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Recombinant human granulocyte–macrophage colony-stimulating factor (rH GM-CSF) regulates f Met-Leu-Phe receptors on human neutrophils

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SUMMARY

The regulation of mature human neutrophil function by recombinant human granulocyte–macrophage colony-stimulating factor (rH GM-CSF) was studied. Preincubation of neutrophils with this CSF did not stimulate superoxide anion directly but enhanced the subsequent release of superoxide anion in response to stimulation with the bacterial product formylmethionylleucyl-phenylalanine (f Met-Leu-Phe). Enhanced superoxide anion production was evident by 5 min and reached a plateau at 30 min. In contrast, neutrophils preincubated with rH GM-CSF exhibited reduced chemotaxis under agarose in response to a gradient of f Met-Leu-Phe. The inhibition of neutrophil migration was dependent on the dose of rH GM-CSF and exhibited a time–course similar to the effect on superoxide production. Binding studies of f Met-Leu-[^{3}H]Phe to purified human neutrophils revealed heterogeneous binding to unstimulated cells. Two affinity components were identified. The high-affinity component consisted of approximately 2000 sites/cell and had an average K_d of 4 ± 2 nM ($n = 6$). The low-affinity component consisted of approximately 40,000 sites/cell and had an average K_d of 220 ± 130 nM ($n = 6$). rH GM-CSF caused conversion to a linear Scatchard plot showing no significant change in total binding sites but a single K_d of 30 ± 10 nM. These data indicate that rH GM-CSF may influence neutrophil responses to f Met-Leu-Phe by regulating the affinity of f Met-Leu-Phe receptors.

INTRODUCTION

Granulocytes play a key role in inflammation as they are the predominant cell type at the sites of infection. The function of these cells can be activated *in vitro* by a number of agents, including cell-derived proteins, known as cytokines. One cytokine that is known to have an important influence on neutrophil function is granulocyte–macrophage colony-stimulating factor (GM-CSF). GM-CSF belongs to a family of cytokines known as colony-stimulating factors (CSF), named originally because of their important role in the proliferation and differentiation of progenitor cells (Metcalf, 1984), but more recently some of these factors have been shown also to functionally activate mature cells in a lineage-specific fashion (Vadas, Nicola & Metcalf, 1983; Lopez *et al.*, 1983; Metcalf *et al.*, 1986).

Abbreviations: CSF, colony-stimulating factors; FCS, fetal calf serum; f Met-Leu-Phe, *N*-formylmethionylleucyl-phenylalanine; HEPES, *N*-2-hydroxethylpiperazine-*N*-2-ethanesulphonic acid; rH GM-CSF, recombinant human granulocyte–macrophage colony-stimulating factor.

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We have shown recently that purified recombinant human (rH) GM-CSF can activate several functions of mature neutrophils and eosinophils. For example, rH GM-CSF stimulated phagocytosis of serum-opsonized yeast, neutrophil-mediated iodination in the presence of zymosan, and the cytotoxic activity of these cells against antibody-coated targets (Lopez *et al.*, 1986). In addition, rH GM-CSF also enhanced *N*-formylmethionylleucyl phenylalanine (f Met-Leu-Phe)-stimulated functions of neutrophils, such as degranulation and superoxide anion production (Lopez *et al.*, 1986).

F Met-Leu-Phe is a member of a family of synthetic formyloligopeptides known to be potent chemo-attractants for neutrophils and macrophages. It is believed that these peptides are related structurally to naturally occurring bacterial products that induce chemotaxis and may be responsible for the functional activation of neutrophils. In particular, f Met-Leu-Phe has been identified as the major chemo-attractant for rabbit neutrophils (Marasco *et al.*, 1984) and is generally known to stimulate a number of neutrophil functions.

The mechanism by which rH GM-CSF enhances the effect of f Met-Leu-Phe is not known, but one possibility is the regulation of f Met-Leu-Phe receptor expression. This may simply involve a change in the number of receptors expressed on the cell

surface. For example, rH GM-CSF has been shown to increase the surface expression of the receptor for C3bi (Lopez *et al.*, 1986) and of the p150,95 molecule, which are members of a family of leucocyte-adhesion molecules (Arnaout *et al.*, 1986). Alternatively, cell surface receptor changes may involve more subtle mechanisms such as phosphorylation of the receptor, resulting in a change in the affinity or function of the receptor. For example, f Met-Leu-Phe triggers the phosphorylation of the C3b receptor on neutrophils (Changelian & Fearon, 1986). It is also known that the formyl peptide receptors on neutrophils are regulated both in their affinity and number (Gallin, Wright & Schiffman, 1978; Fletcher & Gallin, 1980) by secretory stimuli, and also by f Met-Leu-Phe itself (Seligmann, Fletcher & Gallin, 1982).

