fMLP, and peak chemiluminescence recorded. Chemiluminescence in the absence of CSFs was low for MNLs, PMNs, and PMN-MNL. Priming with rhIL-3 had little effect on leukocyte chemiluminescence. Priming with rhG-CSF caused a small but significant increase in fMLP-stimulated PMN chemiluminescence (P < .032) and PMN-MNL chemiluminescence (P = .045). Recombinant human GM-CSF primed PMN (P = .002) and PMN-MNL (P = .006) for an increased oxidative response to fMLP and was the most active of the three CSFs. Only with rhGM-CSF priming was there an indication of interaction between the MNLs and PMNs. The oxidative burst in the PMN-MNL preparation was greater than the sum of the contributions of PMNs and MNLs alone (Fig 1).

When PMN-MNL were primed with growth factors for 45 minutes and then stimulated with fMLP, priming with rhIL-3 (1 to 8 ng/mL) did not affect chemiluminescence, rhG-CSF (4 to 8 ng/mL) slightly increased chemiluminescence (P < .050), and rhGM-CSF and rhGM-CSF (0.2 to 4 ng/mL) markedly increased the fMLP-stimulated oxidative burst (P < .010). There was no significant difference in the chemiluminescence response primed by rhGM-CSF and rhG-CSF at any of the assayed concentrations (Fig 2).

**GM-CSF primes PMNs by 45 minutes for increased chemiluminescence in response to fMLP.** In the absence of rhGM-CSF, PMN-MNL displayed very little chemiluminescence in response to fMLP. Recombinant human GM-CSF (1 ng/mL) primed PMNs for a threefold increase in chemiluminescence in response to fMLP after 45 minutes of incubation (P = .015), and rhGM-CSF increased PMN-MNL fMLP-stimulated chemiluminescence fivefold by 120 minutes of incubation (P = .003). Priming with rhGM-CSF did not affect fMLP-stimulated chemiluminescence of MNLs in the absence of PMNs (Fig 3). There was a dose-response relationship between the number of MNLs (0 to 1 × 10^6 MNLs/mL) and the amount of chemiluminescence, especially in the GM-CSF—primed cell preparations (Fig 4).

The chemiluminescence-enhancing factor(s) appeared to be inherent in MNLs rather than induced by GM-CSF. If the MNLs were primed with rhGM-CSF (1 ng/mL × 90 min) in the absence of PMNs and then the primed MNLs and unprimed PMNs combined, the amount of chemiluminescence in response to fMLP was only half of what was observed when PMN-MNL were primed with GM-CSF (Fig 5).
was observed if combined PMN-MNL had been primed with GM-CSF \((P = .005)\) and equal to the activity when neither the PMNs nor the MNLs had been primed \((P = .646)\) (Fig 5).

Purified lymphocytes \((\sim 94\%)\) had comparable activity to mixed MNL preparations in enhancing rhGM-CSFe-primed fMLP-stimulated PMN chemiluminescence. Chemiluminescence in response to fMLP was 6.96 ± 0.71 AU when PMN-MNL were primed for 120 minutes with rhGM-CSFe (1 ng/mL) and then stimulated with fMLP (1 μmol/L) compared with 6.86 ± 1.12 AU when an equal concentration of purified lymphocytes were substituted for the mixed MNLs \((P = .956)\). Thus, it appears that purified lymphocytes can enhance GM-CSF-primed fMLP-stimulated PMN chemiluminescence.

Cell-associated tumor necrosis factor contributes to GM-CSF priming of pure PMNs and PMN-MNL chemiluminescence. To test for the release of soluble chemiluminescence-enhancing factor(s), we primed PMNs with cell-free conditioned medium derived from PMN-MNL stimulated with 1 ng/mL rhGM-CSFe (GMCM). The amount of PMN chemiluminescence with GMCM priming was equal to that observed with rhGM-CSFe priming \((4.25 ± 0.52 \text{ and } 5.90 ± 1.08 \text{ relative light units, respectively; } P = .116)\), and GMCM priming was eliminated by incubation of the GMCM (18 h at 4°C) with anti-GM-CSF (200 NU/mL) before PMN priming \((0.38 ± 0.03 \text{ relative light units; } P = .005)\). Incubation of GMCM with preimmune serum did not decrease the GMCM priming \((4.43 ± 0.49 \text{ relative light units; } P = .678)\), and control (ie, no GM-CSF) PMN-MNL-conditioned me-
medium did not prime pure PMNs for increased chemiluminescence in response to fMLP (0.18 ± 0.03 relative light units). This indicates that the chemiluminescence-enhancing factor(s) stimulated by GM-CSF are not being released to the medium.

