*Biochemical Pharmacology*, Vol. 44, No. 4, pp. 673–680, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00


# EFFECTS OF TRIPEPTIDES DERIVED FROM MILK PROTEINS ON POLYMORPHONUCLEAR OXIDATIVE AND PHOSPHOINOSITIDE METABOLISMS

DANIÈLE MIGLIORE-SAMOUR, MONIQUE ROCH-ARVEILLER,* MICHÈLE TISSOT,* M'HAMED JAZZIRI, KARIM KEDDAD, JEAN-PAUL GIROUD* and PIERRE JOLLÈS†

Laboratoire des Protéines, CNRS URA 1188, Université de Paris V, 45 rue des Saints-Pères, F-75270, Paris Cedex 06; and *Laboratoire de Pharmacologie, CNRS URA 595, Hôpital Cochin, 27 rue du Faubourg Saint-Jacques, F-75674, Paris Cedex 14, France

(*Received* 14 *January* 1992; *accepted* 29 *May* 1992)

**Abstract**—The tripeptide GLF (glycyl-leucyl-phenylalanine) was isolated from human milk proteins. This peptide increased phagocytosis by human and murine macrophages and protected mice against *Klebsiella pneumoniæ* infection. Specific binding sites on human polymorphonuclear leukocytes (PMNs) have been demonstrated recently. The aim of the present research was to study the action of this peptide on rat and human PMN oxidative burst and to investigate the consequences of cell stimulation on polyphosphoinositide hydrolysis. A biphasic stimulating concentration-dependent effect of GLF on PMN chemiluminescence and superoxide anion generation was demonstrated. One of the peaks of the oxidative response occurred around $10^{-9}$ M, which correlates with the $K_d$ of high affinity receptors of GLF. The other maximum, around $10^{-4}$ M, might be due to the hydrophobic nature of the tripeptide. $O_2^-$ generation mimicked the phorbol myristate acetate response: after a lag period of 2–5 min, $O_2^-$ release gradually increased for 10–15 min until a plateau was reached. Furthermore, GLF enhanced phosphoinositide breakdown with maximal $IP_3$ production at $10^{-7}$ M. Various analogs of GLF were synthesized in order to define the relative importance of the different amino acids and their position in the tripeptide molecule: glycyl-phenylalanine-leucine was devoid of biological properties but enhanced the activity of GLF on the metabolic burst at high concentrations; peptides leucyl-leucyl-phenylalanine and leucyl-leucyl-tyrosine, which displaced GLF from its specific membrane receptors, exerted stimulating effects on PMN oxidative and phosphoinositide metabolisms. It is quite conceivable that these short peptides, which may be generated in the newborn during digestion and which are able to stimulate phagocytic cells, are implicated in the defense of the neonate immature organism against infection.

Besides a nutritional role, milk proteins are of physiological importance as a potential source of numerous peptides endowed with opiate, antihypertensive, antithrombotic [1] and immunomodulating properties [2] among others. These peptides may be implicated in the development of the newborn. As far as immunostimulating peptides are concerned, they may be involved in the stimulation of newborn leukocytes which are less responsive than adult cells. In particular, polymorphonuclear leukocytes (PMNs‡) present significant abnormalities in their chemotactic, chemiluminescence and bactericidal activities [3, 4]. From human and bovine milk proteins we purified several peptides which *in vitro* stimulated phagocytic activities and *in vivo* protected mice against *Klebsiella pneumoniæ* infection [5, 6]. Among them, the following tripeptides were identified: GLF (glycyl-leucyl-phenylalanine, residues 51–53 of human and bovine α-lactalbumins) and LLY (leucyl-leucyl-tyrosine, residues 191–193 of bovine β-casein).

It was shown previously that GLF stimulated human monocyte–macrophage adherence and phagocytosis of human senescent red blood cells (7). Moreover, we recently described specific membrane receptors on human PMNs and monocytes [8].

Some chemotactic peptides such as fMLP (formyl-methionyl-leucyl-phenylalanine)§ have been extensively studied as agents initiating leukocyte activities after binding to specific membrane receptors [9]. Phagocytic responses to chemotactic and other stimuli are vital for host defense and thus substantial interest has been focused on defining the mechanisms of signal transduction within these cells. The central role of calcium, phosphoinositide metabolism and protein kinase C in leukocyte activation is now well established [10]. Although specific receptors for various chemoattractants are present on plasma membranes of phagocytic cells, they appear to utilize a common mechanism to induce cellular responses involving shape change, superoxide production adhesion and degranulation. We demonstrated that GLF receptors differ from those of fMLP since the chemotactic peptide did not displace GLF from its

† Corresponding author.

‡ Abbreviations: DG, 1,2-diacylglycerol; fMLP, formyl-methionyl-leucyl-phenylalanine; GFL, glycyl-phenylalanine-leucine; GFW, glycyl-phenylalanyl-tryptophane; GLF, glycyl-leucyl-phenylalanine; GLY, glycyl-leucyl-tyrosine; $IP_3$, inositol 1,4,5-trisphosphate; LGY, leucine-glycyl-tyrosine; LLF, leucyl-leucyl-phenylalanine; LLY, leucyl-leucyl-tyrosine; OZ, opsonized Zymosan; PBS, phosphate-buffered saline; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PMA, phorbol myristate acetate; PMN, polymorphonuclear leukocyte.

