Studies on Human Neutrophil Biological Functions by Means of Formyl-peptide Receptor Agonists and Antagonists

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Abstract: Phagocytes are activated by several extracellular signals, including formyl-peptides derived from bacterial proteins or disrupted cells. The most intensely studied member of the formylpeptide family is the synthetic tripeptide N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), whose specific receptors have been identified on neutrophil plasma membrane and subsequently cloned. The fMLP-receptor interaction activates multiple transduction pathways responsible for various neutrophil functions such as adhesion, chemotaxis, exocytosis of secretory granules and superoxide anion production, which represent the physiological response to bacterial infection and tissue damage. An unresolved question is whether signaling requirements are identical or specific for each physiological function. The development of fMLP receptor agonists and antagonists has led to an improvement of our knowledge about the above issue. Of particular interest is the possibility that receptorial antagonists, able to transiently inhibit neutrophil responses to formylpeptides, could be therapeutic agents in the treatment of inflammation-related diseases.

Aim of this review is, i) to summarise the current understanding of the series of events that begins at the level of formylpeptide-receptor interaction and is responsible for the activation of transduction pathways, which finally determine neutrophil response; ii) to define the state of art regarding the synthesis as well as the biological actions of fMLP receptor agonists and antagonists.

Keywords: Neutrophils; Formylpeptide receptor; Transduction mechanisms; Neutrophil functions; Formylpeptide receptor agonists; Formylpeptide receptor antagonists

INTRODUCTION

Formylpeptides

It has long been known that bacteria activate human phagocytes by releasing low molecular weight chemoattractants, identified as N-formylpeptides in 1975 [1,2]. Phagocytes, in turn, play a crucial role in host defence against microorganisms, since they are able to destroy invaders by releasing proteolytic enzymes and other damaging proteins and by producing reactive oxygen species. In 1982, Carp demonstrated that formylpeptides, able to serve as potent activators for neutrophils, are also produced in mitochondria of mammalian cells [3], leading to the suggestion that they may be released by disrupted cells at site of inflammation and tissue damage. In particular, it has been postulated that phagocytes, attracted to an inflammatory site and activated by non-mitochondrial peptides, induce cell damage and mitochondrial disruption: the consequent N-formylpeptide release may serve as an amplification signal for further generation of chemotactic factors [4].

The tripeptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) is the major chemotactic factor produced by *Escherichia coli* [5]; this compound, together with its synthetic methyl ester derivative (fMLP-OMe), is a potent chemoattractant for phagocytes [6,7]. Due to the ability to highly activate all neutrophil physiological functions and the relative ease of the synthesis, both fMLP and fMLP-OMe are the most intensely studied members of the formylpeptide family.

Formylpeptide Receptor

Formylpeptides activate phagocyte functions following their binding to a membrane receptor, first cloned by Boulay, Tardif, Brouchon and Vignais in 1990, by screening a mammalian cell expression library for binding to iodinated formylpeptide ligand [8,9]. This is a classical G protein-

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coupled receptor, characterized by seven transmembrane hydrophobic segments connected by hydrophilic domains; potential *N*-linked glycosylation or phosphorylation sites are present in the extracellular or intracellular receptor surface, respectively. Cells transfected with cDNA for formylpeptide receptor display one or two classes of ligand binding sites [9,10]. Binding experiments, carried out on human neutrophil membrane preparations utilising [^{3}H]fMLP, also revealed high and low affinity states for the formylpeptide receptor, depending on the absence or the presence of guanine nucleotides [11,12]. Accordingly, when experiments are performed on the whole neutrophils, only a single class of binding sites is detectable, with low affinity toward formylpeptides, probably due to the interaction of intracellular GTP with the receptor-coupled G proteins [13].

In addition to interacting with G proteins, formylpeptide receptor also rapidly associates with cytoskeleton at 37°C, forming a high-affinity complex with the ligand, which is insensitive to guanine nucleotide. Since this interaction is followed by a rapid internalisation, it has been proposed that receptor-cytoskeleton binding could play a role in the termination of neutrophil responses. These processes are temperature-dependent. In fact, it has been demonstrated that at 15°C, stimulation by fMLP induces a high affinity form of the formylpeptide receptor which becomes associated with the cytoskeleton, but it is not internalised. In contrast, at 4°C, formylpeptide receptor interacts slowly with the cytoskeleton [14-16]. When neutrophils are exposed to photoaffinity ligand at 4°C, a condition which impairs cell activation, formylpeptide receptor is found in a plasma membrane domain enriched in G proteins; on the contrary at 15°C, a condition which causes receptor desensitisation, the receptor is present in a plasma membrane domain enriched in cytoskeleton proteins but depleted in G proteins [17,18]. These observations led to the hypothesis that the segregation of formylpeptide receptors in different membrane domains might control phagocyte physiological functions.

Two human genes, termed *FPR1* and *FPRL1*, encode two formylpeptide receptor subtypes, commonly referred as FPR (formylpeptide receptor) and FPRL1 (formylpeptide receptor-like 1 receptor). These proteins show a high degree of aminoacid sequence identity; both are expressed in phagocytes and bind fMLP, although FPR shows higher affinity [9,19]. Another related human gene (*FPRL2*) encodes a protein which also shows a good degree of identity to FPR and FPRL1; it is expressed in monocytes, but not in neutrophils and its function is undefined [20,21].

