Biochimica et Biophysica Acta, 758 (1983) 168–175
Elsevier

BBA 21494

RELATIONSHIP BETWEEN THE BINDING OF *N*-FORMYLMETHIONYLLEUCYLPHENYLALANINE AND THE RESPIRATORY RESPONSE IN HUMAN NEUTROPHILS

F. ROSSI, P. DE TOGNI, P. BELLAVITE, V. DELLA BIANCA and M. GRZESKOWIAK

Istituto di Patologia Generale, Università di Verona, Strada Le Grazie, Verona (Italy)

(Received December 22nd, 1982)
(Revised manuscript received April 14th, 1983)

Key words: Stimulus-secretion coupling; Chemotactic peptide; Respiratory burst; Myeloperoxidase; (Human neutrophil)

The results presented in this paper demonstrate that the chemotactic peptide *N*-formylmethionylleucylphenylalanine (f-Met-Leu-Phe) is rapidly inactivated by the products of the respiration of human neutrophils stimulated by the peptide itself. The process of inactivation is impeded by the addition of inhibitors of myeloperoxidase (KCN, NaN_3), of catalase, of methionine but not by the addition of superoxide dismutase, indicating that the mechanism of inactivation is the oxidation of methionine residue by myeloperoxidase-H_2O_2-halide system. The oxidation of the peptide causes the rapid cessation of the respiratory burst, since the sulfoxide derivative loses its ability to bind the specific receptors of neutrophil surface and, hence, its biological activity. The comparison between the time course of the binding of f-Met-Leu-[^{3}H]Phe to the specific receptors and the rate of the respiratory response of neutrophils in the presence and in the absence of the process of peptide oxidation was used to investigate the mechanism of the activation of the respiratory burst by the peptide-receptor complexes. In conditions where the inactivation of the stimulatory agent takes place the stimulated respiration slows down and resumes the resting state shortly after the cessation of the binding, although a substantial amount of the peptide remains bound to the specific receptors. In conditions where the degradation of the peptide does not occur the binding of the peptide and the respiratory burst continue for a longer period of time, but the rate of the respiration, calculated in terms of the instantaneous velocity (V_{ist}), is not correlated to the amount of the ligand bound to the membrane receptors measured at various times, indicating that a summation of the effects of the ligand-receptor complexes does not occur as they form. These findings demonstrate, as far as the respiratory response is concerned, that the biological activity of the peptide-receptor complexes is short-lived and that continuous de-novo receptor occupancy is necessary for the maintenance of the activated respiration.

Introduction

Cytotaxin C5a and synthetic formylated peptides, at suitable concentrations, induce in neutrophils an activation of movement and a stimulation of respiration and secretion of the granule constituents [1–4]. As a consequence several enzymes of cytoplasmic granules and intermediates of oxygen reduction, such as O_2^- and H_2O_2, are released outside the cell. It has been shown that these released factors, whose main functions are the killing and digestion of invading organisms and of phagocytosed materials, may play a role in the modulation of inflammation through modifications of cellular responses to the same and to other chemotactic peptides [5–12]. One of the mecha-

nisms of this modulation is the inactivation of the peptides by proteases [5–9,13] and by products of oxygen reduction [14–19]. The action of these last compounds involves H_2O_2, halides and myeloperoxidase secreted by azurophilic granules [14–19], which oxidize the methionine residue of the peptide [14,15].

In the present study data are presented showing that *N*-formylmethionylleucylphenylalanine (f-Met-Leu-Phe) is inactivated by the products of the respiratory burst induced in human neutrophils by the peptide itself and that, as a consequence, the respiratory burst is rapidly extinguished. These findings allowed us to investigate the relationships between the activation of respiration and the binding of f-Met-Leu-Phe to the specific receptors. The results demonstate that (1) the stimulatory action of the peptide-receptor complexes is short-lasting, and (2) the activation of respiration is substained by continuous new bindings.

