CONCISE REPORT

Inhibition of Bone Marrow Myeloid Precursor Cell Proliferation by Chemotactic Oligopeptides

By P. A. Mora, J. Valle, A. Salvado, and D. G. Wright

Three N-formylated oligopeptides with different known activities as chemotactic factors for leukocytes were studied to determine if these mediators affect the in vitro proliferation of myelomonocytic colony-forming cells (CFU-C) recovered from murine bone marrow. All three oligopeptides inhibited CFU-C growth in a dose-dependent fashion that correlated with their relative potencies as chemotactic factors. This inhibition was not altered by growth of CFU-C in the presence of indomethacin, by varying the concentrations of colony-stimulating factor (CSF), or by depleting marrow cell preparations of mature granulocytic elements. These studies indicate that chemotactic factors may mediate myelosuppression through effects on committed myeloid precursor cells in the marrow.

It has been shown that extracellular fluid from acute inflammatory lesions can modulate myeloid precursor cell proliferation and maturation in vitro. Both stimulatory and inhibitory activities have been described. However, the specific mediators in inflammatory fluids that may modulate myelopoietic activity are not well understood. In the present studies, we investigated the effects of three N-formylated, chemotactic oligopeptides on the growth of murine bone marrow myelomonocytic colony-forming cells (CFU-C) to determine if these mediators modify colony growth in ways that are consistent with their relative potencies as chemotactic factors.

MATERIAL AND METHODS

Bone marrow cells were harvested from the femurs of C3H/HeDub (syngeneic) mice. Bone marrow cells (2.2 x 10^7/plate) were grown in 35-mm Petri dishes (Falcon, Cherry Hill, N.J.) using a methylcellulose semisolid matrix (as previously described). One (1.0) milliliter of 0.8% methylcellulose (Fisher Sc. Co., Fairlawn, N.J.) in RPMI 1640 media (Flow Labs., McLean, Va.) was supplemented with 15% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.), 10% deionized bovine serum albumin (Miles Labs. Inc., Elhart, Ind.), 2-mercaptoethanol at 10^-4 M (Calbiochem, La Jolla, Calif.), and 20% L cell (American Type Culture Collection, Rockville, Md.) conditioned media as a source of colony-stimulating factor (CSF). The CSF concentration was adjusted to the linear portion of the dose-growth curve as described. The N-formylated oligopeptides with or without indomethacin (1.4 x 10^-4 M) were added to cultures at final concentrations as follows: N-formyl methionyl leucil phenylalanine (FMLP), N-formyl methionyl phenylalanine (FMP), and N-formyl methionine (FM) inhibited myeloid colony growth in a dose-dependent fashion (Fig. 1). Their relative inhibitory potencies (ED50) corresponded to their reported relative activities as chemotactic factors and secretagogues for neutrophils (Table 1). Neither the proportions of compact and spread colonies, nor the number of clusters were affected by the oligopeptides.

Experiments with granulocyte-depleted bone marrow were performed by fractionation of the bone marrow with Ficoll-Hypaque gradients (Pharmacia, Piscataway, N.J.) as described previously. The mononuclear cells at the interphase were washed 3 times with cold RPMI and resuspended at a final concentration of 2.2 x 10^7 cells/culture plate. The effect of CSF concentrations on the inhibition by the N-formylated peptides was studied by incubating cells with and without the peptides, and with CSF diluted to one-fourth of its optimal concentration. The percent of CFU-C growth inhibition was determined by the formula:

\[ \text{Percent inhibition} = \frac{(\text{CFU-C control group} - \text{CFU-C test group}) \times 100}{\text{CFU-C control group}} \]

RESULTS

Myeloid colony (CFU-C) growth under control conditions in our experimental system was 156 ± 12 CFU-C/2.2 x 10^5 unfractionated bone marrow cells (mean ± SEM; n = 48). Three N-formylated oligopeptides: N-formyl methionyl leucil phenylalanine (FMLP), N-formyl methionyl phenylalanine (FMP), and N-formyl methionine (FM) inhibited myeloid colony growth in a dose-dependent fashion (Fig. 1). Their relative inhibitory potencies (ED50) corresponded to their reported relative activities as chemotactic factors and secretagogues for neutrophils (Table 1). Neither the proportions of compact and spread colonies, nor the number of clusters were affected by the oligopeptides.

