CELL BIOCHEMISTRY AND FUNCTION VOL. 11: 93–100 (1993)

Homologous Priming in Chemotactic Peptide-stimulated Neutrophils

P. BELLAVITE†, S. CHIRUMBOLO, G. LIPPI, P. GUZZO AND C. SANTONASTASO

Istituto di Chimica e Microscopia Clinica, Università di Verona, Ospedale Policlinico, Laboratorio Centrale 37134 Verona, Italy

The kinetics and dose-dependence of activation of human neutrophils exposed to sequential additions of the chemotactic peptide *n*-formyl-methionyl-leucyl-phenylalanine (fMLP) have been investigated by multiwell microplate assays. Treatment of neutrophils with medium–high doses (from 10^{-8} to 5×10^{-7} M) of fMLP caused activation of superoxide anion (O_2^-) production, but prevented further activation by a subsequent addition of an optimal dose (from 10^{-7} M to 5×10^{-7} M) of fMLP. These findings represent an example of cell desensitization, or adaptation. However, neutrophils treated with low, sub-stimulatory doses (from 10^{-10} to 5×10^{-9} M) of the peptide and then treated with optimal doses of fMLP exhibited an O_2^- production that was two to three-fold higher than that induced by the same optimal doses on untreated cells. A similar phenomenon of homologous priming of the oxidative metabolism of neutrophil has not previously been described or characterized. Priming was maximal after about 30 min of incubation with fMLP, which differed from desensitization, which required only a few minutes. Homologous priming was not confined to O_2^- production, but was also observed with the release of the granule enzyme, lysozyme. Low doses of fMLP were also capable of triggering an increase of intracellular free Ca^{2+} and of fMLP membrane receptors, which are possible mechanisms responsible for priming.

KEY WORDS—Priming; desensitization; chemotaxis; superoxide anion; neutrophil modulation.

INTRODUCTION

Desensitization and priming are widespread processes that cause specific dampening and enhancement, respectively, of cellular responses to external signals. Leukocytes are particularly suitable models for studying these phenomena as these cells may be regulated by a large variety of mediators from endogenous (inflammation, cytokines, hormones, etc.) and exogenous (drugs, bacterial products, etc.) sources. It has been shown that pre-incubation of neutrophil granulocytes with the peptide fMLP, or with other chemotactic factors such as C5a, causes dose-dependent inhibition of the subsequent response (in terms of superoxide production and degranulation) to an optimal dose of the same stimulus, a process that has been termed homologous deactivation, or desensitization.[1–9] The opposite of desensitization is priming, namely an increase in responsiveness to a second challenge, exhibited by cells that have been pre-treated with sub-stimulatory doses of chemotactic peptides, endotoxin, or cytokines.

Various combinations of agents capable of reproducing the priming phenomenon *in vitro* have been reported.[10–19] A common feature of these studies is that they utilize priming factors different from those used for the second challenge; in other words, priming is generally referred to as heterologous pre-activation. Therefore, the current view is that repetition of the same stimulus leads to deactivation, while sequential addition of different stimuli leads to an increased response. At variance with this general rule is heterologous desensitization, where different stimulants can deactivate the response to other unrelated factors; this phenomenon is presumably due to complex cross-interactions of the transduction pathways that control both cell activation and receptor function.[20–22] Another exception would be homologous priming, which, however, has never been described as a specific biological response in studies of neutrophil activation. Here we show that priming of neutrophil functions by sequential additions of

† Addressee for correspondence.

0263–6484/93/020093–08$09.00

fMLP is a consistent phenomenon in human neutrophils. We have optimized the conditions for showing homologous priming of neutrophil oxidative metabolism and granule release and we have studied specific mechanisms that may be involved in the cell activation increase.