Materials and Methods

Reagents. f-Met-Leu-Phe, phorbol 12-myristate, 13-acetate, cytochalasin B, catalase, cytochrome *c* and methionine were purchased from Sigma (U.S.A.). Superoxide dismutase was a gift of Dr. J.V. Bannister. f-Met-Leu-[^{3}H]Phe was from New England Nuclear. Preliminary experiments have shown that the biological activity of the labeled peptide is indistinguishable from the unlabeled one. Stock solutions of f-Met-Leu-Phe and phorbol 12-myristate, 13-acetate in dimethylsulfoxide were kept frozen at −20°C until used. Appropriate dilutions in dimethylsulfoxide were made prior to each experiment and added to cell suspensions in very small amounts (2–4 μl/ml of cell suspension).

Cell preparation. Leucocyte suspensions containing 85–90% neutrophils were prepared from venous blood of healthy donors by the standard technique of dextran sedimentation as described by Dunham et al. [20]. The cells, freed from erythrocytes by hypotonic lysis, were washed twice and suspended in Krebs-Ringer phosphate solution (pH 7.4) containing 152 mM [Na^+]; 5 mM [K^+]; 130 mM [Cl^-]; 16.6 mM [PO_4^{3-}];1.22 mM [Mg^{2+}]; 5 mM glucose.

Metabolic studies. The respiratory response of neutrophils to f-Met-Leu-Phe was tested both as O_2 consumption and O_2^- production. O_2 consumption was measured at 37°C with a Clark oxygen electrode connected to a plastic chamber and recorded as previously described [21], using $(2–4)\cdot 10^7$ cells in 2 ml Krebs-Ringer phosphate solution containing 0.5 mM $CaCl_2$ and 5 μg cytochalasin B/ml. O_2^- production was assayed as superoxide dismutase sensitive reduction of cytochrome *c* (200 μM) in the same conditions used for the recording of O_2 consumption. At stated times, aliquots of cell suspension were drawn, added to 1 ml of ice-cold Krebs-Ringer phosphate solution and centrifuged at $8000 \times g$ for 30 s in a microcentrifuge (Eppendorf). The supernatants were assayed for the amount of reduced cytochrome *c* at 550 nm using an extinction coefficient of 21.1 $mM^{-1}\cdot cm^{-1}$. O_2^- production was calculated by subtracting the values obtained by assays carried out in the presence of superoxide dismutase from those in its absence.

V_{ist}, the instantaneous velocity of O_2 consumption at a given time ($V_{ist} = dO_2/dt$), is calculated on the basis of the angular coefficient of the tangent of the respiration trace at the times indicated. V_{max} is the maximum V_{ist} reached by the respiration.

F-Met-Leu-[^{3}H]Phe binding assay. The binding was measured by a rapid filtration technique as described [11,22,23]. Briefly, after the incubation of neutrophils in presence of f-Met-Leu-[^{3}H]Phe, cells were filtered through Whatman (Gf/B) glass fiber filters using a rapid filtration device (Millipore). The filters were washed twice with 5 ml ice-cold Krebs-Ringer phosphate solution, placed in 5 ml of Filter-Count scintillation liquid (Packard) and the samples were counted for [^{3}H] in a Prias CL liquid spectrometer (Packard). Unspecific binding was defined as the amount of binding not inhibited by 500-fold excess of unlabeled f-Met-Leu-Phe. Specific binding was defined as the total amount of f-Met-Leu-[^{3}H]Phe bound minus the unspecific binding.

The time course of the binding was measured at 0 and 37°C in the same conditions of incubation and stirring used for the recording of O_2 consumption or for the assay of O_2^- production. Aliquots of cell suspensions were withdrawn from the in-

cubation chamber at various times, filtered and counted as described above.

Results

The respiratory response to f-Met-Leu-Phe measured as O_2 consumption

Fig. 1 (trace a) shows the time course of the respiratory burst induced in human neutrophils by 50 nM f-Met-Leu-Phe. The increase of O_2 consumption starts about 15 s after the addition of the stimulant, reaches the maximum velocity (V_{max}) in a few seconds, remains linear for less than 1 min and then rapidly decreases.