Because no soluble factor was detected, we examined the possibility that a cell-bound factor was involved. To see if we could maximize priming by increasing cell-to-cell contact, we primed PMNs and PMN-MNL in dispersed suspension (ie, the method employed in the experiments described above) or pelleted by centrifugation into buttons. Following the priming period (60 minutes), all cultures were dispersed by vortex agitation and then chemiluminescence in response to fMLP was measured. We observed that pelleting did not enhance the chemiluminescence response of PMNs with or without GM-CSF, but pelleted PMN-MNL in the presence of rhGM-CSF (1 ng/mL) had a greater chemiluminescence response than cells that had been primed while dispersed (P = .035) (Fig 6). Thus, promotion of cell-to-cell contact further enhances priming by GM-CSF in mixed PMN-MNL cultures.

Because GM-CSF stimulates tumor necrosis factor alpha (TNF-α) production and cell-bound TNF can be biologically active, we examined the role of TNF in GM-CSF-enhanced chemiluminescence. PMNs or PMN-MNL were primed (60 min) with rhGM-CSF (1 ng/mL) in the presence or absence of 20 μL preimmune rabbit serum or rabbit polyclonal anti-human TNF (200 NU/mL) and then chemiluminescence in response to fMLP was measured. Anti-human TNF (but not preimmune serum) markedly decreased (by

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**Fig 3.** Time course of GM-CSF priming of leukocyte chemiluminescence in response to fMLP. PMNs, MNLs, or PMN-MNL were incubated with rhGM-CSF (1 ng/mL) for 15 to 120 minutes at 37°C and then stimulated with fMLP. The results are reported as relative peak fMLP-stimulated chemiluminescence in arbitrary light units. Mean ± SEM (n = six separate experiments). *P < .05 GM-CSF increased chemiluminescence in response to fMLP.

**Fig 4.** MNL increased PMN chemiluminescence in response to fMLP. PMNs (5 × 10⁶/mL) with MNLs (0 to 1 × 10⁶/mL) were incubated with or without rhGM-CSF (1 ng/mL) for 120 minutes at 37°C and then stimulated with fMLP. The results are reported as relative peak fMLP-stimulated chemiluminescence in arbitrary light units. Mean ± SEM (n = five separate experiments). *P < .050 MNL increased chemiluminescence in response to fMLP compared with no MNL.

**Fig 5.** The chemiluminescence-enhancing effect of MNLs is inherent rather than induced by GM-CSF. The leukocytes were primed with rhGM-CSF (1 ng/mL) as follows: A, rhGM-CSF was added to unprimed PMN-MNL; B, MNLs were primed with rhGM-CSF for 90 minutes; C, PMN-MNL were primed with rhGM-CSF for 90 minutes; D, MNLs were primed with rhGM-CSF and then combined with unprimed PMNs. The leukocytes were then stimulated with fMLP and chemiluminescence assayed. The results are reported as relative peak fMLP-stimulated chemiluminescence in arbitrary light units. Mean ± SEM (n = five separate experiments).
GM-CSF, G-CSF, IL-3, and PMN Oxidative Activity

PMN

PMN-MNL

Fig 6. Pelleting of PMN-MNL in the presence of GM-CSF increased priming of fMLP-stimulated chemiluminescence. Pure PMNs (5 x 10^6/mL) or PMN (5 x 10^6/mL) plus MNLs (5 x 10^6/mL) were incubated (37°C for 60 minutes) with rhGM-CSFe (1 ng/mL) either in dispersed suspension or pelleted buttons. All cells were then dispersed and fMLP-stimulated chemiluminescence was assayed. The results are reported as relative peak fMLP-stimulated chemiluminescence in arbitrary light units. Mean ± SEM (n = three to six separate experiments).