It is shown here that rH GM-CSF alters the affinity but not the numbers of f Met-Leu-Phe receptors and that this regulation of receptor expression is consistent with the biological effects of rH GM-CSF on human neutrophils.

MATERIALS AND METHODS

Purification of human neutrophils

Neutrophils were obtained from the peripheral blood of healthy volunteers after dextran sedimentation (Dextran T500, Pharmacia, Uppsala, Sweden) and density gradient centrifugation at 450 *g* on Lymphoprep (Nyegaard, Oslo, Norway) followed by hypotonic lysis of erythrocytes using a 0·2% sodium chloride solution. This solution was brought to the correct osmolarity using a 1·6% sodium chloride solution. The cell preparations, which always consisted of more than 95% neutrophils, were resuspended to 10^7/ml in RPMI-1640 (pH 7·3) and 2% fetal calf serum (FCS), 20 mM HEPES buffer (Sigma, St Louis, MO) and antibiotics. The assays were carried out in this medium unless otherwise stated.

Recombinant human granulocyte–macrophage colony-stimulating factor (rH GM-CSF)

The rH GM-CSF was lot number C01-P0004 containing approximately $4·7 \times 10^6$ U/mg (when tested on Chronic myeloid leukaemic cells), was 97·2% pure and generously provided by Genetics Institute, Cambridge, MA. The protein was obtained from the supernatant of COS cells and purified as described elsewhere (Wong *et al.*, 1985). Dilutions from stock material were made into RPMI-1640 and 2% FCS and kept sterile at 4°. The lipopolysaccharide content of this solution was determined to be less than 0·6 ng/ml, as measured by the limulus amebocyte lysate assay (Levin & Bang, 1968).

Superoxide production

Purified neutrophils (10^7/ml) were incubated with medium or with 10^{-11}M rH GM-CSF for various times at 37°. After this, 150 μl of cells (10^6) were added to a mixture of 100 μl freshly prepared cytochrome C (Sigma, type VI; 12·4 mg/ml), 100 μl f Met-Leu-Phe (10^{-7}M final; Sigma) and made up to 1 ml with medium. The mixtures were then incubated at 37° for 5 min, after which the cells were cooled rapidly, pelleted at 4° and the supernatants transferred to plastic disposable cuvettes. Superoxide production was measured by the reduction of cytochrome C (Weening, Weever & Roos, 1975) using an extinction coefficient of 21·1 nM^{-1} (Van Gelder & Slater, 1962) at 550 nm.

In experiments where the rate of superoxide anion production was measured, cells were preincubated with 10^{-11}M rH GM-CSF for 1 hr at 37°. One-hundred and fifty microlitres of cells were then added to disposable plastic cuvettes containing 100 μl f Met-Leu-Phe (10^{-7}M final), 100 μl freshly prepared cytochrome C and made up to 1 ml with medium. The change in OD 550 nm was continuously monitored in a DU-50 spectrophotometer (Beckman Instruments, Berkeley, CA) at 37° over 10 min and recorded at 30-second intervals.

In control experiments the release of superoxide anion was confirmed by allowing the reaction to f Met-Leu-Phe to proceed in the presence of freshly prepared superoxide dismutase (Sigma; 280 μ/ml final). The release of superoxide anion in response to f Met-Leu-Phe was completely abolished in the presence of superoxide dismutase. In order to ensure the change in OD 550 nm was not limited by cytochrome C concentration, maximum reduction was determined by adding a few grains of sodium dithionite (Sigma) to the final mix.