Until recently it has been thought that formylpeptide receptor is specifically expressed in neutrophils and monocytes. However, in the mid-1990s, the presence of FPR has been demonstrated in different nonhematopoietic cells, such as hepatocytes, dendritic cells and astrocytes, suggesting that formylpeptides may influence cellular mechanisms that are independent of the inflammatory response [22,23]. For this purpose, Buzzi, Vesce, Ferretti, Fabbri and Biondi [24] demonstrated the presence of specific binding sites for [^{3}H]fMLP in human amniotic membranes and that their expression is different in membranes obtained from laboring and nonlaboring women. In particular, high and low affinity binding have been identified in the former, while only low affinity receptors were found in the latter. Moreover, fMLP evokes prostaglandin E_2 release, at higher levels from laboring than nonlaboring tissues. In support to this observation, Biondi, Pavan, Ferretti, Ginanni Corradini, Neri and Vesce [25] demonstrated that in human amnion-derived WISH cells, which appear to behave like nonlaboring amnion tissue, only the low affinity FPRs are expressed. On the basis of these results it has been proposed that fMLP, possibly released following stretching or disruption of intrauterine tissues, exerts a role in both term and premature delivery in women.

Formylpeptide Receptor Signaling

FMLP-receptor interaction generates multiple second messengers in neutrophils, through the activation of phospholipase C, D and A_2, and it rapidly stimulates phosphatidylinositol 3-kinase as well as tyrosine phosphorylation; an increase of cyclic AMP (cAMP) intracellular levels has also been demonstrated [26-29]. The activation of these transduction pathways is responsible for the induction and/or modulation of neutrophil specific responses, i.e. adhesion, chemotaxis, exocytosis of secretory granules, and superoxide anion (O_2^-) production. The mechanism by which a single agonist (i.e. fMLP) is able to activate several transduction pathways and signal the onset of discrete neutrophil functions - which show different dose-response to the agonist itself [30] - has not been completely elucidated. Another unresolved question is whether signaling requirements are identical or specific for each physiological function. Several experiments carried out utilising drugs [31] or pharmacological manipulation of signal transduction pathways [32,33] indicate that distinct mechanisms are responsible for the different neutrophil responses. Moreover, it has been demonstrated that each physiological function shows different requirements for receptor occupancy. O_2^- production needs continuous occupancy of almost 100% of the receptors to achieve and maintain an optimal response, whereas exocytosis of secretory granules or chemotaxis do not [27,34].

Phospholipase C (PLC) activation is one of the earliest events following fMLP-receptor interaction in the neutrophil plasma membrane: as a consequence, an increase in diacylglycerol and inositol phosphates, as well as in Ca^{2+} cytosolic levels, is evoked. Different subtypes of the PLC-β family are involved in neutrophil activation by chemoattractants [26]. Ca^{2+} enhancement appears to occur in two stages: an initial, transient release from intracellular storage sites, followed by a more delayed influx across the plasma membrane [35]. Increase in Ca^{2+} cytosolic levels has been postulated to be a necessary condition for neutrophil response to formylpeptides, because it is one of the earliest detectable events induced by cell exposure to fMLP [36,37]. Of particular interest is the demonstration that, during phagocytosis in human neutrophils, a redistribution of intracellular Ca^{2+} stores occurs, presumably serving to localise functional response within the cell [38]. However, a role for cytosolic free Ca^{2+} concentration, particularly in neutrophil chemotaxis, remains to be established clearly. With regard to this point, Haines, Kolansinski, Cronstein, Reibman, Gold and Weissmann [39] postulated that

chemotactic substances can be divided in "classical" and "pure" chemoattractants. The former group comprises fMLP and other agents (including C5a and interleukin-8), which evoke multiple neutrophil responses, by increasing Ca^{2+} intracellular level. Pure chemoattractants, as substance P and transforming growth factor β1 [40], are molecules that induce chemotaxis but not oxidative burst and granule enzyme release: a common feature of these substances seems to be their inability to influence intracellular calcium concentration. In support to this view, it has been demonstrated that the murine S 100 protein, CP-10, possesses a potent chemotactic activity for phagocytes, but fails to enhance Ca^{2+} levels in human or mouse neutrophils [41]. A similar conclusion has been reached by studying the activation of guinea-pig neutrophils by wasp chemotactic peptides. In fact, *Icaria* chemotactic peptide (I-CP), like fMLP, has been found to induce multiple functional responses by neutrophils as well as very rapid increases in intracellular Ca^{2+} concentration, whereas [Lys7]I-CP, an analog peptide which induces only chemotaxis, provokes slower enhancements of the cation level [42]. The requirement of Ca^{2+} enhancement for chemotaxis has further been questioned following the report by Laffafian and Hallett [43], who demonstrated that the cation levels, measured in human neutrophils moving towards a source of the classical chemoattractant fMLP, do not change significantly. They observed an abrupt rise in Ca^{2+} levels only when cells reach the zone of higher peptide concentration, where chemotaxis stops. Neutrophil chemotaxis seems independent of, not only calcium enhancement, but also of the PLC pathway: as a matter of fact Li, Jiang, Xie, Zhang, Smrcka and Wu [44], utilising mice lacking PLC-β2 and β3, demonstrated that such an enzymatic system has an important role in chemoattractant-mediated production of O_2^-, but not in chemotaxis. In the same experimental model, phosphatidylinositol 3-kinase appears to play an important, although not essential role, in neutrophil chemotaxis, but it is required for O_2^- production [44,45]. Other authors demonstrated that a selective phosphatidylinositol 3-kinase inhibitor has no effect on neutrophil chemotaxis stimulated by fMLP, but completely blocks the chemotactic response evoked by different agonists [46].

Phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), the PLC substrate, has been implicated in the regulation of changes in actin assembly and architecture, involved in neutrophil locomotion [47,48]. For this purpose, it has been hypothesized that fMLP-receptor interaction activates a small G-protein (Cdc42) which, in turn, evokes actin filament barbed-end uncapping and maximal catalytic activity of WASP family proteins, activated by GTP-Cdc42. Active WASP proteins cause the Arp2/3 complex to promote actin nucleation. Rohatgi, Ho and Kirschner [49] recently proposed that $PI(4,5)P_2$ acts in two ways: it activates WASP family proteins, and indirectly stimulates Cdc42.

A phospholipase A_2 (PLA_2) activity has been identified in the cytosol of neutrophils, able to translocate to plasma membrane following an increase in Ca^{2+} cytosolic levels; secretory and membrane-bound forms of the enzyme have also been identified [26]. PLA_2 releases arachidonic acid from phospholipids; this compound represents both a second messenger modulating cellular activities, and the precursor for eicosanoids, which also include prostaglandins whose inhibitory activity on neutrophil responses is well documented [50,51]. Recently, the crucial role of the cytosolic PLA_2 in arachidonate output from differentiated HL-60 cells exposed to fMLP has been questioned by Sternfeld, Thevenod and Schultz. [52], who demonstrated that the bulk of the release originates from activation of PLC and phospholipase D (PLD), but not of cytosolic PLA_2. On the contrary, the essential requirement of cytosolic PLA_2-generated arachidonic acid in the activation of physiological functions by granulocyte-like cells has been evidenced by Pessach, Leto, Malech and Levy [53].

PLD activation results in the production of phosphatidic acid which is both a direct activator of neutrophil responses and a precursor of diacylglicerol, thus sustaining the effects of PLC activation. Like PLA_2, PLD activity has been found both in cytosol and in neutrophil membranes, and the cytosolic enzyme requires a Ca^{2+}-dependent translocation to plasma membrane in order to reach its phospholipid substrate. The Ca^{2+}-dependence of both PLA_2 and PLD activities suggests that these pathways are downstream to PLC stimulation, thus establishing a hierarchy in the mechanisms operated by the three phospholipases [26]. PLD, together with phosphatidylinositol 3-kinase, seems essential for a sustained O_2^- production by human neutrophils stimulated by fMLP [54].

Several studies demonstrated the involvement of tyrosine kinase in phagocyte signal transduction [26,32]; moreover, it has been reported that stimulation of exocytotic activity and O_2^- production by neutrophils and macrophages is impaired by an increase in cell phosphotyrosine phosphatase activity as well as by the presence of tyrosine kinase inhibitors [55,56]. Since neutrophil functionalities in response to distinct agents are differentially affected by tyrosine kinase inhibitors, it has been suggested that multiple tyrosine phosphorylating enzymes may participate in the transduction of the signals originating from the cell environment [33].

It has been reported that fMLP-receptor interaction induces an enhancement of cAMP levels, but the mechanism responsible for this increase is still a subject of debate. No direct coupling between the FPR and adenylyl cyclase could in fact be demonstrated in neutrophil membranes [28] and an inhibitory action of fMLP on cAMP phosphodiesterase activity has never been clearly observed [29]. Nevertheless cAMP elevation by the formylpeptide is counteracted by adenylyl cyclase inhibitors [57], suggesting that the nucleotide synthesizing enzyme is a target for the peptide action. In transient transfection studies a stimulation of type II adenylyl cyclase, mediated by the interaction of the fMLP receptor with a Gi protein, has been evidenced [58]. The role of cAMP enhancement in neutrophil responses has not been completely clarified. Although the nucleotide increase precedes cellular responses, it has been proposed that elevation of cAMP intracellular levels may represent a crucial signal to switch off neutrophil activation [29,59]. However, conditions that inhibit cAMP elevation have been reported to impair fMLP-evoked neutrophil responses [57]. Neutrophil cAMP enhancement, in the presence of formylpeptides, seems a consequence of PLC stimulation. This hierarchy is suggested by the demonstration that the

PLC blocker, U-73122, inhibits the nucleotide increase both in basal and fMLP-activated conditions; on the contrary, the adenylyl cyclase inhibitor MDL 12330A, at doses able to significantly reduce basal or fMLP-enhanced cAMP levels, is completely ineffective on neutrophil PLC activity [60].