MATERIALS AND METHODS

Materials

The chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fMLP) and *Micrococcus lysodeikticus* were purchased from Sigma Chemical Company, St. Louis, MO; fMLP (10^{-4} M) was dissolved in dimethylsulfoxide and stored at −20°C; cytochrome c from Boehringer, Mannheim, Germany; purified human albumin from Behring Institut, Marburg, Germany; Fura-2 acetoxymethylester from Calbiochem, La Jolla, CA; f-Met-Leu-[^{3}H]Phe from NEN-Du Pont, Florence, Italy; fetal bovine serum (FBS) from Flow Laboratories. FBS was inactivated by incubation at 56°C for 30 min, divided into aliquots, stored at −20°C and thawed before use. Percoll was from Pharmacia, Uppsala. Sterile 96-well microtiter plates with flat-bottomed wells (Linbro type) were from Flow Laboratories; the microplates were pre-coated with FBS in order to abolish non-specific cell activation.[23] One hundred μl of 50 per cent FBS in phosphate-buffered saline (PBS) were dispensed into each well and the plate was incubated for at least 2 h at room temperature. Immediately before use, the plates were washed three times with 0·9 per cent NaCl using an automatic plate washer (Easy Washer 2, SLT Labs Instruments). Hank's balanced salt solution (without calcium and magnesium) was from Gibco Ltd, Paisley, Scotland. Other materials and reagents were of the highest purity available. In order to avoid contamination, a possible cause of artifactual activation or priming of the cells, sterile solutions and disposable plasticware were used in all the experiments, which were carried out, whenever possible, under a laminar flow hood. Reagents were prepared using pyrogen-free water or 0·9 per cent NaCl solutions.

Isolation of Neutrophils

Human neutrophils were prepared from ethylene diamine tetraacetate-anticoagulated blood by centrifugation over Percoll gradients.[24,25] Cells were finally suspended in a medium composed of Hank's balanced salt solution containing 5 mM glucose, 0·5 mM $CaCl_2$, 1 mM $MgSO_4$, 0·2 per cent human albumin (H-GCMA), at the concentration of 3×10^6 ml^{-1} and kept at room temperature until used.

Superoxide Anion Production

The superoxide anion was measured by the superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome c[26] modified for microplate-based assays.[23,24,27] The 96-well microtiter plates were prepared according to various schemes and combinations, depending on the test assay to be carried out (e.g. various incubation times or various concentrations of test compounds, etc.) and on the number of compounds to be tested. Cells ($1{\cdot}5 \times 10^5$) were added to triplicate wells of fetal-bovine-coated 96-wells microplates, in a final volume of 150 μl of assay medium composed of H-GCMA containing 0·15 mM cytochrome c as the probe for the detection of O_2^-. The plates were brought to 37°C in a humidified incubator by pre-incubation for 15 min, then incubated at 37°C throughout the experiment. When indicated, the plates were rapidly transferred into a microplate reader (Reader 400, SLT Labs Instruments) and the reduction of cytochrome c was measured at 550 nm using 540 nm as a reference wavelength to avoid interference due to light scattering.[23] In all procedures, care was taken to avoid cooling of the plate when it was taken from the incubator for additions and readings.

Lysozyme Release

Neutrophils were incubated in triplicate wells of microplates essentially under the same experimental conditions as described for the O_2^- assay, with the omission of cytochrome c from the assay medium. The plates were then centrifuged for 5 min at 400 *g* and lysozyme was assayed spectrophotometrically in the supernatants by the hydrolysis of *Micrococcus lysodeikticus*.[24] Aliquots (100 μl) of the sample supernatants or of H-GCMA (as reference blanks) were transferred into new microplate wells and 100 μl of a 0·75 mg ml^{-1} suspension of *Micrococcus lysodeikticus* in 0·1 M Na-phosphate buffer, pH 7·0, were added. Reaction rate was measured with a microplate reader as the decrease of absorbance with respect to blanks at 570 nm

wavelength. Percentage release was calculated on the basis of a standard curve made on a known number of unstimulated neutrophils lysed by a 0·2 per cent Triton X-100.

Intracellular Calcium

The concentration of intracellular free calcium ($[Ca^{2+}]_i$) was measured fluorimetrically with Fura-2.[28] Neutrophils were loaded with 1 mM Fura-2 acetoxymethylester for 45 min at room temperature. After loading and washing, aliquots (2 ml) of cell suspension were placed in a thermostated (37°C) quartz cuvette with magnetic stirring, and Fura-2 fluorescence (excitation 340 nm; emission 500 nm) was recorded with a F2000 Hitachi spectrophotometer. $[Ca^{2+}]_i$ was calculated from the peak following the addition of various doses of fMLP. For fluorescence calibration, F_{min} was obtained by the addition of 5 mM EGTA (final pH, 8·5) and by lysing the cells with 0·1 per cent Triton X-100. F_{max} was determined by addition of 5 mM $CaCl_2$ to the cell lysate.

fMLP Binding

Receptors for fMLP were quantified using radiolabelled f-Met-Leu-[^{3}H]Phe (NEN-Du Pont, Florence, Italy), exactly as described by Metcalf *et al.*[24] Binding was performed at 4°C for 30 min (saturation time) using the final concentration of 10^{-7} M radioactive ligand and 10^{-5} M nonradioactive ('cold') ligand where necessary for assessing nonspecific and displaceable binding.