Chemotaxis

The chemotactic response of neutrophils to a gradient of f Met-Leu-Phe was tested under agarose as described elsewhere (Nelson, Quie & Simmons, 1975). Briefly, 5 ml of 0·5% agarose (type II, Sigma) dissolved in RPMI-1640 and 2% FCS with 20 mM HEPES was set in a plastic petri-dish (50 mm, Kayline, Adelaide) and 2·4 mm diameter wells were formed 2·4 mm apart in a horizontal line from the centre to the edge of the plate. Neutrophils at $2·5 \times 10^7$/ml in medium were preincubated for various times at 37° in the presence or absence of various concentrations of rH GM-CSF. Five microlitres of cells were then added to the centre well, 5 μl of medium added to the inner well and 5 μl of 10^{-7}M f Met-Leu-Phe added to the outer well. The petri-dish was incubated for 2 hr at 37° in 5% CO_2. The cells were fixed with methanol at 4° overnight, followed by 40% formalin for 30 min at 25° before the agarose was removed.

Migration in the absence of any stimuli (random migration), with f Met-Leu-Phe in both cell-containing and adjacent medium-containing wells (chemokinetic stimulus), and with f (Met-Leu-Phe only in the non-cell containing well (chemotactic stimulus) were measured with an ocular micrometer. Results are expressed in arbitrary units as either chemotactic migration, chemokinetic migration or random movement.

High-performance liquid chromatography of f Met-Leu-[^{3}H]Phe

In order to ensure that impurities were not present in the commercially available f Met-Leu-[^{3}H]Phe used, the preparation was analysed by high-performance liquid chromatography. The system consisted of a Beckman 334 gradient system with a variable wavelength detector. Spectrophotometric detection was a 220 nM. The sample was run through a reverse phase, C18, 5 μm particle size Ultrasphere column (Beckman Instruments). Chromatography was run under the following conditions: flow rate 1·0 ml/min, temperature 25°, gradient elution over 20 min from 20% to 60% CH_3CN in 0·1% phosphoric acid. Under these conditions, f Met-Leu-[^{3}H]Phe appeared as a single peak at 16 min, corresponding to the retention time of unlabelled f Met-Leu-Phe. One-minute fractions were collected and radioactive content determined in a Tricarb 2000 CA liquid scintillation counter (United Technologies Packard, Zurich, Switzerland). Greater than 95·5% of radioactivity corresponded to the 16 min peak in all preparations tested.

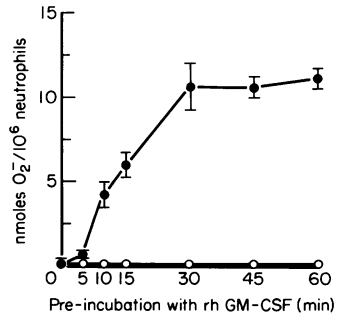

Figure 1. Time-dependent stimulation by rH GM-CSF of superoxide anion production by neutrophils. Cells were incubated in the presence or absence of 10^{-11}M rH GM-CSF for various times at 37° then for 5 min with (closed circles) or without (open circles) 10^{-7}M f Met-Leu-Phe at 37°. Each point represents the difference between unstimulated and rH GM-CSF-treated neutrophils. Each point represents the mean of triplicate determinations ±SEM.

f Met-Leu-[^{3}H]Phe binding to neutrophils

F Met-Leu-[^{3}H]Phe with a specific activity from 50·9 to 55·3 Ci/mmol was purchased from New England Nuclear (Boston, MA). Binding of f Met-Leu-[^{3}H]Phe to purified neutrophils was measured at 4° in siliconized 12×75 mm glass test tubes in a total volume of 105 μl. Cells at a concentration of 10^7/ml in medium were activated with rH GM-CSF for various periods at 37° then washed in cold incubation buffer (Hanks' buffered saline with 1·6 mM CaCl, 10 mM NaAzide, 5 μg/ml cytochalasin B and 0·1% bovine serum albumin; Sigma) and resuspended to 10^7/ml. Sodium azide and cytochalasin B were included in the buffer to minimize receptor-mediated peptide endocytosis (Sullivan & Zigmond, 1980). One-hundred microlitres of cells were added to triplicate tubes containing 5 μl of f Met-Leu-[^{3}H]Phe at various concentrations (100–0·5 nM) in incubation buffer, or to duplicate tubes containing unlabelled f Met-Leu-Phe (100 μM). Bound f Met-Leu-[^{3}H]Phe was measured after 30 min by filtration through glass fiber filters (GF/B, Whatman, Maidstone, U.K.) using Hanks' buffered saline, and quantified by liquid scintillation counting.