§ We adhere to the conventional fMLP abbreviation instead of fMLF corresponding to the one-letter amino acid abbreviation system.

high-affinity binding sites [8]. However, structural similarity between fMLP and the milk-derived tripeptide led us to examine biological activities of GLF and analogs on phagocytes. An oxidative burst has been strongly implicated in specific and non-specific host defense mechanisms. It was characterized by activation of membrane-associated NADPH-oxidase (which is unique to phagocytes) to provide $O_2^-$, one of the crucial oxidant radicals for the microbicidal activity of the phagocytic cells [11]. The binding of agonists to receptors on the cell surface was known to produce the inositol 1,4,5-trisphosphate ($IP_3$) from phosphatidylinositol 4,5-bisphosphate ($PIP_2$) as a second messenger involved in the mobilization of intracellular calcium [12]. The aim of the present research was to study the biological effects of GLF and related analogs after interacting with their receptors, in particular on PMN oxidative metabolism and polyphosphoinositide breakdown.

## MATERIALS AND METHODS

Tripeptides GLF, GFL (glycyl-phenylalanyl-leucine, residues 61–63 of human β-casein), GLY (glycyl-leucyl-tyrosine), LLF (leucyl-leucyl-phenyl-alanine), LGY (leucine-glycyl-tyrosine, residues 92–94 of bovine αs1-casein) and LLY were synthesized by Bachem Fine Chemicals (Bubendorf, Switzerland). GFW (glycyl-phenylalanyl-tryptophane) was synthesized by Appligène (Illkirch, France). Phorbol myristate acetate (PMA), fMLP, luminol and zymosan were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Dowex AG1-X8 ion exchange resin (200–400 mesh, formate form) was from BioRad (Richmond, CA, U.S.A.), *myo*[2-$^3$H]-inositol (740 GBq/mmol) was from Amersham International (Amersham, U.K.). Mono-Poly resolving medium was from Flow Laboratories (Irvine, U.K.).

Heparinized blood samples were obtained from healthy adult volunteers.

Male Sprague–Dawley rats weighing 180–200 g were obtained from Dépré (Saint-Doulchard, France).

To prepare human PMNs 10 mL of blood were carefully layered onto 7 mL of Mono-Poly resolving medium and centrifuged at 800 *g* for 45 min. This resulted in separation of mononuclear cells at the top band and PMNs at a second band [13]. These cells were removed, washed twice and resuspended in phosphate-buffered saline (PBS).

In some experiments (chemiluminescence, phosphoinositide metabolism) rat PMNs were used. They were collected, from the pleural cavity, 4 hr after interpleural injection of 1 mL of isologous rat serum (heated for decomplementation at 56°, 30 min) [14].

The chemiluminescent response of PMN was measured *in vitro* using a Packard Picolite® luminometer [15]. Opsonized Zymosan (OZ) was first of all prepared by incubation of zymosan particles in 10% fresh isologous serum (human or rat) for 30 min at 37°. After centrifugation at 500 *g* for 15 min, the opsonized particles were suspended at a concentration of 5 mg/mL in PBS. Chemiluminescence was measured after introduction of 100 μL of cell suspension ($5 \times 10^6$ cells/mL) into a $6 \times 50$ mm borosilicate tissue culture tube placed in the apparatus where it was kept at 37° for 5 min in the dark. After equilibration, 20 μL of luminol solution were added at a final concentration of $4 \times 10^{-5}$ M. Mean value of 10 measurements (cumulated counts for 20 sec) was considered as basal response of the cells. Thereafter 150 μL of OZ suspension were added and the photoemission recorded for 20 min. The highest response was considered as response of the stimulated cells. Means and SEM of maximal responses of PMNs incubated in PBS (control) and in various concentrations of peptide ($10^{-6}$–$10^{-12}$ M) were calculated and paired samples statistically analysed using Student's *t*-test in comparison with control values.

Superoxide anion generation was evaluated by reduction of ferricytochrome C (horse heart type III) [16]. PMNs ($5 \times 10^5$ cells) and 50 μL of a 1.6 mM solution of ferricytochrome C were incubated with peptide ($10^{-4}$–$10^{-11}$ M) in a final volume of 1 mL adjusted with PBS (treated cells). Controls were prepared without peptide. Absorbance of these two preparations (control and treated cells) was read in parallel at 550 nm in a double beam spectrophotometer Uvikon 810/820 (Kontron, Saint-Quentin-en-Yvelines, France) which subtracted control value from each assay. The specificity of the reaction was checked by the addition of superoxide dismutase which inhibits at least 90% of stimulation.