RESULTS

In the course of studies designed to investigate the mechanisms of respiratory burst priming and deactivation, we were surprised by the finding that treatment of neutrophils with a certain range of concentrations of fMLP did not desensitize the cells, but primed them for an increased fMLP-dependent O_2^- production. As shown in Figure 1, a rapid burst of O_2^- production was elicited in neutrophils by 10^{-7} M fMLP when the peptide was given as the first stimulus to previously untreated cells, while neutrophils treated with 5×10^{-9} M fMLP and untreated cells did not produce significant O_2^-. Thirty minutes after the first addition, a near maximal dose of fMLP (10^{-7} M) was added to all the samples and O_2^- production was measured for further 20 min. Previously activated

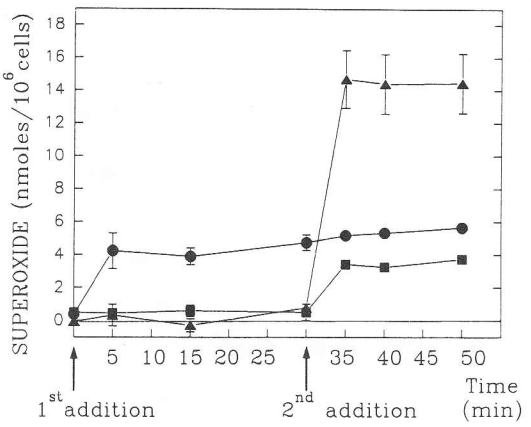

Figure 1. Time-course of O_2^- production by neutrophils treated with sequential additions of fMLP. O_2^- production was assayed with a microplate method. At zero time, cells were supplemented with either 10^{-7} M (●) or 5×10^{-9} M (▲) fMLP; ■: untreated cells. After 30 min, all the cells were supplemented with 10^{-7} M fMLP. The results are mean values ±S.D. for triplicates from a typical experiment, representative of five performed.

neutrophils did not respond to the second addition, while neutrophils which were pre-treated with sub-stimulatory doses of fMLP showed a burst of O_2^- production which was much higher than that of previously untreated cells.

In Figure 2 the extent of the O_2^- burst following

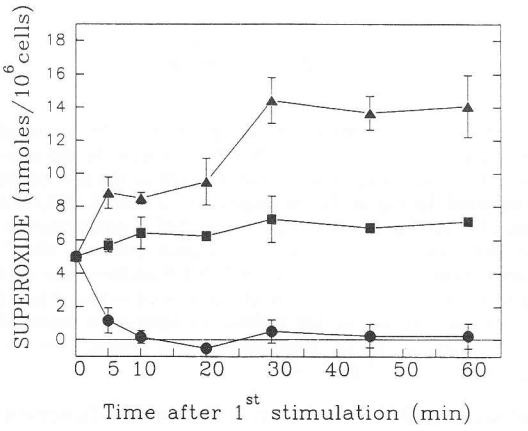

Figure 2. Time-dependence of homologous priming and desensitization. Neutrophils were incubated for various times with different fMLP doses according to the protocol described in the legend of Figure 1, and then treated with a second dose (10^{-7} M) of fMLP. The results represent the O_2^- production in the 10 min following the second addition of fMLP and are mean values ±*S.D.* for triplicates from a typical experiment, representative of three performed.