The binding data were analysed using LIGAND, a weighted non-linear least squares regression analysis computer programme (McPherson, 1985). After subtracting the non-specific binding from total binding, the specific-binding data were fitted to one or more of the appropriate equations from a one-saturable-site model, a two-saturable-sites model, and the Hill equation (Munson & Rodbard, 1980; Colquhoun, 1979; De Lean & Rodbard, 1979). The weighted sums of squared deviations (WSSDs) generated by LIGAND for one saturable site, two saturable sites, and the Hill equation were compared using the *F*-test (McPherson, 1985).

RESULTS

rH GM-CSF alters the production of superoxide anion by neutrophils stimulated with f Met-Leu-Phe

Preliminary experiments showed that rH GM-CSF did not stimulate superoxide anion production by itself but enhanced the production of superoxide anion by neutrophils in response to f Met-Leu-Phe, and 10^{-11} M was the optimal concentration (data not shown).

Table 1. rH GM-CSF inhibits neutrophil migration in a dose-dependent manner

rH GM-CSF conc. (M)	Migration (arbitrary units)				
Conditions of assay	0*	10^{-12}	3×10^{-12}	10^{-11}	3×10^{-11}
Random	15±2†	16±1·5	19±1·5	16±2	14±1·5
Chemotactic	88±1·5	81±3	68±5	64±5	46±5

* Neutrophils were preincubated in the presence or absence of various concentrations of rH GM-CSF for 60 min at 37°.

† Numbers represent the mean of four determinations ±SEM.

Time–course experiments performed by preincubating neutrophils for various times with 10^{-11} M rH GM-CSF to determine the time necessary for rH GM-CSF to prime neutrophils showed that activation had occurred by 5 min and had reached optimal levels by 30 min (Fig. 1). Cells that were preincubated in the presence or absence of rH GM-CSF and not stimulated with f Met-Leu-Phe, exhibited no detectable production of superoxide anion. The amount of superoxide released in response to f Met-Leu-Phe was constant for up to 60 min preincubation with rH GM-CSF. The amount of superoxide released in response to f Met-Leu-Phe from cells that have been treated with rH GM-CSF for 60 min represents a 40% increase over untreated neutrophils.

In order to determine whether the increase in superoxide anion production from neutrophils perincubated with rH GM-CSF was associated with a change in rate of superoxide anion production, neutrophils were incubated with f Met-Leu-Phe in the presence of cytochrome C over a 10-min period. Preincubating neutrophils with 10^{-11} M rH GM-CSF enhanced the rate of production of superoxide anion in their subsequent response to f Met-Leu-Phe. The levels reached a plateau at 5 min. This effect was consistent over seven experiments, where the mean rate of change in OD 550 nm per minute over 5 min for rH GM-CSF-treated cells was 0·086±0·007, whereas the mean rate of change for unstimulated neutrophils was 0·015±0·006.

rH GM-CSF inhibits the chemotactic migration of neutrophils in response to a gradient of f Met-Leu-Phe

In order to determine whether rH GM-CSF influences a different f Met-Leu-Phe-mediated neutrophil response, the chemotaxis of neutrophils towards a gradient of f Met-Leu-Phe was studied. The neutrophils were preincubated with rH GM-CSF then washed and placed in the agarose-containing dishes in the presence or absence of a gradient of f Met-Leu-Phe.

In order to determine the optimal concentration of rH GM-CSF required for this effect, a titration of the factor was performed. This showed that rH GM-CSF inhibited chemotaxis towards a gradient of f Met-Leu-Phe in a dose-dependent manner (Table 1). The inhibition of chemotaxis was also found to be dependent on the time of preincubation with rH GM-CSF (Fig. 2). As with superoxide anion release, the inhibition of neutrophil migration was evident by 5 min and reached a plateau at 30 min.