Phosphoinositide breakdown analysis was performed on rat PMNs which were labelled as described previously [17]: in brief, cells were adjusted to $10^8$ cells/10 mL/tube, labelled with 100 μCi of *myo*[2-$^3$H]inositol/tube and incubated for 16–20 hr. After washing, viability was determined by Trypan blue exclusion test (more than 95%). Cells were counted and adjusted to a concentration of $10^7$ cells/600 μL aliquots, distributed in Eppendorf microtubes and incubated for 10 min at 37°. The agents were then applied to aliquots tested in duplicates, as follows: PBS for 15 sec and 10 min (controls); fMLP at $10^{-6}$ and $10^{-7}$ M for 15 sec as reference; tested peptides GLF, GFL, LLY and LLF at $10^{-5}$–$10^{-9}$ M for 10 min. The reactions were stopped by addition of perchloric acid, followed by three cycles of freezing–thawing. After centrifugation, the hydrosoluble perchloric supernatants containing inositol phosphates were diluted and neutralized. The inositol lipids were extracted from the perchloric insoluble pellets, and deacetylated according to [17]. The [$^3$H]-inositol phosphates and [$^3$H]glycerophosphoryl esters were separated by anion-exchange chromatography on Dowex AG1-X8 columns using the buffer system described by Downes and Michell [18], Berridge *et al.* [19] and Creba *et al.* [20]. We focused particularly on the characterization of $IP_3$ and $PIP_2$.

Statistical analysis was performed using paired Student's *t*-test ± SEM for oxidative metabolism studies. Since tritiated inositol incorporation into phosphoinositides varied from one pool of cells to another, results are expressed as percentages of controls (aliquots incubated with PBS), means ± SEM.

## RESULTS

### *Effects of GLF*

GLF stimulated rat and human PMN basal

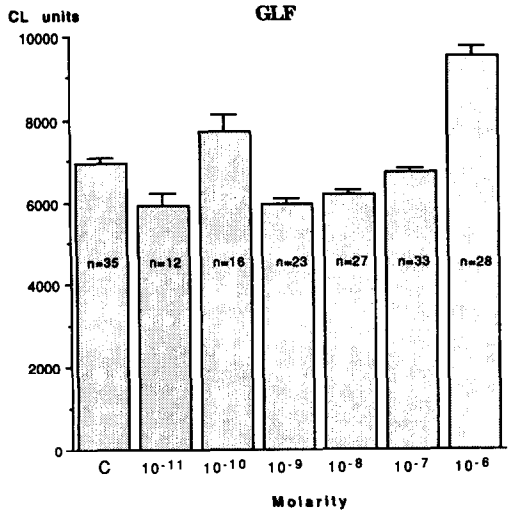


Fig. 1. Basal chemiluminescent response of rat PMN ± SEM in the presence of various concentrations of GLF. C, control values. n, number of experiments in each series. CL, chemiluminescent.

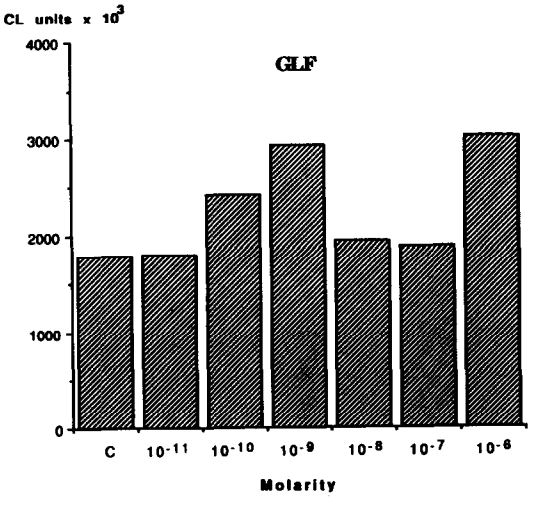


Fig. 2. Chemiluminescent response of rat PMN to OZ in the presence of various concentrations of GLF. C, control values. Data are representative of those obtained in 30 separate experiments. CL, chemiluminescent.

chemiluminescence: similar results were obtained. Figure 1 shows the biphasic response of rat PMNs with peaks reached at $10^{-10}$ and $10^{-6}$ M concentrations. This peptide stimulated also the human and rat PMN response to OZ at the same concentrations. However, the maximal effect depended on the cell reactivity: it could be shifted from $10^{-7}$ to $10^{-6}$ M and from $10^{-10}$ to $10^{-9}$ M depending on the cell batch. Figure 2 shows stimulations observed in a representative experiment of 30 assays.

Generation of superoxide anion by human PMNs stimulated with GLF (difference between treated and control cells) mimicked the PMA response: after a lag time of 2–5 min, according to the concentration used, the $O_2^-$ production increased for up to 10–15 min and was then sustained. Depending on concentrations, the cell response was biphasic (Fig. 3): maximal effects were observed at $10^{-9}$ M ($0.8 \pm 0.1$ mM $O_2^-/10^6$ cells/min) and $10^{-4}$ M ($4.8 \pm 1.0$ mM $O_2^-/10^6$ cells/min). This effect was totally inhibited by superoxide dismutase. Under our experimental conditions $5 \times 10^{-7}$ M PMA generated a mean value of 6 mM $O_2^-/10^6$ cells/min.

GLF stimulated phosphoinositide metabolism according to a bell-shaped response curve. The liberation of $IP_3$ (Fig. 4) was maximal at $10^{-7}$ M. The $PIP_2$ level was not modified.