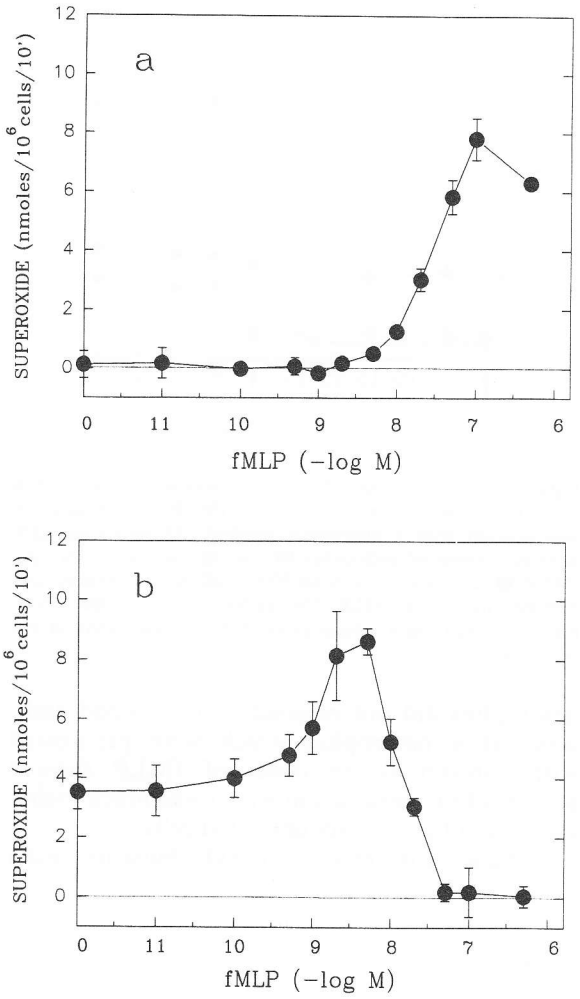

Figure 3. Dose-response of O_2^- production by neutrophils treated with various doses of fMLP. In Figure 3a the activity elicited by increasing doses of fMLP given as the first addition is reported. In Figure 3b the response to a second fMLP addition (5×10^{-7} M), depending on the fMLP concentration of the first addition (i.e. the homologous priming effect) is shown. From the first to the second fMLP addition neutrophils were incubated for 30 min. the O_2^- produced in the 10 min following the first (a) and the second (b) addition is reported as mean values ±*S.D.* for triplicates from a typical experiment, representative of 12 performed.

the second fMLP addition is shown as a function of the incubation time between the first and the second addition. The incubation time necessary for optimal homologous priming was about 30 min. On the other hand, the kinetics of desensitization induced by high fMLP doses were much faster, requiring only 5–10 min to completion. Cells desensitized by pre-treatment with 10^{-7} M fMLP were highly responsive to other stimulants such as serum-treated zymosan or phorbol-myristate acetate, indicating that deactivation is stimulus-specific (not shown).

Figure 3 reports the dose-dependence of homologous priming and desensitization. In Figure 3a it can be seen that direct stimulation (first addition) was caused by an fMLP concentration of greater than 10^{-8} M and peaked at about 10^{-7} M. Homologous priming of the response to the second addition (Figure 3b) was caused by pre-treatment with doses from 10^{-10} to 10^{-8} M. In a series of 12 experiments, the homologous priming peak was reached at $3{\cdot}7 \times 10^{-9}$ M fMLP ($\pm 1{\cdot}5 \times 10^{-9}$ M S.D.). Desensitization was caused by doses of 10^{-8} M and upwards. It is worth noting that desensitization of the secondary response was proportional to the extent of the first stimulation.

Homologous priming was not confined to the respiratory burst response, but was also observed with the release of the granule enzyme lysozyme (Figure 4). The dose-dependence of the priming of lysozyme release was similar to the dose-dependence of the priming of O_2^- formation.

Priming of two distinct functions such as oxidative metabolism and degranulation suggests some general modification of cell sensitivity rather than an up-regulation of specific effector mechanisms. Neither homologous priming nor desensitization were affected by 20 μg ml^{-1} cycloheximide, indicating that protein synthesis is not involved in these processes (not shown). We then quantified membrane fMLP receptors of neutrophils after challenge with various concentrations of fMLP. Treatment of neutrophils with 10^{-9} M fMLP for 15 min at 37°C raised the specific binding of f-Met-Leu-[^{3}H]Phe from 42 ± 2 fmoles $(10^6$ cells$)^{-1}$ to 67 ± 6 fmoles $(10^6$ cells$)^{-1}$ while treatment with 10^{-7} M fMLP reduced the binding to 3 ± 1 fmoles $(10^6$ cells$)^{-1}$.

We also determined the intracellular calcium concentration in neutrophils treated with increasing doses of fMLP (Figure 5). $[Ca^{2+}]_i$ increased following the addition of doses as low as 5×10^{-10} M and plateaued at 10^{-8} M. Therefore, fMLP concentrations which were capable of eliciting a substantial increase in $[Ca^{2+}]_i$ corresponded to the priming concentrations.