Superoxide Release

Superoxide release was assayed from pelleted PMN, MNL, and PMN-MNL cell preparations that had been incubated with or without growth factors (1 ng/mL x 60 min). Priming pelleted pure PMNs with rhGM-CSFe resulted in a sevenfold increase in fMLP-stimulated superoxide release (P = .004), but the addition of MNLs to the PMNs did not further enhance superoxide release (P = .004 compared with PMN-MNL in the absence of GM-CSF, and P = .132 compared with pure PMN primed with GM-CSF) (Table 2).

Recombinant human IL-3 and rhG-CSF did not significantly prime pure PMNs for increased superoxide release in response to fMLP (P = .316 and P = .321, respectively) nor mixed PMN-MNL (P = .480 and P = .185, respectively) (Table 2).

Leukocyte MPO Release

Because luminol-enhanced chemiluminescence is dependent upon myeloperoxidase mobilization,13,14 we assayed the effect of MNLs and rhGM-CSF on PMN degranulation by measuring the release of MPO from the leukocytes into the surrounding medium after stimulation with fMLP. MNL preparations did not release MPO either in the presence or the absence of rhGM-CSF (10 ng/mL). fMLP-stimulated PMN alone released 2.6 μg MPO; MNLs increased this by ~70%) both GM-CSF–primed pure PMNs (P = .014) and mixed PMN-MNL (P = .030) chemiluminescence in response to fMLP (Table 1).

GM-CSF can stimulate leukocyte production of IL-1.22 Anti–IL-1β (200 NU/mL) decreased rhGM-CSFe (8 ng/mL)–primed mixed PMN-MNL chemiluminescence 18% from 5.06 ± 1.45 AU to 4.13 ± 1.3 AU (P = .018). Therefore, IL-1 could be a contributing factor to GM-CSF–primed PMN-MNL oxidative activity.

Table 1. Effect of Polyclonal Anti-TNF on Priming of PMN by rhGM-CSFe

<table>
<thead>
<tr>
<th>Condition</th>
<th>PMN</th>
<th>PMN-MNL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No GM-CSF</td>
<td>0.17 ± 0.03</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>Preimmune serum</td>
<td>0.17 ± 0.07</td>
<td>2.27 ± 0.89</td>
</tr>
<tr>
<td>Anti-TNF (200 NU/mL)</td>
<td>0.10 ± 0.06</td>
<td>0.47 ± 0.14</td>
</tr>
<tr>
<td>rhGM-CSFe (1 ng/mL)</td>
<td>3.05 ± 0.47</td>
<td>5.82 ± 1.30</td>
</tr>
<tr>
<td>Preimmune serum</td>
<td>3.63 ± 0.42</td>
<td>6.37 ± 0.88</td>
</tr>
<tr>
<td>Anti-TNF (200 NU/mL)</td>
<td>1.02 ± 0.24*</td>
<td>1.75 ± 0.35*</td>
</tr>
</tbody>
</table>

PMNs or PMN plus MNL were incubated 60 minutes with or without rhGM-CSFe and preimmune rabbit serum or rabbit polyclonal anti-human TNF and then chemiluminescence in response to fMLP was measured. The results are reported as mean relative peak chemiluminescence ± SEM (n = three to six separate experiments).

*P < .05 Anti-TNF decreased chemiluminescence compared with no antibody.