*Effects of other tripeptides*

Among the other tripeptides whose effects on the chemiluminescence of OZ-stimulated PMNs were tested, only LLF manifested a biphasic activity like that of GLF with two maxima at $10^{-10}$ and $10^{-7}$ M; the two peptides GFW and LLY increased cell response only at $10^{-7}$ and $10^{-6}$ M. GLY, LGY and GFL did not show any significant effect. Figure 5 represents the effects (in percentages compared to control values) obtained in each experimental series.

Basal stimulating effects (in the absence of any other stimulant) were observed on superoxide anion generation with LLY and LLF (Fig. 6). LLY stimulated $O_2^-$ production with a maximal effect (around 1.5 mM $O_2^-/10^6$ cells/min) at low concentrations ($10^{-10}$–$10^{-9}$ M). LLF was less active and maximal stimulation occurred at higher concentrations ($10^{-7}$–$10^{-6}$ M). The other analogous tripeptides tested were inactive.

An important enhancement of $IP_3$ generation, especially at $10^{-7}$ and $10^{-6}$ M, was induced by LLY and LLF (Fig. 4). The $PIP_2$ level did not change. Other tripeptides tested had no effect.

*Effect of associations*

Among the stimulating peptides the association of LLY ($10^{-9}$ M) with GLF ($10^{-7}$ M) enhanced 3-fold the activity of GLF in the chemiluminescent assay after stimulation by OZ (Fig. 7). The effect was lower (2-fold enhancement of GLF activity) if the concentration of LLY was lower ($10^{-10}$ M). In contrast, the association of GFW or LLF with GLF, whatever the concentration, did not change the activity of GLF.

In contrast, the analogous peptide GFL ($10^{-6}$ M), inactive by itself, potentiated 2-fold the activity of GLF ($10^{-6}$ M) after stimulation of rat PMNs by OZ. Among the other non-stimulating peptides, GLY did not modify the activity of GLF on rat OZ-stimulated cells. However, the simultaneous addition of inactive GLY ($10^{-6}$ M) and GLF ($10^{-4}$ M) to human PMNs delayed the basal induced $O_2^-$ generation of the latter peptide (Fig. 8).

## DISCUSSION

This study demonstrates that GLF exerts a

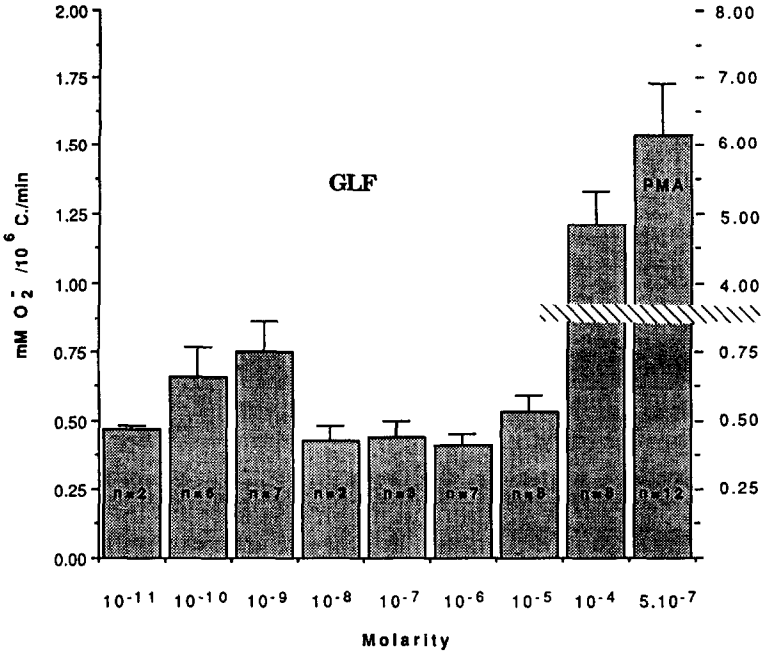


Fig. 3. Superoxide anion production by human PMN after stimulation by GLF: difference in activity (mM $O_2^-/10^6$ cells/min) between control and treated cells. n, number of assays with different cell batches.

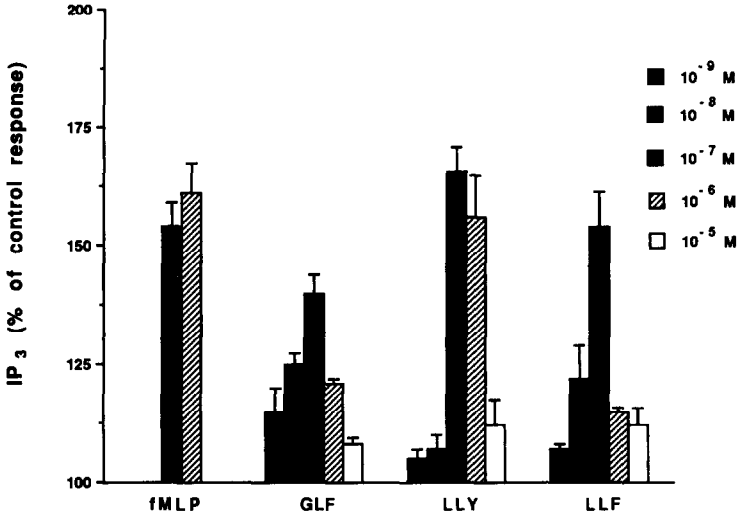


Fig. 4. $IP_3$ release after PMN stimulation by various concentrations of fMLP, GLF, LLY, LLF (controls values = 100%). Values represent the means ± SEM of duplicate determinations. Data are representative of those obtained in four separate experiments.

stimulating effect on oxidative and phosphoinositide metabolisms of both human and rat PMNs.