After determining optimal rH GM-CSF concentrations and time of preincubation, all three kinds of neutrophil locomotion

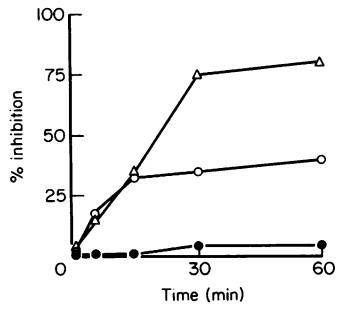

Figure 2. Time-dependent inhibition by rH GM-CSF of neutrophil migration towards a gradient of f Met-Leu-Phe. Following incubation with or without 10^{-11}M rH GM-CSF for various times at 37°, the cells were washed and incubated in the presence or absence of a gradient of 10^{-7}M f Met-Leu-Phe for 2 hr at 37°. Random (closed circles), chemotactic (open triangles) and chemokinetic (open circles) migration were determined in duplicate. Results are expressed as percentage inhibition of unstimulated neutrophil migration.

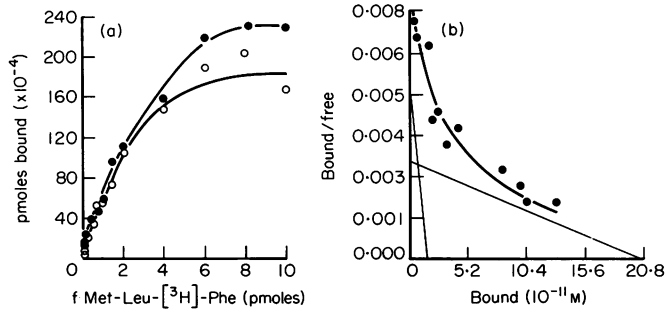

Figure 3. F Met-Leu-[^{3}H]Phe binding isotherms of unstimulated and rH GM-CSF-stimulated neutrophils. (a) Following 60 min incubation at 37° in the presence (open circles) or absence (closed circles) of 2×10^{-11}M rH GM-CSF, neutrophils were washed then incubated 30 min at 4° with increasing concentrations of f Met-Leu-[^{3}H]Phe. Non-specific binding in the presence of 1000-fold or more excess of f Met-Leu-Phe was determined in duplicate and subtracted from total binding which was determined in triplicate. (b) Computer-generated Scatchard analysis of f Met-Leu-[^{3}H]Phe binding to unstimulated neutrophils.

Table 2. rH GM-CSF inhibits neutrophil migration under agarose

Conditions of assay	Migration (arbitrary units)	
	Nil*	rH GM-CSF†
Random	17·3 ± 1·8‡	14·5 ± 0·9
Chemokinetic	38·8 ± 10·3	26·9 ± 4·2
Chemotactic	80·2 ± 6	60 ± 5·7

* Neutrophils were preincubated in the presence or absence of 10^{-11}M rH GM-CSF for 60 min at 37°.

† In the presence of superoxide dismutase and catalase chemotaxis was reduced from 80 ± 5 by unstimulated cells to 60 ± 2·5 by GM-CSF-stimulated cells. Both random and chemokinetic migration were similarly unaffected by the presence of superoxide dismutase and catalase in this assay.

‡ These are pooled data from duplicate determinations of nine individuals ± SEM.

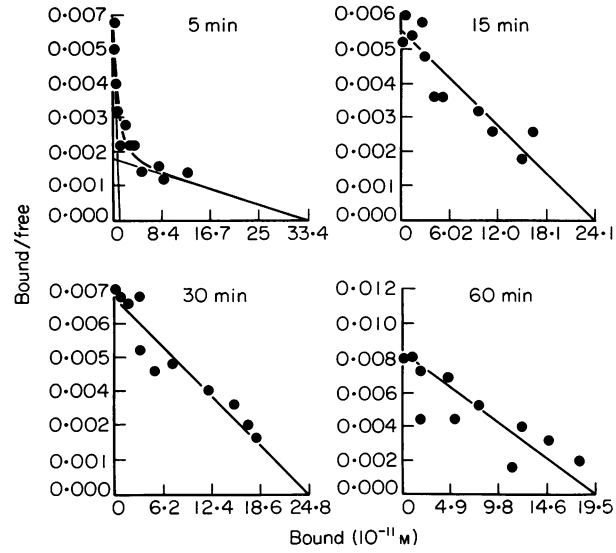

Figure 4. Time-dependent change by rH GM-CSF in the characteristics of f Met-Leu-[^{3}H]Phe binding to neutrophils. Computer-generated Scatchard analysis of steady-state binding of f Met-Leu-[^{3}H]Phe to neutrophils stimulated with rH GM-CSF for 5, 15, 30 and 60 min.

