Myeloperoxidase-Mediated Modulation of Chemotactic Peptide Binding to Human Neutrophils

By Thomas A. Lane and Geraldine E. Lamkin

Methionine-containing chemotactic peptides, such as formyl-methionyl-leucyl-phenylalanine (FMLP), are inactivated via a neutrophil-derived, myeloperoxidase-mediated oxidation of the methionine residue.[7] We report that extracellular inactivation of FMLP by myeloperoxidase modulates the apparent binding of methionine-containing chemotactic peptides to their surface receptors. Inhibitors of myeloperoxidase enhanced FMLP binding. At subsaturating concentrations of ^{3}H-FMLP (20 n*M*), 1 m*M* cyanide (KCN) increased the binding of ^{3}H-FMLP to human neutrophils (PMN) by 51% ± 12%. Similar increases occurred with 0.1 m*M* azide and 10 m*M* aminotriazole (ATZ). KCN had little effect on maximal ^{3}H-FMLP binding to PMN at saturation (control—17,040 ± 910 receptors/PMN; KCN—16,820 ± 1,940 receptors/PMN), but decreased the concentration of ^{3}H-FMLP required to half-saturate the PMN receptors (control—39 ± 3 n*M*; KCN—17 ± 1 n*M*). ATZ gave similar results. The binding to PMN of the non-methionine-containing chemotactic peptide ^{125}I-formyl-norleucyl-leucyl-phenylalanyl-norleucyl-tyrosyl-lysine (^{125}I-FNLPNTL) was unaltered by KCN. Also, the binding of ^{3}H-FMLP to myeloperoxidase-deficient PMN was unaltered by KCN. Both KCN and ATZ decreased the oxidation of FMLP by PMN. Finally, ATZ (but not KCN) enhanced the chemotactic migration of PMN in response to submaximal concentrations of FMLP. These studies show that intact PMN inactivate methionine-containing chemotactic peptides by a pathway that is sensitive to myeloperoxidase inhibitors and is absent in myeloperoxidase-deficient PMN. This action results in an apparent decrease in the affinity of the chemotactic peptide receptor for methionine-containing chemotactic peptides, which may modulate chemotatic events in inflammatory loci.

NEUTROPHIL CHEMOTAXIS is mediated by the interaction of certain chemotactic agents with specific membrane receptors. Receptors have been identified for C5a,[1] bacterial chemotactic factor,[2,3] and others.[4] There are several potential control mechanisms, both intra- and extracellular, through which the response of the neutrophil to a defined chemotactic stimulus may be modified. Chemotactic factor inactivator, described by Ward and coworkers,[5] inactivates fluid-phase C5a. Likewise, Clark and coworkers[6,7] and Tsan and Denison[8] have demonstrated that the bacteria-derived F-met peptides may be inactivated by neutrophil myeloperoxidase. Myeloperoxidase inactivates the F-met peptides by oxidation of the sulfur atom in the methionine group to its sulfoxide derivative, which binds poorly to the F-met receptor.[6,8] The binding of F-met peptides to neutrophil membrane receptors may also be modulated by degranulation,[9] neutrophil storage,[10] phagocytosis,[11] or exposure of neutrophils to the chemotactic peptide itself,[12,13] or to certain chemicals.[14-17] In this article we demonstrate that neutrophils modulate the binding of methionine-containing chemotactic peptides to their surface receptors by a mechanism that is dependent on myeloperoxidase. This results in an apparent decrease in ^{3}H-formyl-methionyl-leucyl-phenylalanine (^{3}H-FMLP) receptor affinity in vitro. A similar mechanism may take part in the modulation of neutrophil chemotaxis at sites of inflammation.

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MATERIALS AND METHODS

Cell Preparation

Granulocytes were prepared as previously described.[11] PMN were suspended in basal Eagle's medium with Earl's salts (BME, Microbiological Associates, Bethesda, MD) for chemotaxis studies and in phosphate-buffered saline, pH 7.3, with 10 m*M* glucose, 5 m*M* KCl, 1.5 m*M* $CaCl_2$, and 0.5 m*M* MgC12 at 10^7 polymorphonuclear cells (PMN)/ml for receptor-binding assays. Blood from a myeloperoxidase-deficient patient[18] was generously supplied through the cooperation of Michael F. Parry, M.D., Stamford Hospital, Stamford, CT.

Myeloperoxidase (MPO) was assayed as described by Babior and Cohen.[19] All normal cells in this study were myeloperoxidase-sufficient.

We prepared ^{125}I-formyl-norleucyl-leucyl-phenylalanyl-norleucyl-tryosyl-lysine (^{125}I-FNLPNTL) (Penninsula Labs, San Carlos, CA) by the lactoperoxidase method,[20] according to the manufacturer's instructions (BioRad Technical Bulletin 1071, Richmond, CA) and isolated the pure radiolabeled peptide as described by Niedel and coworkers.[13] The purified peptide exhibited one peak of activity on thin-layer chromatography as described by Niedel.[13] ^{3}H-FMLP was obtained from New England Nuclear, Boston, MA.

The binding of ^{3}H-FMLP to PMN was examined as previously described.[11,14] The binding of ^{125}I-FNLPNTL was examined as described by Niedel[13] with minor modifications. Briefly, to 200 μl of PMN (2.5×10^6 PMN/ml) was added 36 μl of ^{125}I-FNLPNTL (final concentration 2 n*M*, specific activity 350 μCi/nmole) with or without 100 n*M* unlabeled FNLPNTL. At the end of incubation, 1 ml of iced buffer was added, and duplicate 0.5-ml samples were filtered and washed.[13] The filters were counted on a Beckman Gamma 300.