Table 2. Effect of Growth Factor Priming on PMNs, MNLs, and PMN-MNL Superoxide Release

<table>
<thead>
<tr>
<th>Condition</th>
<th>PMN</th>
<th>MNL</th>
<th>PMN-MNL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>2.1 ± 1.3</td>
<td>2.1 ± 0.5</td>
<td>3.9 ± 1.0</td>
</tr>
<tr>
<td>rhGM-CSFe</td>
<td>15.3 ± 0.9*</td>
<td>2.7 ± 0.5</td>
<td>18.8 ± 1.2*</td>
</tr>
<tr>
<td>rhG-CSF</td>
<td>3.5 ± 1.8</td>
<td>2.6 ± 1.1</td>
<td>8.2 ± 1.15</td>
</tr>
<tr>
<td>rhIL-3</td>
<td>4.6 ± 2.7</td>
<td>1.6 ± 0.6</td>
<td>7.1 ± 1.8</td>
</tr>
</tbody>
</table>

PMNs (5 x 10^6/mL), MNLs (5 x 10^6/mL), or PMNs (5 x 10^6/mL) plus MNLs (5 x 10^6/mL) were primed with or without growth factors (1 ng/mL) for 60 minutes with the cells in a pellet. The cells were then resuspended and stimulated with fMLP (1 μmol/L x 10 min) and assayed for SOD-inhibitable superoxide release. Results are expressed as superoxide released (nmol/10 min). Mean ± SEM (n = four to 10 separate experiments).

*P < .050 compared with no growth factor.
46% \( (P = .002) \). Recombinant human GM-CSF (8 ng/mL) increased the release of MPO from PMN 169% \( (P = .013) \), and rhGM-CSF increased MPO release from PMN-MNL 103% \( (P = .007) \) compared with PMN-MNL (Table 3).

**DISCUSSION**

Although GM-CSF does not stimulate superoxide release directly from nonadherent PMNs, it primed PMNs for enhanced oxidative activity in response to a second stimulus, including fMLP, the complement component C5a, leukotriene B4, phorbol myristate acetate, and opsonized zymosan.\(^{3,5,9} \) GM-CSF directly stimulates the oxidative activity of PMNs adhered to surfaces coated with extracellular matrix proteins.\(^{8} \) Similar to GM-CSF, G-CSF primes PMNs for increased oxidative activity in response to a second stimulus or adherence.\(^{3,8} \) Cloned gibbon IL-3 has been reported to stimulate eosinophil superoxide release.\(^{37} \)

As has been previously reported,\(^{3,4,6,8,26} \) we observed that pharmacologic concentrations of rhGM-CSF—primed mature human PMNs for increased oxidative activity in response to fMLP, with peak activity occurring after 45 minutes of priming. The greatest oxidative activity was observed in PMN-MNL preparations in which cell-to-cell interaction was maximized. The chemiluminescence was almost threefold higher in pelleted PMN-MNL compared with suspended pure PMNs primed with GM-CSF and then stimulated with fMLP. The role of cell-to-cell contact in cell-to-cell communication (in addition to soluble factors) is becoming evident. For example, it has been observed that live and fixed activated lymphocytes can stimulate PMN superoxide release when cell-to-cell contact is promoted.\(^{38} \)

Recently, it has been reported that in vitro incubation with GM-CSF stimulates the expression of IL-1 receptors on PMNs. Thus, GM-CSF not only stimulates the production of the inflammatory cytokines TNF and IL-1,\(^{1,3,32} \) but also can increase PMN sensitivity to IL-1.\(^{39} \) Therefore, the inflammatory action of GM-CSF may be magnified by both increased production and enhanced sensitivity to other inflammatory cytokines.

GM-CSF increases the expression of the adherence factor Mac-1 on PMNs,\(^{25} \) and stimulates leukocyte aggregation.\(^{4,6} \) Increased cell-to-cell contact through cell aggregation may make the PMNs more sensitive to the enhancing factor(s) on MNLs.