DISCUSSION

The neutrophil is a complex cell that can be present in various activity states: (1) Resting, or dormant,

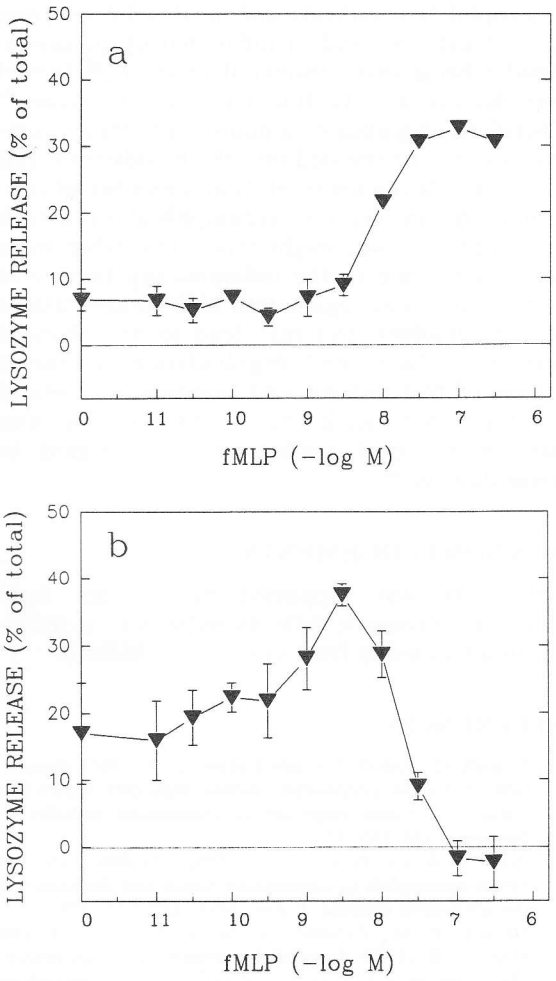

Figure 4. Dose-response of lysozyme release by neutrophils treated with various doses of fMLP. Neutrophils were incubated in triplicate wells of microplates essentially as in the protocol described in the legend of Figure 3. Cells in Figure 4a received only the first fMLP addition and the lysozyme release was determined after 40 min of incubation; in Figure 4b the cells were treated for 30 min with the indicated doses of fMLP, and then were supplemented with a second fMLP addition (5×10^{-7} M) and the lysozyme was determined 10 min after the second addition. Mean values ±*S.D.* for triplicates from a typical experiment, representative of four performed, are reported.

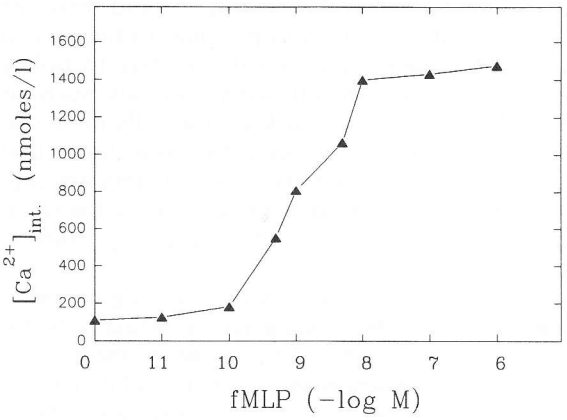

Figure 5. Effect of increasing doses of fMLP on the intracellular free calcium concentration of human neutrophils. Values from a typical experiment, representative of six performed, are shown.

where metabolic and secretory functions are absent; (2) activated, where the cell functions are triggered as a consequence of interaction with particulate or soluble membrane-perturbing agents; (3) primed, where the cell, after contact with sub-stimulatory doses of agonists, exhibits an increased responsiveness to a subsequent stimulation; (4) deactivated, or adapted, or de-sensitized, or inactivated, which are low-response states, induced by various mechanisms that may down-regulate either receptors or the effector systems.

The various activity states of neutrophils are relevant for the non-specific immunological surveillance against microbial invaders and also for the potentially harmful role played by these cells in pathological processes such as rheumatoid arthritis, adult respiratory distress syndrome, post-ischemic tissue injury, immune complex diseases, etc.