A reasonable explanation for this very short duration of the respiratory burst is that the products of O_2 reduction inactivate the chemotactic peptide. The experimental evidence for this explanation has recently been provided in different laboratories [14–19]. Thus, assuming that the burst stops as a consequence of the inactivation of the peptide through the myeloperoxidase-H_2O_2-halide system, an inhibitor of myeloperoxidase should preserve the stimulants and modify the time course of the respiratory burst. The results of the experiments reported in Fig. 1 (traces b and c) show that this is the case. In presence of KCN or NaN_3 the kinetic of the respiration activated by f-Met-Leu-Phe is substantially different from that in absence of inhibitors of myeloperoxidase. The main difference is that the duration of the burst is greatly increased, while its start and V_{max} are practically unmodified by the presence of the inhibitors. A similar lengthening of the burst has been obtained by addition of catalase (trace e), while that of superoxide dismutase (trace d) is without effect, confirming that the mechanism responsible for the shortening of the burst required the presence of H_2O_2. It is worth pointing out that the lower rate of O_2 consumption in presence of catalase, in comparison to the rate in presence of KCN or NaN_3, is due to the reaction of degradation of H_2O_2 by catalase, that gives back O_2 ($H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2$).

An alternative explanation of the very short length of the respiratory burst by f-Met-Leu-Phe could be that a very rapid inactivation of all the respiratory system takes place with the mechanisms postulated for the termination of the activated respiration and also involving the endogenous myeloperoxidase system [24]. The data of Fig. 2 demonstrate that this explanation is not

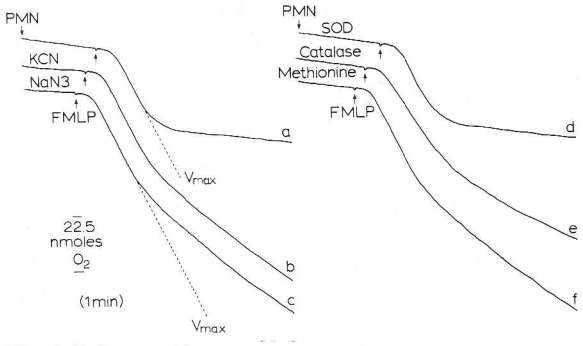

Fig. 1. Polarographic recording of the stimulation of O_2 consumption induced by $5 \cdot 10^{-8}$ M f-Met-Leu-Phe (FMLP) in $4 \cdot 10^7$ human neutrophils (PMN) suspended in 2 ml of Krebs-Ringer phoshate solution. Where indicated the assay mixture contained; 1 mM KCN; 2 mM NaN_3; 30 μg/ml sodium oxide dismutase (SOD); 2600 U/ml catalase and 1 mM methionine.

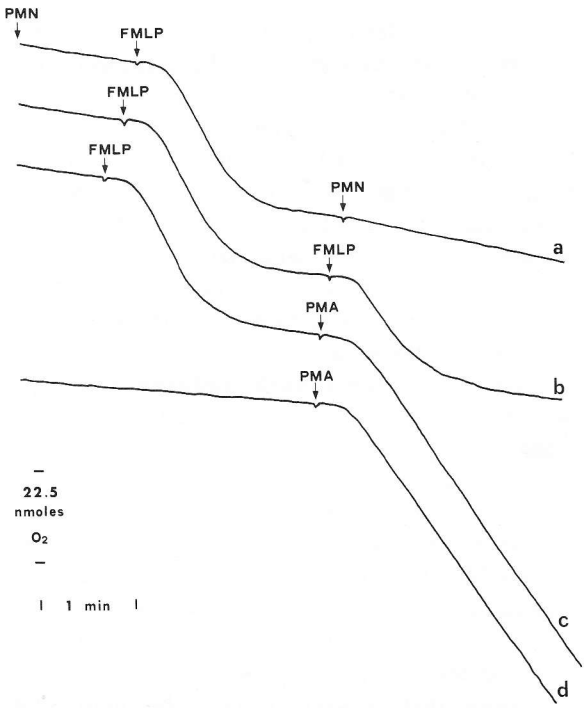

Fig. 2. Polarographic recording of the stimulation of O_2 consumption induced by $5 \cdot 10^{-8}$ M f-Met-Leu-Phe (FMLP) or 100 ng/ml phorbol 12-myristate, 13-acetate (PMA) in $4 \cdot 10^7$ human neutrophils.