were studied. Table 2 shows the summary data from nine different individuals. It was found that preincubating neutrophils with rH GM-CSF significantly inhibited both chemokinetic ($P < 0.01$) and chemotactic ($P < 0.001$) movement, as determined by the Student's two-tailed ± t-test. In contrast, the random migration of unstimulated neutrophils was not significantly ($P > 0.05$) altered by treatment with rH GM-CSF. As with superoxide anion release, the response varied between different individuals. In order to ensure rH GM-CSF did not inhibit neutrophil migration due to oxidant toxicity, this assay was carried out in the presence of 100 μg/ml superoxide dismutase and 70 μg/ml catalase. No change in the pattern of inhibition caused by rH GM-CSF was observed.

Binding of f Met-Leu-[^{3}H]Phe to neutrophils

In order to determine the characteristics of f Met-Leu-Phe receptor binding to neutrophils following stimulation with rH GM-CSF at 37°, cells were cooled to 4° and steady-state receptor-binding experiments performed. A typical specific binding saturation curve for unstimulated neutrophils and for neutrophils incubated with 2×10^{-11} M rH GM-CSF for 1 hr is shown in Fig. 3.

Statistical analysis of the binding data indicated that the two-saturable-sites model was preferable to the one-saturable-site model for unstimulated neutrophils, and Scatchard analysis of these same data was curvilinear (Fig. 3b); neutrophils that had been incubated with rH GM-CSF for 60 min, however,

Table 3. rH GM-CSF alters the affinity of f Met-Leu-Phe receptors on human neutrophils

Unstimulated neutrophils	K_{d1} (nM)	4±2*
	No. sites/cell	2,000±830
	K_{d2} (nM)	220±130†
	No. sites/cell	40,000±13,000
	Significance	$P<0.01$‡
Neutrophils+rH GM-CSF	K_d	30±10
	No. sites/cell	26,000±5000§
	Significance	$P>0.05$¶

* Arithmetic means±standard error of computer-estimated values from six separate experiments each with a curvilinear Scatchard plot for normal neutrophils and a linear Scatchard plot for rH GM-CSF-stimulated neutrophils.

† The K_d of the low affinity population was found to be significantly different from the K_d of the homogeneous receptor population of rH GM-CSF-stimulated neutrophils ($P=0.004$).

‡ The two site model was statistically preferred to the one site model as calculated by the F test.

§ The number of sites per cell was compared to those on normal neutrophils from six separate experiments and no significant change was found ($P>0.1$).

¶ The one-site model was statistically preferred to the two-site model as calculated by the F test.

fitted better to a one-saturable-site model and the Scatchard plot for these neutrophils was linear.

In order to determine what period of exposure to rH GM-CSF was necessary for a change in the characteristics of f Met-Leu-[^{3}H]Phe binding to occur, cells were incubated with 2×10^{-11} M rH GM-CSF at 37° for 5, 15, 30 and 60 min. The cells were then cooled to 4° and the binding assay was performed. The data in Fig. 4 are representative of two experiments where each point was determined in triplicate. The appropriateness of each fit was determined using the F test as described in the Materials and Methods. The characteristic curvilinear Scatchard plot obtained without stimulation remained at 5 min ($P=0$, 0·039) but had changed to a straight line Scatchard plot by 15 min ($P=0.483$, 0·807) and remained straight for longer preincubation times (30 min, $P=0.259$, 0·445; 60 min, $P=0.362$, 0·384).

A summary of the analysis of the binding models from six separate experiments, both for unstimulated and rH GM-CSF-stimulated neutrophils, is shown in Table 3. The characteristics of the two-saturable-sites model were a high affinity site with a K_d value of 4±2 nM and a low affinity site with a K_d value of 230±130 nM. Two to seven percent of the total number of sites detected were of the higher affinity. In addition, these same data when applied to the Hill equation were characterized by a Hill coefficient of 1·0±0·02, suggesting the curvilinearity of the Scatchard plot is not due to site–site interactions. Although the affinity was altered by rH GM-CSF stimulation, the total number of receptors remained significantly unchanged, as determined by the Student's two-tailed ±t-test.