In our experiments, neutralizing polyclonal antibody to TNF decreased the amount of chemiluminescence in both PMN and PMN-MNL cultures primed with rhGM-CSF, indicating that TNF may play a role in the enhancing effect of GM-CSF. Similar to GM-CSF, TNF primes PMNs for increased oxidative activity in response to a second stimulus\(^{41-45} \) and stimulates the oxidative burst of PMNs adherent to biological surfaces.\(^{4,6} \) Biologically active cell-associated TNF has been found on activated leukocytes.\(^{19-22} \) Our data suggest that there is PMN-associated TNF and this TNF is induced by GM-CSF because anti-TNF equally inhibited PMN chemiluminescence in both pure PMN and mixed PMN-MNL preparations and the antibody had little effect in the absence of GM-CSF. It has been reported that production of TNF by PMN is induced by GM-CSF.\(^{47} \)

![Table 3. Effect of rhGM-CSF Priming on PMNs, MNLs, and PMN-MNL MPO Release](attachment://table3.png)

Because the anti-TNF preparation used in these experiments was polyclonal whole rabbit serum, there is the possibility that steric hindrance from the relatively large immunoglobulin molecules binding to the PMNs may contribute to the observed oxidative burst by blocking GM-CSF and/or fMLP binding to PMNs.

We observed no difference in priming for the oxidative burst by two recombinant forms of GM-CSF. One was expressed in yeast and the other in *Escherichia coli*. Native GM-CSF is fully glycosylated; yeast-expressed recombinant GM-CSF is partially glycosylated; and *E coli*—expressed GM-CSF is nonglycosylated. *E coli*—expressed recombinant GM-CSF has the same potency as native GM-CSF in stimulating blood progenitor cells.\(^{48,49} \)

Like rhGM-CSF, rhG-CSF at achievable concentrations primed PMNs for increased superoxide release in response to fMLP and did not affect MNL superoxide release in response to fMLP. This was similar to results with chemiluminescence. In contrast to what was found with chemiluminescence, the presence of MNLs did not further increase superoxide release from GM-CSF—primed PMNs. Luminol-enhanced chemiluminescence is not directly related to superoxide release.\(^{50,52} \) Chemiluminescence differs from superoxide release in that the chemiluminescence response is dependent, in part, upon MPO mobilization that occurs subsequent to superoxide release.\(^{5,14} \) As we (Table 3) and others\(^{7} \) have observed, GM-CSF increases PMN mobilization of MPO. The greater effect of MNLs on GM-CSF—primed PMN oxidative activity when assayed by chemiluminescence (compared with superoxide release) thus appears to be related to stimulation of MPO mobilization from the PMNs.

Like rhGM-CSF, rhG-CSF at achievable concentrations primed PMNs for increased chemiluminescence in response to fMLP, but the effect of rhG-CSF was less than with rhGM-CSF. There was no significant effect of rhIL-3 on PMN, MNL, or PMN-MNL chemiluminescence or superoxide release. This is in agreement with Lopez et al,\(^{37} \) who reported that although recombinant gibbon IL-3 stimulates mature human eosinophil function, it has little effect on mature human PMN activity. In addition, our data indicate that unlike GM-CSF, the presence of MNLs does not increase G-CSF— or IL-3—primed PMN oxidative activity.

Recombinant human growth factors have been reported to be effective in promoting leukocyte proliferation in patients.
with a wide range of diseases, including acquire immune deficiency syndrome (AIDS) and aplastic anemia, and in patients receiving chemotherapy followed by bone marrow transplantation. Not only the presence, but also the functional state of the PMN will decide the degree of benefit to the patient. Because the list of growth factors is growing, it is necessary to keep aware of the effects of these factors on mature leukocytes and realize that no single cytokine acts alone but rather works within a complex network of inherent and induced factors that may be expressed on the cell surface or released to the surrounding environment. From our experiments, it appears that PMNs are the principal targets of GM-CSF direct action. In addition, GM-CSF, by making the PMNs more responsive to factor(s) in the environment, indirectly increases PMN oxidative activity.

ACKNOWLEDGMENT

We thank Steven Gillis of Immunex Corp (Seattle, WA) and Dorothée Krumwiek of Beringwerke AG (Marburg, Germany) for supplying the growth factors and Bonnie Pobiner and William Novick of Hoechst-Roussel Pharmaceuticals (Somerville, NJ) for helpful information and ideas.

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The effect of three human recombinant hematopoietic growth factors (granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and interleukin-3) on phagocyte oxidative activity

GW Sullivan, HT Carper and GL Mandell