applicable to our case. Traces c and b show in fact that the addition of a new stimulant or of a second aliquot of f-Met-Leu-Phe to neutrophil suspension after the termination of a first burst by f-Met-Leu-Phe, induces a further stimulation of the respiration. This finding indicates that the respiratory system of neutrophils has not been completely destroyed and is potentially functioning after the first activation. However this result does not rule out the possibility that the second burst is due to the activation of new molecules of NADPH oxidase, not recruited during the first stimulation.

The trace a of Fig. 2 shows that the addition of new neutrophils when the burst has ceased is not followed by an increase in the oxygen consumption indicating that at the time of the addition of new cells the chemotactic peptide of the medium was completely inactivated.

If oxidation of the methionine residue of the peptide was responsible for its inactivation, free methionine would be expected to inhibit the process by serving as a competitive substrate for oxidation [17–19] and, hence, to preserve the stimulatory activity of the peptide. The experiments reported in Fig. 1 (trace f) show that this is the case, because in presence of exogenous methionine the respiratory response of neutrophils to f-Met-Leu-Phe is similar to that in presence of the inhibitors of myeloperoxidase-H_2O_2-halide system.

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

Fig. 3 presents the data of six experiments on the time courses of the binding of f-Met-Leu-[^{3}H]Phe to neutrophils incubated at 37°C in the same conditions used for the study of the respiratory response. It can be seen that the time courses of the binding are markedly influenced by the presence of inhibitors of myeloperoxidase. The binding in the presence or absence of KCN is fairly rapid in both cases and reaches the same value in 30 s. In the presence of KCN the binding reaches equilibrium in about 6–8 min; in its absence no further binding occurs after the initial 30 s and, after a slight decrease, possibly due to a partial dissociation of the peptide-receptor complex, a substantial amount of f-Met-Leu-[^{3}H]Phe remains specifically associated with cell membrane.

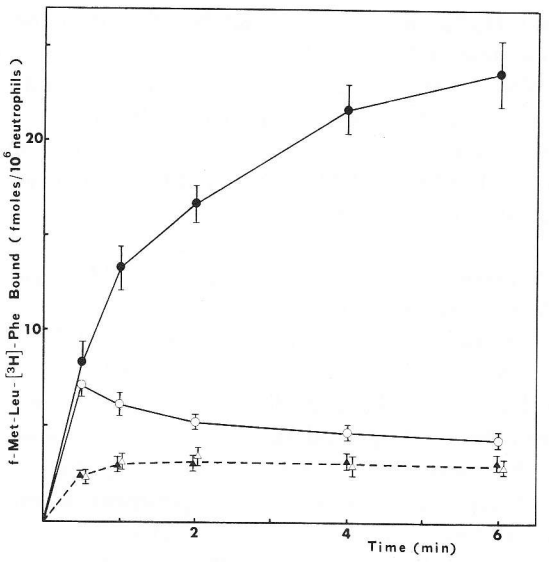

Fig. 3. Kinetics of the binding of $5 \cdot 10^{-8}$ M f-Met-Leu-[^{3}H]Phe to human neutrophils at 37°C. Total binding in absence (○———○) and in presence of 1 mM KCN (●———●). Unspecific binding, determined with 500-fold excess of f-Met-Leu-Phe, in absence (△------△) and in presence of 1 mM KCN (▲------▲). Mean values ± S.E. for six separate experiments performed on different batches of cells.