The oxidative burst response was concentration dependent and biphasic: one maximum correlated with the $K_d$ ($2.3 \pm 1\ 10^{-9}$ M) of the high-affinity GLF binding sites (8) and the other was obtained with a high GLF concentration ($10^{-4}$ M). Stimulation by GLF of superoxide anion generation mimicked that obtained by PMA. Recently, studies on the kinetics of fMLP receptor up-regulation [21] showed that the receptors are rapidly up-regulated in native cells from an intracellular localization. A possible up-regulation might also explain the progressive increase in superoxide anion generation observed

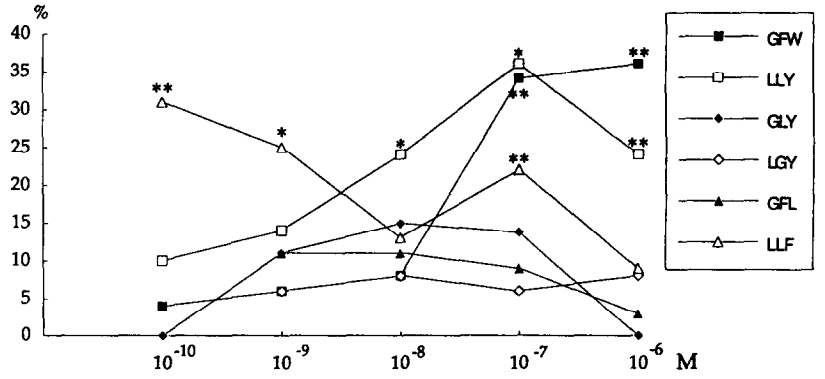


Fig. 5. Comparative chemiluminescent response to OZ of rat PMN incubated with various concentrations of different peptides. Data are expressed as percentage of enhancement above control values. * $P < 0.05$, ** $P < 0.01$.

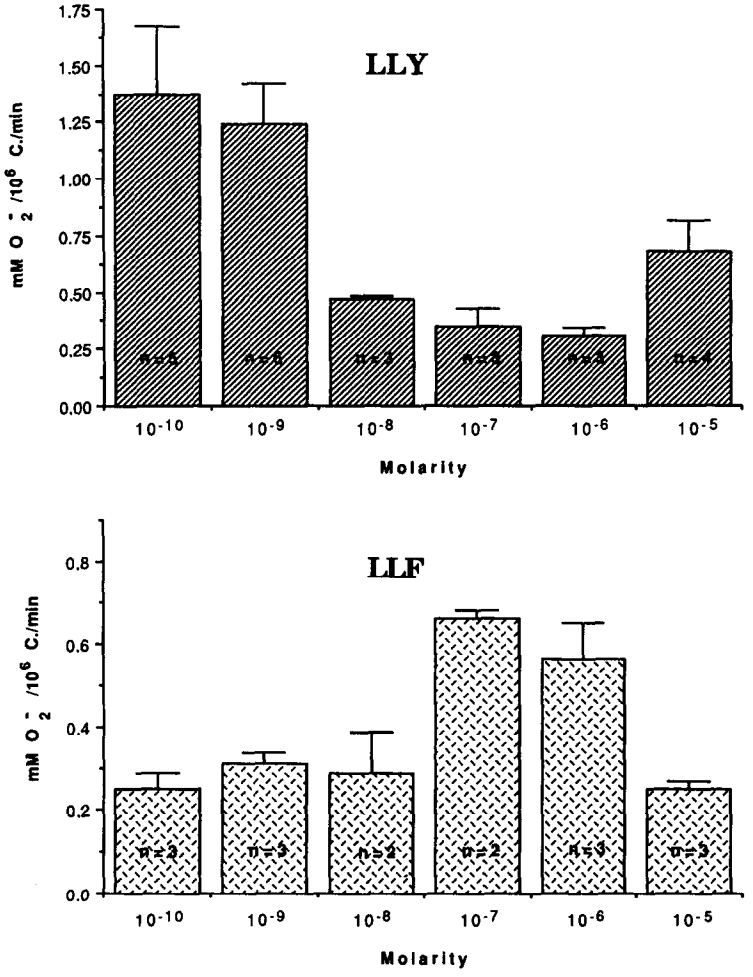


Fig. 6. Superoxide anion production by human PMN after stimulation by two GLF analogous tripeptides, LLY and LLF: difference between control and treated cells. n = number of assays with different cell batches.