FORMYLPEPTIDE RECEPTOR AGONISTS

As previously mentioned, the tripeptide methyl ester fMLP-OMe is a potent chemoattractant and is also the generally adopted molecule, together with the natural counterpart fMLP, as a reference model for evaluating the activity of newly synthesized analogs [6,7]. In addition to the high lipophilic character, due to the presence of the three hydrophobic side chains, this compound is characterised by a pronounced backbone conformational flexibility, which seems an important feature for establishing efficient interactions with the formylpeptide receptors [61].

Several fMLP-OMe analogs have been synthesised and utilised in different laboratories, with the aim to characterise formylpeptide-receptor interaction in phagocytes as well as in cellular activation.

Some of these synthetic peptides, whose structure is depicted in Fig. (**1**), are characterised by the presence of unnatural α-aminoacids; in particular, in for-Thp-Leu-Ain-OMe [Thp^1, Ain^3] analog the native Met and Phe external residues have been replaced by 4-amino-tetrahydrothiopyran-4-carboxylic acid (Thp) and 2-aminoindane-2-carboxylic acid (Ain), respectively [62], whereas in for-Thp-Leu-Phe-OMe [Thp^1] [62] or in for-Met-Leu-Ain-OMe [Ain^3] [63], only one of the two residues has been replaced. In for-Met-Δ^zLeu-Phe-OMe [$\Delta^z Leu^2$], the central Leu has been replaced by (*Z*)-2,3-didehydroleucine (Δ^zLeu) [64]. Although the three synthetic residues maintain close structural similarity with the corresponding natural aminoacids, they can determine a significant reduction of peptide backbone flexibility inducing, at the same time, the adoption of preferred conformations. The resulting "conformationally constrained" analogs of the natural peptide ligand are expected to show selective activity and are useful tools to collect information on the nature of the bioactive, receptor-bound conformations.

In accordance with the above considerations, it has been found that fMLP-OMe, which has an identical behavior to that of fMLP, is able to induce a full response by neutrophils. On the contrary, some of the constrained analogs exhibit a different and selective behavior. In particular, [Thp^1, Ain^3] and [Thp^1], both characterized by the presence of the Thp residue at the N-terminal position, are pure chemoattractants [62] while [$\Delta^z Leu^2$], which is conformationally constrained at the central position, is only

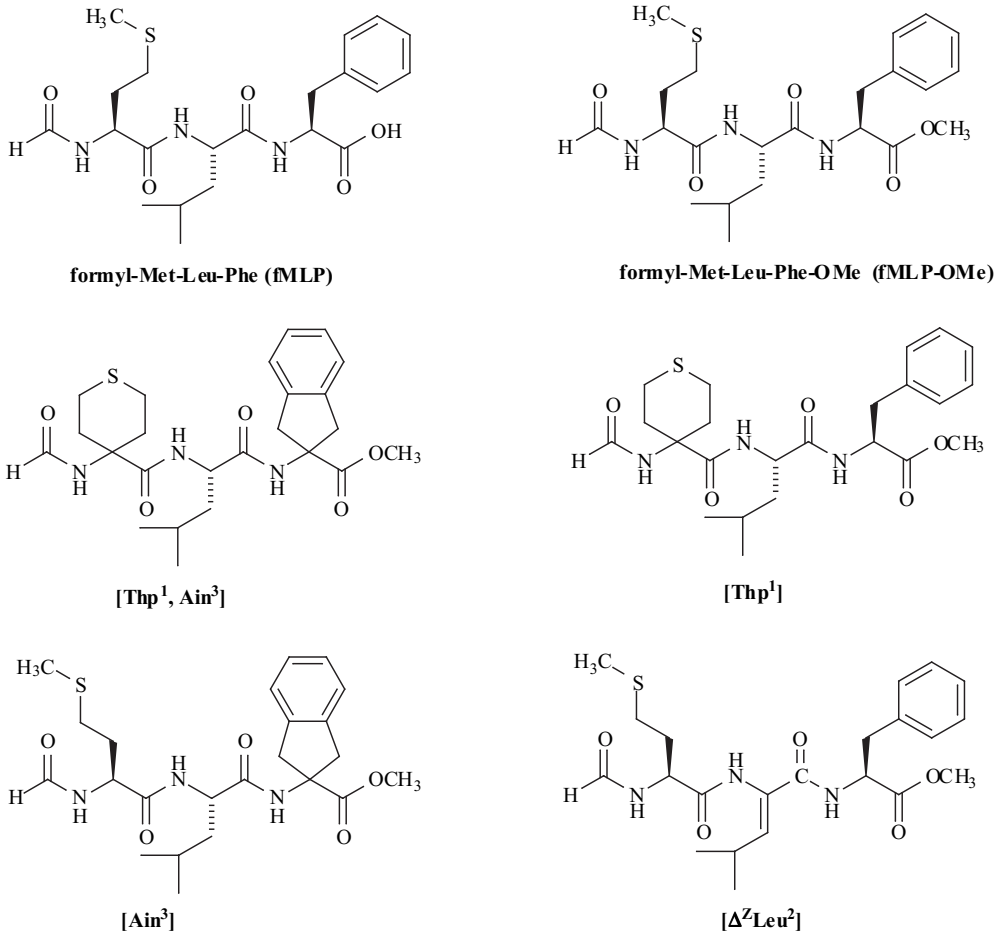

Fig. (1). Chemical formulas of fMLP, fMLP-OMe and synthetic peptides which are characterised by the presence of unnatural α-aminoacids.