A time course of binding similar to that in presence of KCN was found when other inhibitors of the process of inactivation were added. Fig. 4 shows a typical experiment of binding of f-Met-

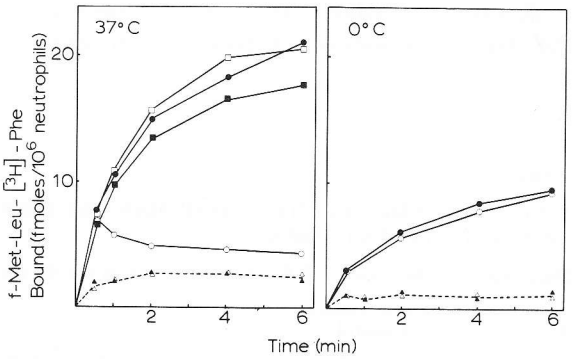

Fig. 4. A representative experiment of the kinetic binding of $5 \cdot 10^{-8}$ M f-Met-Leu-[^{3}H]Phe to human neutrophils at 37°C and 0°C. Total binding in absence (○———○) and in presence of KCN (●———●); of 2600 U/ml catalase (■———■); of 1 mM methionine (□———□). Unspecific binding in absence (△------△) and in presence of KCN(▲------▲). The values of unspecific binding in presence of catalase or methionine, not shown in the figure, are similar to those in presence and in absence of KCN.

Leu-[^{3}H]Phe in presence of KCN, of methionine, of catalase and in the absence of inhibitors.

When measured at 0°C (Fig. 4), i.e. in conditions where the ligand-receptor complexes do not induce a metabolic response in neutrophils, the specific binding of f-Met-Leu-[^{3}H]Phe in the presence and in the absence of KCN was similar.

Relationships between binding of f-Met-Leu-[^{3}H]Phe and respiratory response

The traces of Figs. 1 and 3 show that at 37°C the time courses of the respiration parallel those of the binding of the peptide. The comparison between the bindings and the respiratory responses can be more clearly seen by examining Fig. 5, which reports a representative experiment, where the binding and the respiratory activation were simultaneously measured on the same batch of neutrophils. In the absence of KCN the rapid extinction of the activated O_2 consumption is strictly linked to the cessation of the binding. It is worth pointing out that while the cessation of binding occurs around 30 s after the addition of the peptide, the slowing down and the cessation of respiration are slightly delayed, taking place about 20–30 and 60–70 s later, respectively.

In the presence of KCN both the binding of the peptide and the respiratory burst continue for a longer period of time. However, while the amount of f-Met-Leu-[^{3}H]Phe bound to the membrane receptors progressively increases, the velocity of the respiration does not increase but remains linear and then decreases. Table I reports the data of fmol of f-Met-Leu-[^{3}H]Phe bound at 37°C and the instantaneous velocity (V_{ist}) of oxygen consumption of neutrophils at various times. The results indicate that summation of the effect of the ligand-receptor complexes as they form does not happen. Thus, it seems that the rate of the respiration induced by the stimulant is not dependent on the total number of the receptors occupied but on the rate of receptor occupation.

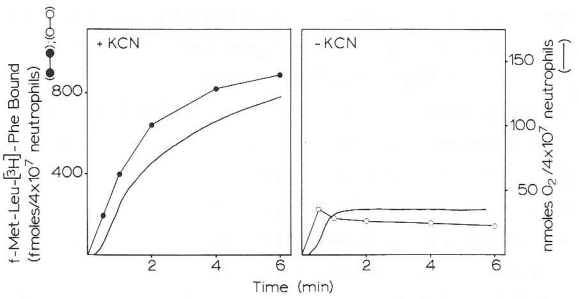

Fig. 5. Comparison between the specific binding of $5 \cdot 10^{-8}$ M f-Met-Leu-[^{3}H]Phe to human neutrophils and the stimulation of O_2 consumption.