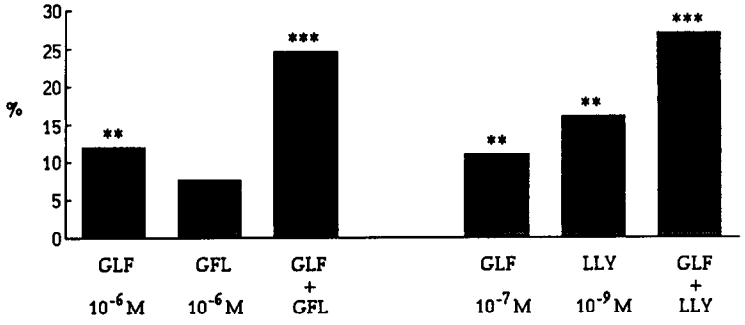


Fig. 7. Chemiluminescent response to OZ of rat PMN incubated with GLF, GFL, LLY or the combination of GLF+GFL or GLF+LLY. Data are expressed as percentage of enhancement above control values. Significances were calculated on real values using the paired Student's *t*-test, in comparison with control values. n = 6. ** $P < 0.01$, *** $P < 0.001$.

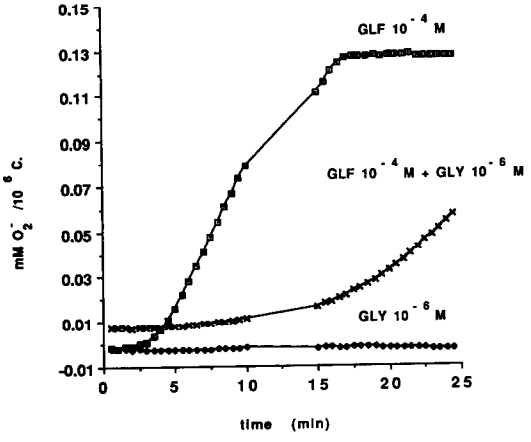


Fig. 8. Effect of association of the two tripeptides GLF and GLY on superoxide anion production by human PMN compared with the activity of each peptide.

after GLF stimulation with appearance or reappearance of new sites of binding to the cell surface as suggested for *N*-formyl peptide [22]. However, after stimulation by fMLP the kinetics of superoxide anion generation by human PMNs are more rapid and transient [23]. This difference corroborated the lack of competitiveness of fMLP for high-affinity binding sites of GLF and thus confirmed the different nature of GLF and fMLP receptors, in spite of the structural similarity of these two peptides. Moreover, GLF failed to stimulate PMN chemotaxis (unpublished data). This might be related to the absence of the methionine residue which seems to be required for chemotactic activity [24].

The occupancy by the tripeptide of its receptors initiated the phosphoinositide breakdown and the formation of the second messenger $IP_3$. Signals from various stimuli transmitted across the cell membrane via specific receptors, such as the chemoattractant receptors, are initiated with a common mechanism via a guanine nucleotide regulatory G protein [25, 26]. It is now well established that the breakdown of $PIP_2$ by phospholipase C into the $Ca^{2+}$ mobilizer $IP_3$ and the protein kinase C activator 1,2-diacylglycerol (DG) represents an early biochemical event initiated by the ternary complex agonist–receptor–regulatory G protein. PMA activation of superoxide production takes place through a direct activation of protein kinase C which is translocated from the cytoplasm to plasma membrane [27, 28]. Protein kinase C contains two functionally distinct domains: a hydrophobic phospholipid-, phorbol ester-, DG-binding domain and a hydrophilic catalytic domain [29]. *In vitro* experiments suggested that the fatty acid composition of membrane phospholipids may be one of the regulatory factors in the activation of protein kinase C [30]. Thus, perturbation of the hydrophobic part of the plasma membrane could influence cellular responses to stimulation of protein kinase C. The maximal oxidative response of PMNs to stimulation by $10^{-4}$ M GLF might be due in part to the hydrophobic nature of this tripeptide which may act, at this high concentration, on membrane fluidity.

The biphasic concentration dependence of PMN metabolic burst induced by GLF may also be related to the existence of two classes of GLF receptors with high and low affinity [8]. Studies of formylpeptide binding to PMN membranes suggested a model with two classes of independent receptors with high and low affinity binding sites [31]. It has, first of all, been suggested that the difference in affinity was not due to intrinsically different receptors but rather to interactions between the receptor and the regulatory G protein [10]. Recent studies suggested that the fMLP receptor complex was composed of more than one component, and that the different affinity states which have been described may be associated with distinct ligand binding sites [32].

The appearance of biphasic activity was observed by chemiluminescence without or with addition of OZ. Only this technique demonstrated a direct activation of the cells, up to a concentration of $10^{-11}$ M. It may be noticed that low concentrations did not prime PMNs since chemiluminescence with Zymosan reflected only an additive effect and not a potentiation of Zymosan stimulation. However this

is not inconsistent with other data obtained with the two other techniques. While $O_2^-$ generation is measured at the same time as chemiluminescence, the indirect method used for the measurement of oxygen radicals did not allow the same sensitivity. On the other hand, $IP_3$ generation is determined after a long period of incubation and cells might be less responsive. Moreover, a greater dose of agonist might be necessary to observe a noticeable increase in $IP_3$ generation compared with that used for $O_2^-$ production since other sources of DG (not only from activation of phospholipase C, but also from that of phospholipases D and $A_2$) are mobilized [33]. In any case, in each assay, GLF action is related to control values obtained from cells of the same batch.