able to elicit O_2^- production and degranulation [64]. [Ain^3], which contains a conformational constraint at the C-terminal residue, behaves as a full agonist, but is less efficacious than the parent fMLP-OMe in evoking O_2^- production [62]. The activation of specific transduction pathways seems to underlie the different biological activities exhibited by the above described fMLP-OMe constrained analogs. In fact [$\Delta^z Leu^2$], like to fMLP and fMLP-OMe, induces a considerable enhancement of PLC activity as well as of Ca^{2+} and cAMP intracellular levels; on the other hand, [Thp^1, Ain^3] and [Thp^1] are unable to significantly influence the enzyme or the second messengers' intracellular levels. As for [Ain^3], it enhances the concentration of Ca^{2+} and cAMP at the same high doses that evoke O_2^- production [29,60,65,66]. The constrained, fMLP-OMe analogs also show a different affinity towards the FPR. In particular, the full agonist fMLP-OMe and the constrained analog [$\Delta^z Leu^2$] are both effective in displacing the labelled peptide from its binding sites, while [Thp^1, Ain^3] and [Thp^1] are much less efficacious [66].

Other fMLP-OMe analogs under study are the [cyclodimer Phe(Lys)], for-Met-Leu-Pheol-COMe [$(Pheol\text{-}Ac)^3$], and for-Nle-Leu-Δ^2 Phe-OMe [Nle^1, $\Delta^Z Phe^3$] Fig. (**2**). The [cyclodimer Phe(Lys)], which contains a 20-membered ring made up of two Phe(Lys) fragments, behaves as full agonist towards FPR and this can be related to the capability of the for-Met moiety, which is not part of the ring, to adopt a correct spatial orientation [67]. The for-Met-Leu-Pheol-COMe [$(Pheol\text{-}Ac)^3$] analog contains the (S)-phenylalaninol (Pheol) acetate fragment in place of the Phe-OMe residue. Thus, this ligand, as compared to the parent reference peptide, maintains the C-terminal ester function although it is translated and reversed in its direction [-O-C(=O) *versus* -C(=O)-O]. However, the key H-bond interaction of the C-terminal carbonyl group with the appropriate receptor area [68] can still be operative [69]. This ligand is a selective activator of O_2^- production and lysozyme release but is inactive as chemoattractant; at present we have no obvious explanation for such a selectivity. Finally, the for-Nle-Leu-Δ^2 Phe-OMe [Nle^1, $\Delta^Z Phe^3$] ligand contains the (S)-norleucine (Nle) and the *Z*-2,3-didehydrophenylalanine (Δ^Z Phe) in place of the native Met and Phe external residues; this analog is unable to evoke neutrophil responses and this behavior has been related with the unfavorable spatial orientation of the aromatic ring with respect to the backbone adjacent atoms [70]. The [cyclodimer Phe(Lys)] and for-Met-Leu-Pheol-COMe [$(Pheol\text{-}Ac)^3$] induce a consistent enhancement of Ca^{2+} and cAMP intracellular levels in human neutrophils, whereas for-Nle-Leu-Δ^2 Phe-OMe [Nle^1, Δ^Z Phe^3] is completely ineffective in this regard [60].