The respiratory response to f-Met-Leu-Phe assayed as O_2^- production

Fig. 6 reports a representative experiment where the binding and the O_2^- production by human neutrophils were measured. It can be seen that when the respiratory response of neutrophils to f-Met-Leu-Phe is measured as O_2^- production no

TABLE I

BINDING OF f-Met-Leu-[^{3}H]Phe TO HUMAN NEUTROPHILS AND INSTANTANEOUS VELOCITY (V_{ist}) OF RESPIRATION AT DIFFERENT TIMES

Data represent the mean ± S.E. of six experiments for the binding and seven experiments for the O_2 consumption.

Time	Control		Control + 1 mM KCN	
	f-Met-Leu-[^{3}H]Phe [a] bound	V_{ist} of O_2 [b] consumption	f-Met-Leu-[^{3}H]Phe bound	V_{ist} of O_2 consumption
30 s	278 ± 24.7	58.1 ± 7.6	344 ± 39.4	65.8 ± 5.1
1 min	244 ± 24.0	31.2 ± 4.2	536 ± 53.7	54.1 ± 5.4
2 min	211 ± 12.4	4.7 ± 1.7	672 ± 41.1	30.0 ± 4.3
4 min	188 ± 12.9	2.6 ± 1.3	872 ± 53.7	20.8 ± 2.8

[a] fmol f-Met-Leu-[^{3}H]Phe bound/4×10^7 neutrophils at the time indicated.
[b] nmol O_2/min per 4×10^7 neutrophils at the time indicated.

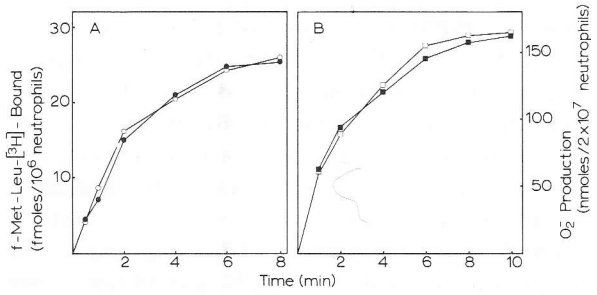

Fig. 6. (A) Specific binding of 5×10^{-8} M f-Met-Leu-[^{3}H]Phe to human neutrophils at 37°C in presence of cytochrome *c* with (●———●) and without KCN (○———○). (B) O_2^- production by human neutrophils stimulated with $5 \cdot 10^{-8}$ M f-Met-Leu-Phe in presence (■———■) and in absence of KCN (□———□).

difference is observed in the presence and in the absence of KCN. This apparent discrepancy with the behaviour of the respiratory response measured as O_2 consumption is due to the fact that the measurement of O_2^- production is made in presence of cytochrome *c*, which is reduced by superoxide anion and, hence, prevents its dismutation to H_2O_2 and the oxidative degradation of the chemotactic peptide. This is confirmed by the finding that in presence of cytochrome *c* (Fig. 6) the binding of f-Met-Leu-[^{3}H]Phe is similar to that in presence of inhibitors of the myeloperoxidase-H_2O_2-halide system (see Fig. 4).

Discussion

It has been shown by Clark et al [16,19] and by Tsan and Denison [17] that products of O_2 reduction released by leucocytes stimulated by phagocytosis and by phorbol 12-myristate, 13-acetate are able to inactivate chemotactic peptides C5a and f-Met-Leu-Phe. The inactivation is due to the oxidation of methionine by the myeloperoxidase-H_2O_2-halide system with formation of the sulfoxide derivative and loss of the ability to bind to intact leucocytes and, hence, of the biological activity [14–19]. The results presented in this paper demonstrate that a rapid inactivation of the peptide can be caused by the products of the respiration of neutrophils stimulated by the peptide itself, and that this inactivation induces a rapid cessation of the respiratory burst. The process of inactivation of the peptide and the consequent termination of its effect on cellular response are impeded by the addition of KCN, NaN_3, catalase and methionine, confirming that the mechanism of inactivation is the oxidation of methionine residue by myeloperoxidase-H_2O_2-halide.

The analysis that we have done of the consequences of this phenomenon on the time courses of the formation of the peptide-receptor complexes and of the associated respiratory response provides us with useful information concerning; (1) the validity and the limits of the use of the model leucocyte-chemotactic peptide to study the stimulus-response coupling; (2) the control of the respiratory response by chemotactic peptides; (3) the molecular mechanisms of the activation of the respiratory burst by the ligand-receptor complexes.