Neither synergism nor competition was observed at respective receptors between OZ and GLF, but only additive activation, indicating different pathways of action at distinct receptors.

The analogous peptide GFL, which did not displace GLF from its specific receptors [8], was devoid of biological activity. Similarly, it is noteworthy that fMPL (formyl-methionyl-phenylalanyl-leucine), analog of the chemotactic peptide fMLP, was practically inactive [34]. This result emphasizes the strict structural specificity of the respective receptors for biological activity. However, added to an equimolar concentration of GLF ($10^{-6}$ M), GFL greatly enhanced the stimulating effect of GLF on PMN chemiluminescent response to OZ. This additive effect might be due to the hydrophobic nature of both peptides. The potentiation might be related to extensive changes in membrane composition similar to that evoked for the potentiation of fMLP superoxide production enhanced by cytochalasin treatment [35].

Two other peptides, LLF and LLY, were found to have an effect on PMN oxidative burst, with or without OZ stimulation. This activity, like that of GLF, was paralleled by the activation of phosphoinositide turnover, using similar concentrations. LLF and LLY recognized GLF high-affinity binding sites with a similar affinity ($K_i$ around 2.5 nM). The presence of a leucine residue as central amino acid seems to be of importance for the affinity of the peptides but not for expression of their biological activity: GLY was inactive though it competed with strong affinity with GLF for binding sites ($K_i = 10^{-9}$ M). This may be considered as an antagonist since, at 100 times lower concentrations it delayed the oxidative response of GLF; the only difference between GLF and GLY is the hydroxyl radical of tyrosine which might play an important role in the specific site of the receptor. The elongation of the peptide by replacement of N-terminal glycine by a leucine seems to modify strongly the structural relationship between ligand and active site of the receptor since LLY was more active than GLF in stimulating oxidative burst and phosphoinositide breakdown.

GLF and some analogs exert a stimulating effect on human adult PMNs. The natural peptides may be released during the digestive process and they may stimulate cells of the immune system of the gut of adults and also of that of newborns for whom milk represents the exclusive nutritive source. It can be speculated that around $10^{-5}$ mol of GLF may be released from human or bovine $\alpha$-lactalbumine (2.6 or 1.2 g/L of milk, respectively) after one natural milk intake of 100 mL. Under these conditions, it is conceivable that these peptides may play a physiological role and favor the development of immature PMNs of the neonates.

*Acknowledgements*—The excellent technical assistance of Mrs M. Gelman, M. Lenoir, M. Semichon and Mr A Thuret is gratefully acknowledged.

## REFERENCES

1. Fiat AM and Jollès P, Caseins of various origins and biologically active casein peptides and disaccharides: structural and physiological aspects. *Mol Cell Biochem* **87**: 5–30, 1989.
2. Migliore-Samour D and Jollès P, Casein, a prohormone with an immunomodulating role for the newborn? *Experientia* **44**: 188–193, 1988.
3. Mills E, Thompson T, Bjorksten B and Quié P, The chemiluminescent response and bactericidal activity of polymorphonuclear neutrophils from newborns and their mothers. *Pediatrics* **63**: 429–434, 1979.
4. Anderson DC, Abbassi O, Kishimoto TK, Koenig JM, McIntire LV and Smith CW, Diminished lectin–, epidermal growth factor–, complement binding domain–cell adhesion molecule-1 on neonatal neutrophils underlies their impaired CD18-independent adhesion to endothelial cells *in vitro*. *J Immunol* **146**: 3372–3379, 1991.
5. Parker F, Migliore-Samour D, Floc'h F, Zerial A, Werner GH, Jollès J, Casaretto M, Zahn H and Jollès P, Immunostimulating hexapeptide from human casein: amino acid sequence, synthesis and biological properties. *Eur J Biochem* **145**: 677–682, 1984.
6. Berthou J, Migliore-Samour D, Lifshitz A, Delettré J, Floc'h F and Jollès P, Immunostimulating properties and three-dimensional structure of tripeptides from human and cow caseins. *FEBS Lett* **218**: 55–58. 1987.
7. Gattegno L, Migliore-Samour D, Saffar L and Jollès P, Enhancement of phagocytic activity of human monocytic-macrophagic cells by immunostimulating peptides from human casein. *Immunol Lett* **18**: 27–32, 1988.
8. Jazziri M, Migliore-Samour D, Casabianca-Pignède MR, Keddad K, Morgat JL and Jollès P, Specific binding sites on human phagocytic blood cells for Gly-Leu-Phe and Val-Glu-Pro-Ile-Pro-Tyr, immunomodulating peptides from human milk proteins. *Biochim Biophys Acta*, submitted.
9. Sha'afi RI and Molski TFP, Activation of the neutrophil. *Prog Allergy* **42**: 1–64, 1988.
10. Snyderman R and Uhing R, Phagocytic cells: stimulus–response coupling mechanisms. In: *Inflammation Basic Principles and Clinical Correlates* (Eds. Gallin JI, Goldstein IM and Snyderman R), pp. 309–223. Raven Press, New York, 1988.
11. Babior BM, Oxidants from phagocytes: Agents of defense and destruction. *Blood* **64**: 959–966, 1984.
12. Berridge MJ, Inositol trisphosphate and diglycerol as second messengers. *Biochem J* **220**: 345–360, 1984.
13. Ferrante A and Thong YH, Optimal conditions for the purification of neutrophils and mononuclear cells from human blood. *J Immunol Method* **36**: 109–114, 1980.
14. Giroud JP, Roch-Arveiller M and Muntaner O, Prélèvement répété des polynucléaires dans la cavité pleurale du rat. Application à l'étude du chimiotactisme. *Nouv Rev Fr Hematol* **20**: 535–543, 1978.
15. Allen RC, Sternholm L and Steel RH, Evidence for the generation of an electronic state(s) in human