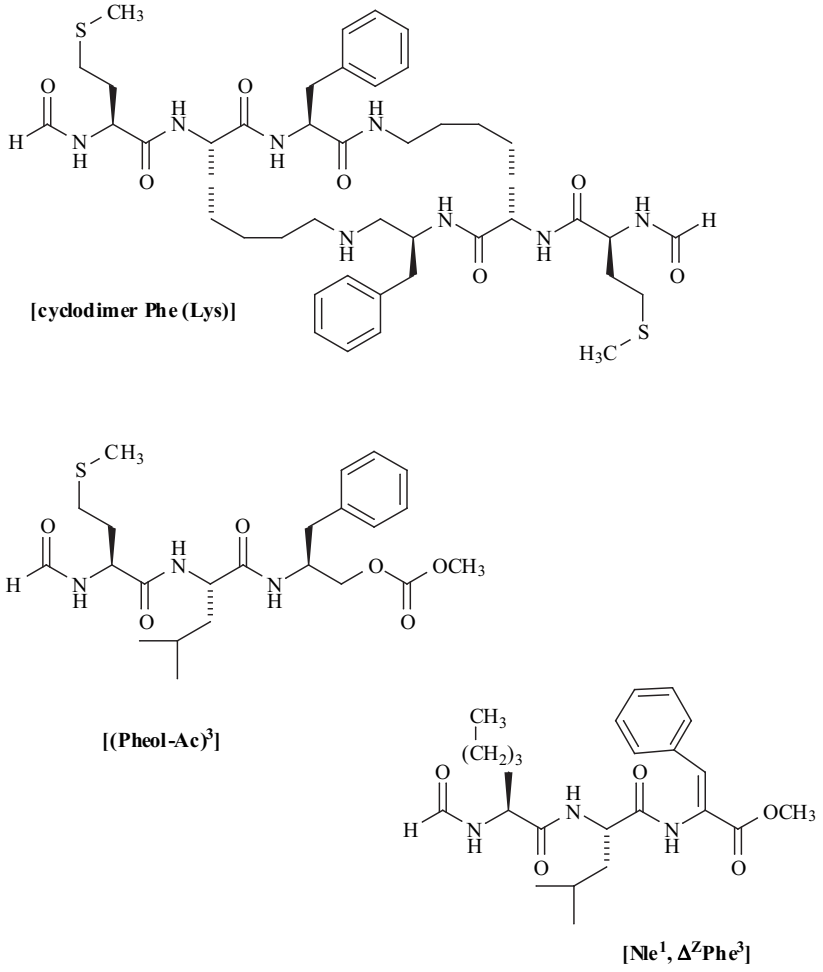

Fig. (2). Chemical formulas of fMLP-OMe analogs: [cyclodimer Phe(Lys)], for-Met-Leu-Pheol-COMe [$(Pheol\text{-}Ac)^3$], and for-Nle-Leu-Δ^2 Phe-OMe [Nle^1, $\Delta^Z Phe^3$]

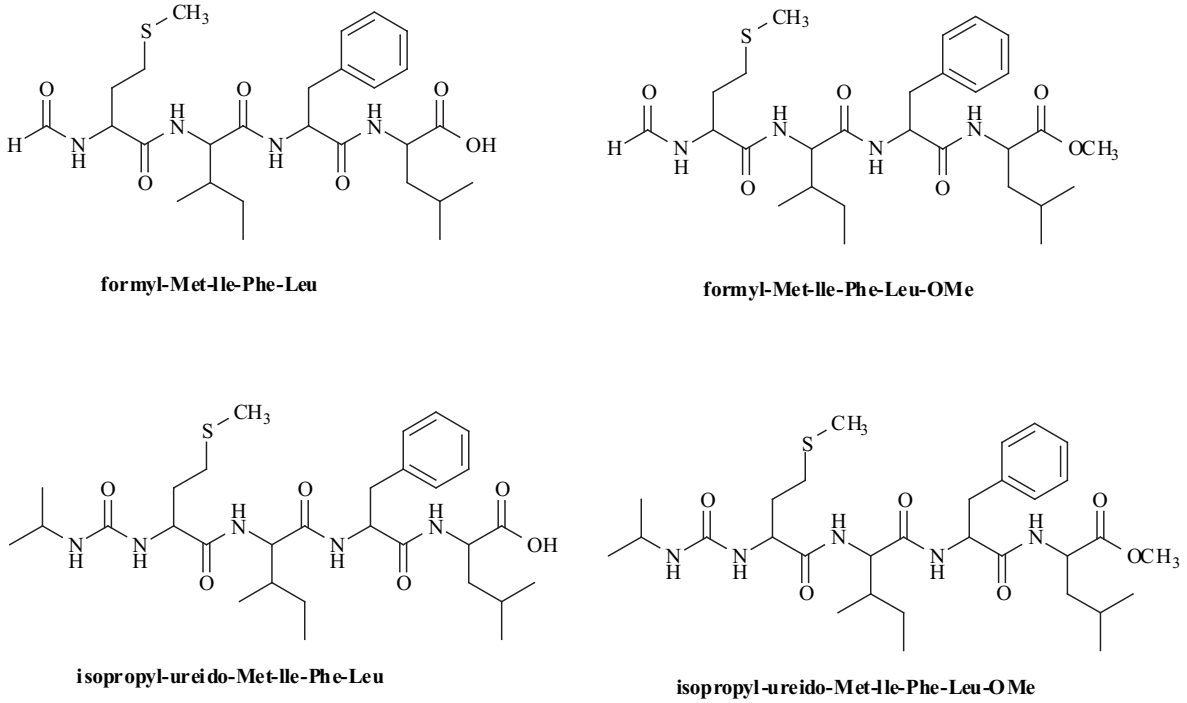

Fig. (3). Chemical formulas of tetrapeptide derivatives which are full (f-Met-Ile-Phe-Leu) or partial (isopropylureido-Met-Ile-Phe-Leu) agonists of formyl peptide receptor.

Two N-formylated tetrapeptides with phenylalanine in position 3 (fMet-Ile-Phe-Leu and fMet-Leu-Phe-Ile) act as full chemotactic agonists on human monocytes [71]. A similar behavior toward human neutrophils has recently been confirmed for the free acid peptide derivative fMet-Ile-Phe-Leu, and it has been shown that the C-terminal methyl ester fMet-Ile-Phe-Leu homologs have an agonist power similar to that of the free acid derivative; moreover, the FPR affinity and activity values of these N-formyl-tetrapeptides are one order of magnitude higher than those of fMLP [72]. N-ureido isopropyl-Met-Ile-Phe-Leu derivatives have been shown to be weak partial agonists toward FPR on human neutrophils. It has, moreover, been demonstrated that the agonist properties of these tetrapeptide derivatives are not noticeably influenced by C-terminal methyl esterification or by conversion to the corresponding amide [72].

In Fig. (**3**), the chemical structure of the above mentioned peptides is reported.