As regard the first point our results although obtained in conditions of extreme O_2^- production suggest that the use of inhibitors of the toxic actions of oxygen radicals and of H_2O_2 should be carefully considered in the studies concerning the effects of methionyl peptides, the properties of the specific receptors, the turnover, the effects and the fate of the peptide-receptor complexes. It is likely that many results so far reported are influenced or distorted by the effects that we have presented. The second point regards the biological meaning of this oxygen-dependent inactivation of the chemotactic peptides. As suggested by others it could be one of the mechanisms of control and modulation of the inflammatory process [15–19], a sort of self-saving process against too high exposure to stimulating peptides and to the consequent toxic effects of oxygen radicals, and also a manner of preserving the recognition apparatus from useless occupation.

The last point to be discussed is the relevance that our finding may have to understand the mechanism of the stimulus-response coupling, at least as far the respiratory burst is concerned. By comparing the time courses of the binding of f-Met-Leu[^{3}H]Phe and of the respiration in absence of KCN it can be seen that they are very similar. The binding and the respiratory burst reach the maximum values around 30 s. Then, the increase of the binding stops and the intensity of the respiration slows down and resumes the resting value within seconds. These findings clearly dem-

onstrate that when the binding of new ligand ceases the respiration also stops indicating that (1) the peptide that remains associated with membrane receptors is ineffective in the activation of the respiration, (2) the continuation of the burst is dependent on the formation of new ligand-receptor complexes.

The reversibility of the respiratory burst and the need for continuous binding of the stimulatory agent for the continuation of the burst, has been previously demonstrated in our laboratory [25] by stopping the activated respiration induced by concanavalin A in neutrophils with the addition of the competing sugar α-methylglucopyranoside. The transient nature of the respiratory response of neutrophils has been recently shown also by Sklar et al [26] and by Radin et al. [27] by interrupting with different procedures the binding of the stimulatory peptide to the specific receptors.

The meaning of the reversibility of the respiration of whole cells can be analyzed in terms of the activity of the NADPH oxidase (or of the respiratory system) responsible for the burst of phagocytes. It is likely, that the respiration ceases because new molecules of the oxidase are not recruited and activated, due to the fact that new ligand-receptor complexes are not formed. If this is the case, the molecules of the oxidase, previously activated following the formation of the ligand-receptor complexes, remain in an activated state for a short time. We have seen in our experiments that the slowing down and the cessation of the respiration stimulated by f-Met-Leu-Phe in the absence of KCN are slightly delayed with respect to the cessation of the formation of the new peptide-receptor complexes. The time that elapses between the cessation of binding and the decrease and cessation of respiration could be taken as the time (in seconds) that the respiratory enzyme remains in an activated state. Thus the de-novo receptor occupancy is necessary for the maintenance of the burst since the activation of the oxidase by the ligand-receptor complex lasts only a few seconds.

This transient activation could depend on the particular experimental conditions employed here, which cause an inactivation of the peptide which remains bound to the membrane receptors. Alternatively it could be a physiological phenomenon due to a rapid deactivation of the receptor-ligand complexes or of the coupling mechanism whereby receptor occupation is translated into the respiratory response or of the respiratory enzyme. In favour of this second possibility is the finding that the short duration of the effects of the ligand-receptor complexes is also shown by the relationships between the kinetics of binding and of the respiration in presence of KCN, a condition where the oxidative inactivation of the chemotactic peptide does not occur. The traces of Figs. 1, 3 and 5 and the data of Table I show in fact that there is a dissociation between the amount of the peptide bound to the membrane receptors and the rate of O_2 consumtion by neutrophils. In fact while the former increases for some minutes, the latter remains linear during the first minute after its start and then progressively decreases, indicating that a summation of the effects of the ligand-receptor complexes does not occur as they form.