polymorphonuclear leukocytes and its participation in bactericidal activity. *Biochem Biophys Res Commun* **47**: 679–684, 1972.
16. Johnston R, Kell B B, Misra HP, Lehmeyer JE, Webb LS, Baehner RL and Rajacopalan KV, The role of superoxide anion generation in phagocytic bactericidal activity. *J Clin Invest* **55**: 1357–1372, 1975.
17. Tissot M, Mathieu J, Mirossay L, Thuret A and Giroud JP, Polyphosphoinositide metabolism in polymorphonuclear cells from healthy and thermally injured rats: effect of the immunomodulator RU 41740. *J Leuk Biol* **50**: 607–614, 1991.
18. Downes CP and Michell RH, The polyphosphoinositide phosphodiesterase of erythrocyte membranes. *Biochem J* **198**: 133–140, 1981.
19. Berridge MJ, Dawson RMC, Downes CP, Heslop JP and Irvine RF, Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. *Biochem J* **212**: 473–482, 1983.
20. Creba JA, Downes CP, Hawkins PT, Brewster G, Michell RH and Kirk CJ, Rapid breakdown of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate in rat hepatocytes stimulated by vasopressin and other $Ca^{2+}$-mobilizing hormones. *Biochem J* **212**: 733–747, 1983.
21. Norgauer J, Eberle M, Fay SP, Lemke HD and Sklar LA, Kinetics of *N*-formyl peptide receptor up-regulation during stimulation in human neutrophils. *J Immunol* **146**: 975–980, 1991.
22. Zigmond SH, Sullivan SJ and Lauffenburger DA, Kinetic analysis of chemotactic peptide receptor modulation. *J Cell Biol* **92**: 34–43, 1982.
23. Sklar LA, Ligand–receptor dynamics and signal amplification in the neutrophil. *Adv Immunol* **39**: 95–143, 1986.
24. Harvath L, Neutrophil chemotatic factors. In: *Cell Motility Factors* (Ed. Goldberg ID), pp. 35–52. Birkhaüser, Basel, Switzerland, 1991.
25. Snyderman R, Regulatory mechanisms of a chemoattractant receptor on leukocytes. *Fed Proc* **43**: 2743–2748, 1984.
26. Smith CD, Lane BC, Kusaka I, Verghese MW and Snyderman R, Chemoattractant-receptor induced hydrolysis of phosphatidylinositol 4,5-bisphosphate in human polymorphonuclear leukocyte membranes: requirement of a guanine nucleotide regulatory protein. *J Biol Chem* **260**: 5875–5878, 1985.
27. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U and Nishizuka Y, Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* **257**: 7847–7851, 1982.
28. Christiansen NO, Larsen CS, Juhl H and Esmann V, Membrane-associated protein kinases in phorbol ester-activated human polymorphonuclear leukocytes. *Biochim Biophys Acta* **884**: 54–59, 1986.
29. Cabot MC and Jaken S, Structural and chemical specificity of diacylglycerols for protein kinase C activation. *Biochem Biophys Res Commun* **125**: 163–169, 1984.
30. Snoek GT, Feijen A, Hage WJ, Van Rotterdam W and De Laat SW, The role of hydrophobic interactions in the phospholipid-dependent activation of protein kinase C. *Biochem J* **255**: 629–637, 1988.
31. Snyderman R and Pike MC, Chemoattractant receptors on phagocytic cells. *Annu Rev Immunol* **2**: 257–281, 1984.
32. Mc Phail L, Clayton CC and Snyderman R, A potential second messenger role for unsaturated fatty acid activation of $Ca^{2+}$ dependent protein-kinase. *Science* **225**: 622–624, 1984.
33. Morel F, Doussière J and Vignais P, The superoxide-generating oxidase of phagocytic cells. Physiological, molecular and pathological aspects. *Eur J Biochem* **201**: 523–546, 1991.
34. Showell HJ, Freer RJ, Zigmond SH, Schiffmann E, Aswanikumar S, Corcoran B and Becker EL, The structure–activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. *J Exp Med* **143**: 1154–1169, 1976.
35. Jesaitis AJ and Allen RA, Activation of the neutrophil respiratory burst by chemoattractants: regulation of the *N*-formyl peptide receptor in the plasma membrane. *J Bioenerg Biomembr* **20**: 679–707, 1988.