FORMYLPEPTIDE RECEPTOR ANTAGONISTS

It has been reported that the *t*-Boc peptide derivative *t*-Boc-Phe-D-Leu-Phe-D-Leu-Phe, Fig. (**4A**), displays FPR antagonist activity on rabbit neutrophils [73]. In this context it has been suggested that the *t*-Boc group on peptide derivatives is essential for imparting FPR antagonist activity to rabbit neutrophils, even if it causes a loss in binding potency [74]. It has been reported that the ability to antagonise rabbit neutrophil functions is not greatly dependent on the primary sequence (from tri- to penta-peptides) or chirality of peptide derivatives. On the other hand, the antagonist's ability to interact with FPR has been shown to be much more influenced by such structural characteristics [75-78]. In particular, it has been demonstrated that the affinity for FPR of the derivative *t*-Boc-Phe-D-Leu-Phe-D-Leu-Phe is one order of magnitude higher than that of *t*-Boc-Phe-Leu-Phe-Leu-Phe [78]. Results derived from further and more detailed studies on the rabbit neutrophils indicate that the *t*-Boc-Phe-Leu-Phe-Leu-Phe-OMe peptide derivative can show a definite agonist activity, while the homologous *t*-Boc-Phe-D-Leu-Phe-D-Leu-Phe-OMe is a full antagonist [79]. Moreover, it has been demonstrated that the C-terminal methyl esterification reduces the ability of the penta-peptide derivative to inhibit the release of glucosaminidase [80].

A series of amino-terminal carbamate analogs of fMLP, in particular branched carbamates such as *i*-Boc, Fig. (**4B**), *t-Boc* and bezyloxycarbonyl, have been demonstrated to be FPR antagonists on human neutrophils. The peptide antagonists were found to be more potent inhibitors of superoxide anion release than of cell adhesion [81].

Aminoterminal urea-substituted modified MLP peptides have been shown to be FPR antagonists on human neutrophils. This is true for *N*-ureido substituents such as methyl-, ethyl-, *n*-propyl-, *iso*-butyl-, *tert*-butyl- and benzyl-ureido [82]. Moreover, it has been reported that *N*-ureido-Phe-D-Leu-Phe-D-Leu-Phe peptide derivatives, Fig. (**4C**) and (**4D**), show an enhanced FPR affinity and antagonist power on human neutrophils, with respect to the tripeptide MLP homologs [82].

It has been investigated whether *t*-Boc or *N*-ureido–aliphatic substituents in the Met-Ile-Phe-Leu chain (HCO-Met-Ile-Phe-Leu is a potent FPR full agonist) can induce an antagonist behavior on human neutrophils [72]. In this context, the presence of *N*-isopropylureido substituent