In this report we have shown that pre-treatment of neutrophils with a certain range of fMLP concentrations did not de-sensitize the cells, but instead primed them for a higher fMLP-dependent O_2^- production. We have called this phenomenon homologous priming of neutrophil activation. Homologous priming was not previously observed in our[29] and other[1–9] studies which showed only densitization following repeated stimulation. To the best of our knowledge, priming of fMLP-triggered responses by fMLP has been reported as a collaterial finding in the context of other studies in a couple of papers only[30,31] but the specific features of this phenomenon were neither noted nor characterized. These apparent discrepancies may be explained partially by the different experimental systems used (stirred cell suspensions or stationary cultures, different incubation time, cells from blood or from exudates, etc.). Another possibility is that the methods of cell preparation may

inadvertently prime the neutrophils and therefore interfere with homologous priming. In the present series of studies, we took extreme care to isolate the cells with non-activating methods such as Percoll gradients and to keep the cells in sterile, apyrogenic solutions, since it has been shown that trace amounts of bacterial contaminants are capable of priming the macrophages during the isolation procedures and of preventing further priming.[32]

The mechanisms underlying homologous priming have yet to be investigated, although a tentative interpretation may be possible on the basis of our current knowledge of neutrophil reeptor dynamics. It is known that the respiratory burst is a function requiring high receptor occupancy (> 30 per cent) and is induced only by relatively high fMLP concentrations,[30,33] while other responses such as membrane depolarization and elevation of intracellular calcium are elicited by 1–3 per cent occupancy, and actin polymerization by < 0·1 per cent occupancy.[33] It has been also shown that fMLP receptors, once they have bound ligand, shift to a high affinity state and become associated with the cytoskeleton ('receptor sequestration'), and this association correlates with inhibition of O_2^- generation.[7,34] Here we have shown that: (a) Homologous priming occurs at doses which are more than one order of magnitude lower than those causing the activation; (b) priming doses of fMLP are capable of triggering elevations of $[Ca^{2+}]_i$ and membrane receptor up-regulation, in accord with previous reports, showing that both *in vitro*[30,35] and *in vivo*[36,37] priming is associated with rapid up-regulation of a number of receptors, including those for formyl peptides; (c) activation of the respiratory burst and enzyme release appear to be closely associated with deactivation. On the basis of these findings, the following hypothesis may be advanced: Low doses of formyl peptides occupy only a minor fraction of the receptors, but are capable of triggering intracellular transduction pathways associated with the recruitment of new receptors for both homologous and heterologous stimulants and possibly with other biochemical modifications responsible for priming. High doses of formyl peptides fully activate cell responses, but at the same time induce homologous desensitization due to high receptor occupancy and sequestration.

Like heterologous priming, homologous preactivation may also be of pathophysiological significance, in the light of the dual role played by neutrophil free radicals and neutrophil enzymes in host defences and in inflammatory processes. Besides being major bacterial products,[38] formyl peptides can also be found in necrotic tissues.[39] Therefore, significant amounts of these compounds may be released into the bloodstream and prime circulating neutrophils in a number of conditions. As an obvious oversimplification of such *in vivo* process, one might expect that when these cells accumulate in the inflammatory focus and come into contact again with high concentrations of the stimulant, this may lead to an enhanced respiratory burst and degranulation. Increased release of free radicals and enzymes is beneficial because it increases killing efficiency, but can also have pathological implications with regard to tissue damage.[40]

ACKNOWLEDGEMENTS

This work was supported by a grant from Consiglio Nazionale delle Ricerche. S.C. is recipient of a fellowship from Guna S.r.l., Milano.

REFERENCES

1. English, D., Roloff, J. S. and Lukens, J. N. (1981). Regulation of human polymorphonuclear leukocyte superoxide release by cellular responses to chemotactic peptides. *J. Immunol.*, **126,** 165–171.
2. Ward, P. A. and Becker, E. L. (1968). The deactivation of rabbit neutrophils by chemotactic factor and the nature of the activatable esterase. *J. Exp. Med.*, **127,** 643–709.
3. Henson, P. M., Zanolari, B., Schwartzman, N. A. and Hong, S. R. (1978). Intracellular control of human neutrophil secretion. I. C5a-induced stimulus-specific desensitization and the effects of cytochalasin B. *J. Immunol.*, **121,** 851–855.
4. Showell, H. J., Williams, D., Becker, E. L., Naccache, P. H. and Sha'afi, R. (1979). Desensitization and deactivation of the secretory responsiveness of rabbit neutrophils induced by the chemotactic peptide, formyl-methionyl-leucyl-phenylalanine. *J. Reticuloendothel. Soc.*, **25,** 139–150.
5. Simchowitz, L., Atkinson, J. P. and Spilberg, I. (1980). Stimulus-specific deactivation of chemotactic factor-induced cyclic AMP response and superoxide generation by human neutrophils. *J. Clin. Invest.*, **66,** 736–747.
6. O'Flaherty, J. T., Kreutzer, D. L., Showell, H. J., Vitkauskas, J., Becker, E. L. and Ward, P. A. (1980). Selective neutrophil desensitization to chemotactic factors. *J. Cell Biol.*, **80,** 564–572.
7. Jesaitis, A. J., Tolley, J. and Allen, R. A. (1986). Receptor–cytoskeleton interactions and membrane traffic may regulate chemoattractant-induced superoxide production in human granulocytes. *J. Biol. Chem.*, **261,** 13662–13669.
8. Lee, G. H. D., Kaptein, J. S., Scott, S. J., Niedzin, H., Calunta, C. I. and Lad, P. M. (1989). Desensitization of calcium mobilization and cell function in human neutrophils. *Biochem. J.*, **262,** 165–172.