The cellular composition of CFU-C grown with FMLP at 1.5 x 10^-9 M (ED50 for CFU-C growth inhibition) for 7 days (mean values ± SEM, n = 3) was: undifferentiated blasts 7.3% ± 1.8%, granulo-
cytic cells $33.2\% \pm 2.0\%$, macrophages $54.6\% \pm 1.3\%$, and mononuclear cells $4.9\% \pm 1.1\%$—values that were not different from those of control untreated CFU-Cs.

Inhibition of CFU-C growth with maximal and submaximal concentrations of CSF were studied. In these studies, optimal concentrations of CSF added to culture conditions (0.16 ml LCCM/ml culture media) resulted in growth of $212 \pm 3$ CFU-C at 7 days (mean CFU-C/$2.2 \times 10^5$ nucleated bone marrow cells $\pm$ SEM, $n = 3$). Addition of $1.0 \times 10^{-6}M$ FMP resulted in $27\%$ inhibition of CFU-C growth. When one-quarter of the optimal CSF concentration was added to the culture conditions, CFU-C growth at 7 days was reduced to $82 \pm 2$ (mean CFU-C/$2.2 \times 10^5$ nucleated bone marrow cells). However, inhibition of CFU-C growth by $1.0 \times 10^{-6}M$ FMP under these conditions remained the same ($29\%$).

In separate studies, it was found that the addition of an inhibitor of prostaglandin synthetase (indomethacin $1.3 \times 10^{-7}M$) to the bone marrow cultures did not modify the inhibitory effects of oligopeptides on CFU-C growth. Inhibition of CFU-C growth by $1.5 \times 10^{-9}M$ FMLP was $12.2\% \pm 2.0\%$ (mean $\pm$ SEM, $n = 3$) under control conditions of CFU-C growth and $11.7\% \pm 1.5\%$ ($n = 3$) when indomethacin was present in the cultures. Similarly, inhibition of CFU-C growth by the oligopeptide FMLP was not affected by depletion of the marrow cell preparations of mature granulocytic elements prior to culture.

**DISCUSSION**

These studies demonstrate that the N-formylated peptides FMLP, FMP, and FM inhibit in vitro growth of myelomonocytic colony-forming cells (CFU-C) present in murine bone marrow. This inhibition is dose-dependent and corresponds to the relative activities of these compounds as chemotactic factors and secretagogues for mature neutrophils. Studies of possible mechanism(s) for this inhibition indicate that CFU-C growth inhibition is not mediated through the induction of prostaglandin E (PGE) synthesis by bone marrow macrophages, for a potent inhibitor of PGE synthetase (indomethacin) did not alter the effects of the oligopeptides on CFU-C growth. Also, varying concentrations of CSF had no effect on the inhibition of CFU-C growth by an oligopeptide (FMP) when expressed as percent of controls. This finding indicates that oligopeptide inhibition is relatively independent of CSF concentrations, whether inhibition reflects a direct effect of this oligopeptide on early myelomonocytic precursors (CFU-C) and/or indirect effects mediated through bone marrow cells that are distinct from CFU-C. Studies with the human myeloid cell line, HL-60, have shown that receptors for FMLP are detectable on the surface of these immature cells, and so, it is not unreasonable to speculate that the N-formylated oligopeptides may interact directly with CFU-C or their immediate progeny through specific receptors.

Table 1. Peptide Concentrations That Caused 50% of Maximal CFU-C Growth Inhibition (ED<sub>50</sub>) Compared to Their Reported ED<sub>50</sub> for Leukocyte Chemotaxis

<table>
<thead>
<tr>
<th>Peptide</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; Growth Inhibition</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; Chemotaxis*</th>
</tr>
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<tbody>
<tr>
<td>FMLP</td>
<td>$4.4 \times 10^{-9}$</td>
<td>$7.0 \times 10^{-11}$</td>
</tr>
<tr>
<td>FMP</td>
<td>$6.4 \times 10^{-7}$</td>
<td>$4.1 \times 10^{-7}$</td>
</tr>
<tr>
<td>FM</td>
<td>$1.1 \times 10^{-3}$</td>
<td>$2.1 \times 10^{-3}$</td>
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*See reference Showell et al."
Our findings support the concept that chemotactic factors involved in inflammatory processes may influence the proliferation and differentiation of myeloid precursor cells in the bone marrow and may thus affect the regulation of myelopoiesis. Furthermore, these findings suggest that the generation and circulation of chemotactic molecules in systemic inflammatory diseases may have a role in the pathogenesis of myelosuppression that is occasionally a feature of these diseases.

REFERENCES

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