The nature and the level of the mechanism responsible for the short-lasting efficacy of the receptor-ligand complexes are under investigation.

To explain the transient nature of the responses to chemotactic peptides Sklar et al. [26] have advanced the hypothesis of the existence of a mechanism which can inhibit cellular triggering at a stage beyond the formation of the ligand-receptor complex.

Addendum

After this manuscript had been submitted, R.A. Clark published a paper (J. Immunol. (1982) 129, 2725–2728) showing that chemotactic methionyl-peptides trigger their own oxidative inactivation by human neutrophils.

Acknowledgement

This work was supported by Grant No. 81.01803.04 from the C.N.R.

References

1 Showell, H.J., Freer, R.J., Zigmond, S.H., Schiffmann, E., Aswanikumar, J., Corcoran, B.A. and Becker, E.L. (1976) J. Exp. Med. 143, 1154–1169

2 Becker, E.L. (1976) Am. J. Pathol. 85, 385–394

3 Lehmeyer, J.E., Snyderman, R. and Johnson, R.B. Jr. (1979) Blood 54, 35–45
4 Smith, R.J., Wierenga, W. and Iden, S.S. (1980) Inflammation 4, 73–88
5 Ward, P.A. and Hill, J.H. (1970) J. Immunol. 104, 535–543
6 Venge, P. and Olsson, I. (1975) J. Immunol. 115, 1505–1508
7 Wright, D.G. and Gallin, J.I. (1977) J. Immunol. 119, 1068–1076
8 Wright, D.G. and Gallin, J.I. (1975) Inflammation 1, 23–39
9 Brozna, J.P., Senior, R.M., Kreutzer, D.L. and Ward, P.A. (1977) J. Clin. Invest. 60, 1280–1288
10 Gallin, J.I., Wright, D.G. and Schiffmann, E. (1978) J. Clin. Invest. 62, 1364–1374
11 Fletcher, M.P. and Gallin, J.I. (1980) J. Immunol. 124, 1585–1588
12 Fletcher, M.P., Seligmann, B.E. and Gallin, J.I. (1982) J. Immunol. 128, 941–948
13 Aswanikumar, S., Schiffmann, E., Corcoran, B.A. and Wahl, S.M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 2439–2442
14 Clark, R.A. and Klebanoff, S.J. (1979) J. Clin. Invest, 64, 913–920
15 Tsan, M.F. and Chen, J.W. (1980) J. Clin. Invest. 65, 1041–1050
16 Clark, R.A. (1980) Fed. Proc. 39, 699, (abstr. 2275)
17 Tsan, M.F. and Denison, R.C. (1981) J. Immunol. 126, 1387–1389
18 Clark, R.A., Szot, S., Venkatasubramanian, K. and Schiffmann, E. (1980) J. Immunol. 124, 2020–2026
19 Clark, R.A. and Szot, S. (1982) J. Immunol. 128, 1507–1513
20 Dunham, P.B., Goldstein, I.M. and Weissmann, G. (1974) J. Cell. Biol. 63, 215–226
21 Rossi, F., Della Bianca, V. and Davoli, A. (1981) Febs Lett. 132, 273–277
22 Williams, L.T., Snyderman, R., Pike, M.C. and Lefkowitz, R.J. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 1204–1208
23 Niedel, J., Wilkinson, S. and Cuatrecasas, P. (1979) J. Biol. Chem. 254, 10700–10706
24 Jandl, R.C., André-Schwartz, J., Borges-DuBois, L., Kipnes, R.S., McMurrich, B.J. and Babior, B.M. (1978) J. Clin. Invest. 61, 1176–1185
25 Romeo, D., Zabucchi, G. and Rossi, F. (1973) Nature 243, 111–112
26 Sklar, L.A., Oades, Z.G., Jesaitis, A.J., Painter, R.G. and Cochrane, C.G. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 7540–7544
27 Radin, A.R., Korchak, H.M., Wilkenfeld, C., Rutherford, L.E. and Weissmann, G. (1982) Clin. Res. 30, 520 A