DISCUSSION

In this paper we show that rH GM-CSF modulates the affinity but not the total number of f Met-Leu-Phe receptors on human neutrophils. These data may explain the biological findings that rH GM-CSF enhanced superoxide anion production by neutrophils in response to f Met-Leu-Phe but inhibited their chemotaxis towards a gradient of f Met-Leu-Phe, since it has been reported that these important functional roles of neutrophils are stimulated by f Met-Leu-Phe at greatly different concentrations.

Our study of the equilibrium binding of f Met-Leu-[^{3}H]Phe to unstimulated neutrophils revealed a curvilinear plot when analysed as described by Scatchard (Scatchard, 1949). A non-linear Scatchard plot is consistent with a heterologous population of f Met-Leu-Phe receptors, and the binding of formyl peptides to the receptors of human neutrophils (Williams *et al.*, 1977; Koo, Lefkowitz & Snyderman, 1982; Mehta & Spilberg, 1983) has been shown previously to result in curvilinear Scatchard plots. These investigations have been widely interpreted as due to the presence of two binding sites, one of higher affinity and one of lower affinity (Koo *et al.*, 1982). There are several possible explanations for the curvilinearity of the Scatchard analysis of f Met-Leu-Phe receptors on normal neutrophils. The most favoured one is that there are two sites that are interconvertible states of the same receptor (Koo, Lefkowitz & Snyderman, 1983; Lohr & Snyderman, 1982; Yuli, Tomonaga & Snyderman, 1982), although other workers have suggested that the two sites may be independent (Mackin *et al.*, 1983) or that the curvilinear Scatchard plot is due to negative cooperativity (Seligmann *et al.*, 1982).

LIGAND analysis of our binding data reveals a high affinity and a low affinity component. When these data were fitted to the Hill equation, a Hill coefficient of 1 was observed, suggesting a two-site binding model was more appropriate than negative site–site interactions. Despite the various interpretations placed on a curvilinear Scatchard plot, our observations thus far suggest the existence of two components of the f Met-Leu-Phe receptor population on normal resting neutrophils.

The stimulation of cells with 2×10^{-11} M rH GM-CSF caused a time-dependent modulation of the f Met-Leu-Phe receptor population. Unstimulated neutrophils, and neutrophils incubated with rH GM-CSF for 5 min at 37°, exhibited curvilinear Scatchard plots, indicative of two-affinity binding sites as described above. After 15 min incubation with rH GM-CSF, however, the Scatchard plot was linear, indicating a homogenous population of receptors. The Scatchard plot remained linear over 30 min and 60 min of stimulation with 2×10^{-11} M rH GM-CSF. In addition, the affinity of the resulting receptor population was invariably intermediate between the affinities of the high and low binding component of unstimulated neutrophils. Although the affinity of the receptors was altered, total receptor number did not change significantly following stimulation with rH GM-CSF. It appears that the relative numbers of lower affinity receptors are increased upon stimulation by rH GM-CSF. This suggests either that the high affinity receptors are internalized and lower affinity ones expressed from the intracellular pool (Fletcher & Gallin, 1983) or that the formyl peptide receptor exists in an interconvertible state, as is the case for interleukin-2 receptors. It was also observed that the affinity of the homogeneous receptor population increased slightly over the 45 min following the conversion: the reason for this is unknown but may be due to the progressive expression of more functionally relevant receptors.

The priming effect of rH GM-CSF on the ability of

neutrophils to produce superoxide anion in response to f Met-Leu-Phe was time-dependent, with activation being virtually maximal at 30 min, and maintained over the time period studied. It is notable that the change in the characteristics of the f Met-Leu-Phe receptors after 15 min paralleled the enhancement of superoxide anion production by rH GM-CSF. Thus it appears that cells pre-exposed to rH GM-CSF are primed and their subsequent response to a bacterial product such as f Met-Leu-Phe is both more efficient and of greater magnitude; such a priming event caused by a natural cytokine might be expected to be physiologically significant.