9. McLeish, K. R., Gierschik, P. and Jakobs, K. H. (1989). Desensitization uncouples the formyl peptide receptor–guanine nucleotide-binding protein interaction in HL60 cells. *Mol. Pharmacol.*, **36,** 384–390.
10. Van Epps, D. E. and Garcia, M. L. (1980). Enhancement of neutrophil function as a result of prior exposure to chemotactic factor. *J. Clin. Invest.*, **66,** 167–175.
11. English, D., Roloff, J. S. and Lukens, J. M. (1981) Chemotactic factor enhancement of superoxide release from fluoride and phorbol myristate acetate stimulated neutrophils. *Blood*, **58,** 129–134.
12. Bender, J. C., McPhail, L. C. and Vann Epps, D. E. (1983). Exposure of human neutrophils to chemotactic factors potentiates activation of the respiratory burst enzyme. *J. Immunol.*, **130,** 2316–2323.
13. Dewald, B. and Baggiolini, M. (1985). Activation of NADPH oxidase in human neutrophils. Synergism between fMLP and the neutrophil products PAF and LTB4. *Biochem. Biophys. Res. Commun.*, **128,** 297–304.
14. Fu, Y. K., Arkins, S., Shang Wang, B. and Kelley, K. W. (1991). A novel role of growth hormone and insulin-like growth factor-1. Priming neutrophils for superoxide anion secretion. *J. Immunol.*, **146,** 1602–1608.
15. Bellavite, P., Serra, M. C., Bazzoni, F., Miron, S. and Dusi, S. (1990). Triggering and regulation of the free radical production by phagocytes. In: *Free Radicals, Lipoproteins and Membrane Lipids* (Crastes de Paulet, A., Douste Blazy, L. and Paoletti, R. eds.) Plenum Press: New York, pp. 23–44.
16. Cassatella, M. A., Bazzoni, F., Flynn, R. M., Dusi, S., Trinchieri, G. and Rossi, F. (1990). Molecular basis of interferon-gamma and lipopolysaccharide enhancement of phagocyte respiratory burst capability. Studies on the gene expression of several NADPH oxidase components. *J. Biol. Chem.*, **265,** 20241–20246.
17. Weisbart, R. H., Kwan, L., Golde, D. W. and Gasson, J. C. (1987). Human GM-CSF Primes neutrophils for enhanced oxidative metabolism in response to the major physiological chemoattractants. *Blood*, **69,** 18–21.
18. Guthrie, L. A., McPhail, L. C., Henson, P. M. and Johnston, R. B. (1984). Priming of neuthrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme. *J. Exp. Med.*, **160,** 1657–1671.
19. McColl, S. R., Beauseigle, D., Gilbert, C. and Nacchache, P. H. (1990). Priming of the human neutrophil respiratory burst by granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha involves regulation at a post-cell surface receptor level. *J. Immunol.*, **145,** 3047–3053.
20. O'Flaherty, J. T., Jacobson, D. P. and Redman, J. P. (1989). Bidirectional effects of protein kinase C activators. Studies with human neutrophils and platelet-activating factor. *J. Biol. Chem.*, **264,** 6836–6843.
21. Naum, C. C., Kaplan, S. S. and Basford, R. E. (1991). Platelets and ATP prime neutrophils for enhanced O_2^- generation at low concentrations but inhibit O_2^- generation at high concentrations. *J. Leukoc. Biol.*, **49,** 83–89.
22. Didsbury, J. R., Uhing, R. J., Tomhave, E., Gerard, C., Gerard, N. and Snyderman, R. (1991). Receptor class desensitization of leukocyte chemoattractant receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **88,** 11564–11568.
23. Bellavite, P., Chirumbolo, S., Mansoldo, C., Gandini, G. and Dri, P. (1992). A simultaneous assay for oxidative metabolism and adhesion of human neutrophils. Evidence for correlations and dissociations of the two responses. *J. Leukoc. Biol.*, **51,** 329–335.
24. Metcalf, J. A., Gallin, J. I., Nauseef, W. M. and Root, R. K. (1986). *Laboratory Manual of Neutrophil Function.* Raven Press: New York.