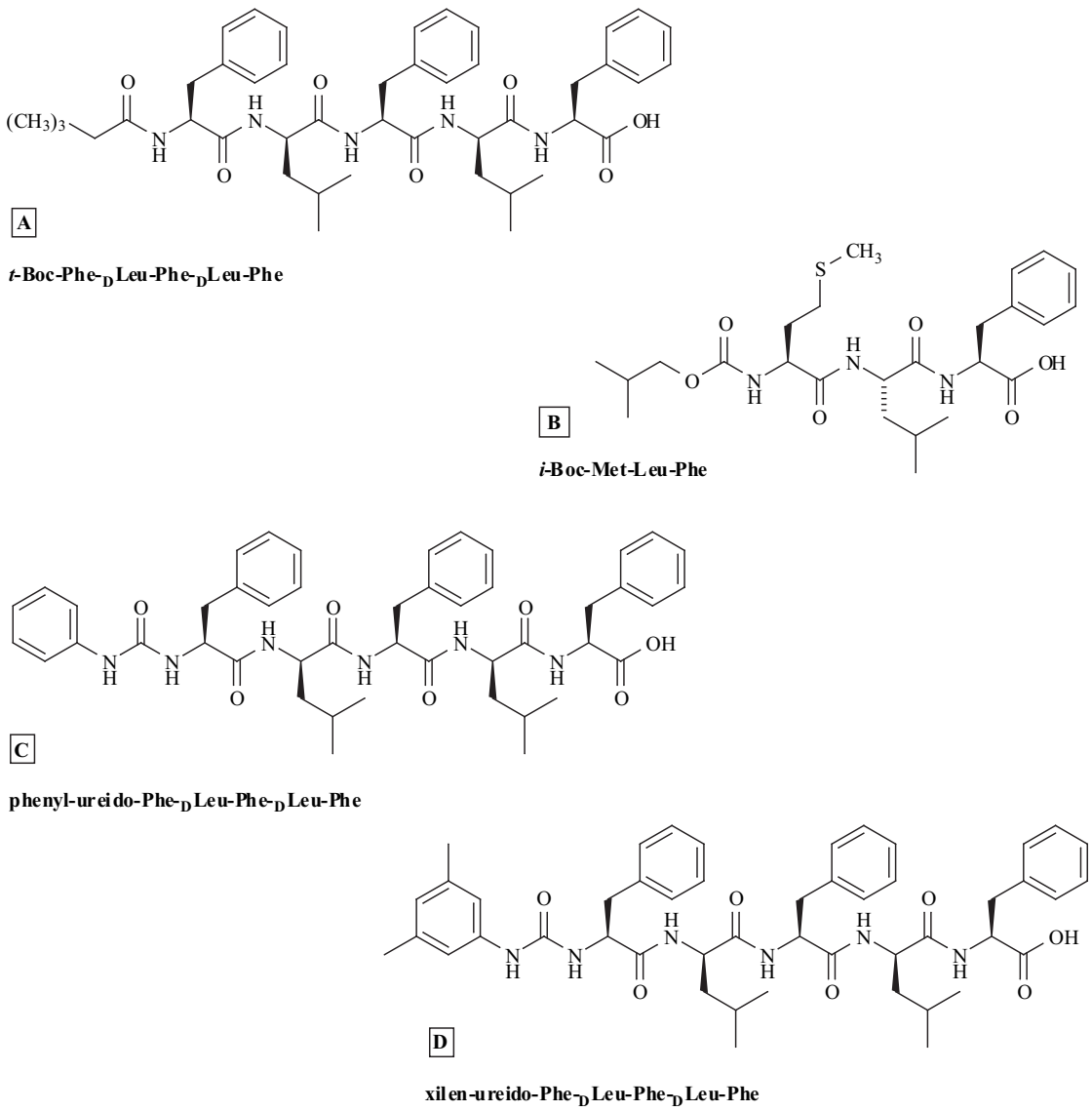

Fig. (4). Chemical formulas of tri and pentapeptide derivatives which are antagonists of formyl peptide receptor.

within the tetrapeptide chain has been found to impart weak partial agonist properties, whereas the *t*-Boc-Met-Ile-Phe-Leu derivative does not appear able to interact with FPR [72].

A series of free acid and methyl-ester Phe-D-Leu-Phe-D-Leu-Phe analogs, including either *N*-*t*-Boc or four different *N*-ureido substituents, were analysed in detail on human neutrophils [13,83]. It has been demonstrated that these peptide derivatives are able (*i*) to interact with FPR, (*ii*) to reduce fMLP effectiveness in enhancing the cytosolic level of Ca^{2+} and (*iii*) to antagonize the multiple neutrophil functions evoked by fMLP, i.e. chemotaxis, O_2^- production and secretagogue activity. Also in this case, these peptide antagonists were found to be more potent inhibitors of superoxide anion release than of cell adhesion. Moreover, it has been shown that C-terminal methyl-esterification is detrimental to the FPR affinity and antagonist activity of these pentapeptide derivatives [13,83].

On the basis of these observations, further investigations were performed with the aim to evaluate the effect of an hydrophilic environment in the C-terminal position of N-ureido pentapeptide derivatives. It has been observed that only pentapeptide derivatives with the free acid C-terminal or "olo" groups show an appreciable affinity toward FPR, whereas the analogs with the C-terminal methylester or amido groups scarcely bind to it. According to these results it has been hypothesised that, at the C-terminal level, a hydroxyl function is essential to impart affinity to FPR, whereas the carbonyl group does not appear to be directly involved in this interaction. It has also been observed that the substitution of Phe with more hydrophilic aminoacid at the C-terminal pentapeptide chain produces weak effects on the affinity and antagonist power toward FPR [84].

As far as the N-terminal position of Phe-D-Leu-Phe-D-Leu-Phe analogs is concerned, the N-terminal thiazolyl-ureido has been found to greatly contribute to the affinity and antagonist power toward FPR [84].

All the pentapeptides investigated have been shown to inhibit O_2^- production and lysozyme release more efficaciously than neutrophil chemotaxis. According to these

results it has been hypothesised that this different antagonist activity executed by the pentapeptide derivatives towards the various neutrophils responses could be caused by their interaction with different states and/or with different subtypes of FPR [84].

Annexin I peptides have recently been reported as novel, endogenous FPR ligands able to induce anti-inflammatory effects [85]. Moreover, the immunomodulatory activity of cyclosporins, proposed as cancer chemotherapeutic drugs, has been related to the inhibition of FPR functions [86].

CONCLUDING REMARKS

Several lines of evidence suggest that the specificity of neutrophil functions in response to stimulating agents, such as formylpeptides, could be determined by a series of events just beginning at the level of the receptor-formylpeptide interaction. In particular low doses of peptides, sufficient to induce the chemotactic response, are supposed to interact with a high-affinity receptor isoform that activates transduction pathways without involving phospholipase C, Ca^{2+} or cAMP enhancements; higher formylpeptide concentrations - typical of infection sites - could allow the binding of peptides with a different receptor isoform, able to activate the transduction pathways leading to phospholipase C activation, or Ca^{2+} as well as cAMP enhancement, responsible for O_2^- production and lysozyme release.

It is well known that, in several pathological conditions, the inappropriate activation of neutrophils can cause tissue damage. Many drugs exist, able to inhibit neutrophil responses, acting by impairing some of the different steps of the transduction pathways which are activated by formylpeptides. A major limitation in their use as therapeutic agents for the treatment of inflammation-related diseases is that these drugs are not selective, and therefore other cellular responses could also be inhibited at the same time. The development of new FPR antagonists, able to transiently inhibit neutrophil specific functions, is therefore highly desirable.

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