One of the salient features of the activation of neutrophils by rH GM-CSF is that no detectable release of superoxide anion occurs in the presence of rH GM-CSF alone. This is not surprising as rH GM-CSF does not translocate protein kinase *C* in mature neutrophils (our unpublished observations), which is a mediator in responses to f Met-Leu-Phe (Naccache *et al.*, 1985). Previous reports have associated the formyl peptide receptor with a family of proteins that regulate andenylate cyclase, known as the guanine nucleotide-binding proteins or G proteins (Gilman, 1984). Evidence suggesting that the G proteins modulate the affinity of the formyl peptide receptor has been presented for human neutrophils (Koo *et al.*, 1983; Lad, Olsen & Smiley, 1985; Goldman *et al.*, 1985). Considering this, it is feasible that rH GM-CSF modulates the affinity of f Met-Leu-Phe receptor via the G proteins.

We found that rH GM-CSF influences neutrophil function in a dose-dependent fashion, as shown previously (Lopez *et al.*, 1986). Preincubating neutrophils with rH GM-CSF caused an inhibition of both chemokinetic and chemotactic migration in response to f Met-Leu-Phe. Although rH GM-CSF inhibited random movement as suggested previously (Gasson *et al.*, 1984), the effect was not significant. One possible reason for this discrepancy is that in our experiments the neutrophils were washed following preincubation with rH GM-CSF. This was done in order to eliminate the possibility of negative gradient effects, as Wang *et al.* (1987) have shown that rH GM-CSF is chemotactic by itself; indeed, without the washing step we did find that random movement was reduced to a greater extent (Y.H. Atkinson and C.M. Lucas, unpublished data).

A study of the regulation of f Met-Leu-Phe receptors by biosynthetic human GM-CSF has been published recently (Weisbart, Golde & Gasson, 1986). These data differ from ours on several points. Firstly, they observe that preincubating neutrophils with rH GM-CSF enhances chemotaxis whereas we have shown here that rH GM-CSF inhibits both the chemokinesis and chemotaxis of neutrophils. This discrepancy may be due to the fact that Weisbart *et al.* (1986) have expressed migration as a 'chemotactic index'. This is defined as the ratio of migration towards a chemo-attractant to migration away from the chemo-attractant. Expressing chemotaxis in such a way may lead to misinterpretation of the data, as illustrated by Nelson (Nelson *et al.*, 1975). If one migration component in the chemotactic index is altered, whereas the other remains unaffected, the chemotactic index is altered and subsequently not representative of the observations.

Secondly, Weisbart *et al.* (1986) present binding data that represent f Met-Leu-Phe receptors as a homogenous population on unstimulated neutrophils. This is in disagreement with previous observations by others (Williams *et al.*, 1977; Koo *et al.*, 1982; Mehta & Spilberg, 1983). Furthermore, it is possible that the binding assays were performed under suboptimal conditions for a number of reasons: (i) the concentration of Ca^{2+} and Mg^{2+} were not specified and these are important in f Met-Leu-Phe binding to neutrophils (Niedel, Wilkinson & Cuatrecasas, 1979) as it has been reported that millimolar concentrations of Ca^{2+} and Mg^{2+} enhanced binding from four to six-fold, although the requirement for Mg^{2+} was not as important as for Ca^{2+}. (ii) Metabolic inhibitors were not used and these are essential to minimize internalization that can occur even at 4° (Sullivan & Zigmond, 1980).

The changes in the affinity of the FMLP receptor paralleled changes in the biological response studied. A decrease in the high-affinity f Met-Leu-Phe receptor population was accompanied by an enhanced superoxide production but a decreased neutrophil chemotaxis. This is consistent with the previous findings (Sklar, 1986) showing that the chemotactic reponse is most sensitive to low formyl peptide concentrations, whereas the generation of superoxide anion requires higher concentrations of formyl peptide. The evidence above implies that the high-affinity formyl peptide receptors may mediate neutrophil chemotaxis, whereas the lower affinity receptors mediate superoxide anion generation.

Such an interpretation of our data would make considerable physiological sense. Thus neutrophils far from the site of invading bacteria would encounter very low concentrations of f Met-Leu-Phe detectable only by high affinity receptors. Upon migrating towards the source of infection (i.e. up the f Met-Leu-Phe gradient), the low affinity receptors would become responsive and superoxide production would be induced. CSF, produced locally, would assist in maintaining the neutrophils at the site of inflammation and optimize superoxide production where it is required most. By contrast, the administration of GM-CSF by the intravenous route, as is being suggested for clinical trials, might lead, at least transiently, to deleterious effects.

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