25. Pertoft, H., Laurent, C., Laas, T. and Kagedal, L. (1978). Density gradients prepared from colloidal silica particles coated with polyvinyl pyrrolidone (Percoll). *Anal. Biochem.*, **66,** 271–282.
26. Babior, B. M., Kipnes, R. S. and Curnutte, J. T. (1973). Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J. Clin. Invest.*, **52,** 741–744.
27. Pick, E. and Mizell, D. (1981). Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic immunoassay reader. *J. Immunol. Methods*, **46,** 211–226.
28. Grynkiewicz, G., Poenie, M. and Tsien, R. Y. (1985). A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.*, **260,** 3440–3450.
29. De Togni, P., Bellavite, P., Della Bianca, V., Grzeskowiak, M. and Rossi, F. (1985). Intensity and kinetics of the respiratory burst of human neutrophils in relation to receptor occupancy and rate of occupation by formyl-methionyl-leucyl-phenylalanine. *Biochem. Biophys. Acta*, **838,** 12–22.
30. Norgauer, J., Eberle, M., Fay, S. P., Lemke, H. D. and Sklar, L. A. (1991). Kinetics of *n*-formyl peptide receptor up-regulation during stimulation in human neutrophils. *J. Immunol.*, **146,** 975–980.
31. Karnad, A. B., Hartshorn, K. L., Wright, J., Myers, J. B., Schwartz, J. H. and Tauber, A. I. (1989). Priming of human neutrophils with *n*-formyl-methionyl-leucyl-phenylalanine by a calcium-independent, pertussis toxin-insensitive pathway. *Blood*, **74,** 2519–2526.
32. Ding, A. H. and Nathan, C. F. (1987). Trace levels of bacterial lipopolysaccharide prevent interferon-gamma or tumor necrosis factor-alpha from enhancing mouse peritoneal macrophage respiratory burst capacity. *J. Immunol.*, **139,** 1971–1977.
33. Sklar, L. A., Hyslop, P. A., Oades, Z. G., Omann, G. M., Jesaitis, A. J., Painter, R. G. and Cochrane, C. G. (1985). Signal transduction and ligand-receptor dynamics in the human neutrophil. Transient responses and occupancy-response relations at the formyl peptide receptor. *J. Biol. Chem.*, **260,** 11461–11467.
34. Jesaitis, A. J., Bokoch, G. M., Tolley, J. O. and Allen, R. A. (1988). Lateral segregation of neutrophil chemotactic receptors into actin- and fodrin-rich plasma membrane microdomains depleted in guanyl nucleotide regulatory proteins. *J. Cell Biol.*, **107,** 921–928.
35. Zimmerli, W., Reber, A. M. and Dahinden, C. A. (1990). The role of formylpeptide receptors, C5a receptors, and cytosolic-free calcium in neutrophil priming. *J. Infect. Dis.*, **161,** 242–249.
36. Follin, P., Briheim, G., Sandstedt, S. and Dahlgren, C. (1989). Human neutrophil chemiluminescence and f-Met-Leu-Phe receptor exposure in bacterial infections. *APMIS*, **97,** 585–590.
37. Zimmerli, W., Seligmann, B. and Gallin, J. I. (1986). Exudation primes human and guinea pig neutrophils for subsequent responsiveness to the chemotactic peptide *N*-formylmethionylleucylphenylalanine and increases complement component C3bi receptor expression. *J. Clin. Invest.*, **77,** 925–933.

38. Marasco, W. A., Phan, S. H., Krutzsh, H., Showell, H. J., Feltner, D. E., Nairn, R., Becker, E. L. and Ward, P. A. (1984). Purification and identification of Formyl-Methionyl-Leucyl-Phenylalanine as the major peptide neutrophil chemotactic factor produced by *Escherichia coli*. *J. Biol. Chem.*, **259,** 5430–5439.
39. Carp, H. (1982). Mitochrondrial *N*-formylmethionyl proteins as chemoattractants for neutrophils. *J. Exp. Med.*, **155,** 264–275.
40. Weiss, S. J. (1989). Tissue destruction by neutrophils. *N. Engl. J. Med.*, **320,** 365–376.

Received 14 September 1992
Accepted 5 October 1992