Table 1. Effect of MPO Inhibitors on ^{3}H-FMLP Binding to Neutrophils

	Percent FMLP Bound ± SE	*n*
Control	100*	9
KCN, 1m*M*	151 ± 12†	9
Azide, 0.1m*M*	149 ± 17†	4
Aminotriazole, 10m*M*	199 ± 27†	5

Neutrophils (10^7 PMN/ml) were incubated with saline (control) or the indicated agent for 5 min at 37°C, followed by ^{3}H-FMLP binding analysis (see Materials and Methods)

n: Refers to number of experiments.

*0.142 ± 0.065 pmole/10^7 PMN; 20 n*M* ^{3}H-FMLP.

†$p < 0.01$ compared to the control.

In FMLP oxidation experiments, PMN (10^7 PMN/ml) were incubated with or without cyanide (KCN) for 15 min at 37°C, followed by incubation for 30 min at 37°C with 20 n*M* ^{3}H-FMLP. The cells were placed on ice for 5 min and spun at 3,000 *g* for 5 min. The supernatants were then examined for ^{3}H-FMLP oxidation.

FMLP oxidation was measured using a modification of the thin-layer chromatography technique described by Tsan and coworkers.[8] Using precoated silica plates (Silica Gel-60, Fisher Scientific) we obtained R_f values of 0.37 and 0.59 for FMLP and the sulfoxide derivative of FMLP (FM_sLP), respectively, using t-butanol:acetic acid:water of 6:1:1, and R_f values of 0.72 and 0.62 for FMLP and FM_sLP, respectively, using a solvent of t-butanol: methylethylketone:water:ammonia of 4:3:2:1. Using this system, between 4.4% and 10% of added FMLP was spontaneously oxidized of FMLP after incubation (see above) in the absence of neutrophils. For each experiment, the value for that day's spontaneous oxidation was considered as background and was subtracted from each experimental value.

Chemotaxis was examined by a modified Boyden chamber technique as described by Cates,[21] using a 48-well microchemotaxis chamber (Neuroprobe, Bethesda, MD) as described by Falk and coworkers.[22] Values for chemotaxis were expressed as the number cells migrating to the bottom of the filter per high power field. Ten high power fields were examined for each well, and each experimental point was run in triplicate. There was no crosscontamination between wells containing buffer and those containing chemotactic agent, FMLP.

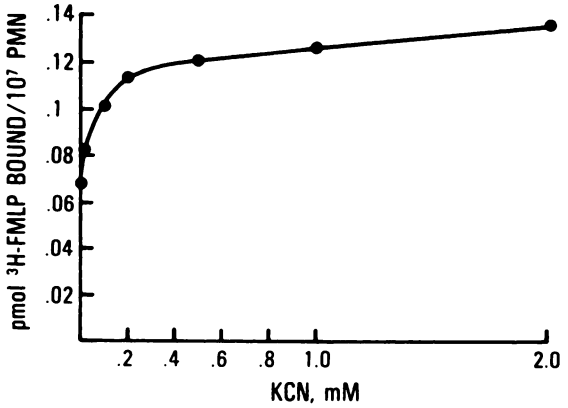

Fig. 1. Effect of KCN on binding of ^{3}H-FMLP to neutrophils. Neutrophils were incubated with KCN for 15 min at 37°C, followed by ^{3}H-FMLP binding analysis as described in Materials and Methods. One of three experiments.

The mean ± standard error of the data are presented, and statistical evaluations were performed using a two-tailed t test.

RESULTS

Incubation of neutrophils for 15 min at 37°C with cyanide (KCN), azide, or aminotriazole (ATZ) enhanced the binding of subsaturating (20 n*M*) concentrations of ^{3}H-FMLP to neutrophils (Table 1). The increase in binding due to KCN was dose-dependent (Fig. 1). The concentration of KCN producing 50% of the maximal effect (EC_{50}) was 0.1 m*M*. Similar dose–response curves were obtained for azide (EC_{50}–0.04 m*M*) and aminotriazole (EC_{50}–0.2 m*M*).

Scatchard analysis of ^{3}H-FMLP binding to PMN incubated with or without KCN showed that KCN had little effect on total receptor numbers at saturation, but substantially enhanced the apparent receptor affinity at subsaturating FMLP concentrations (Fig.

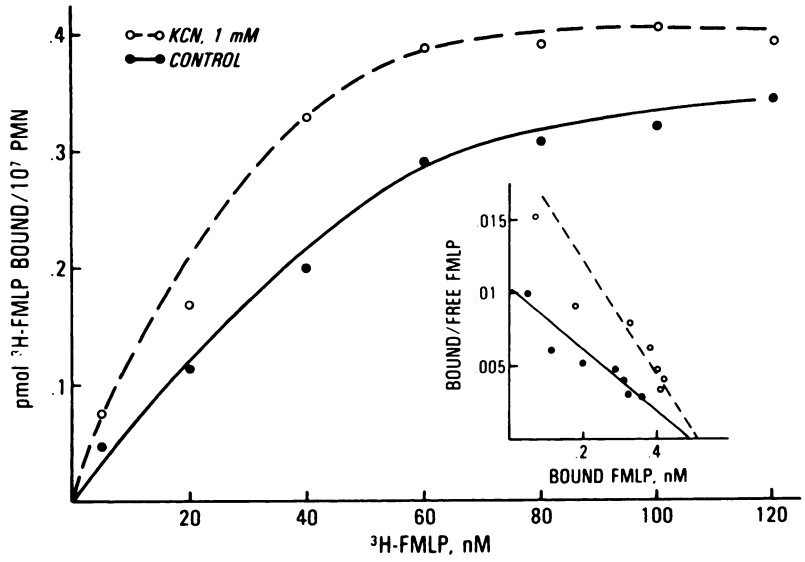

Fig. 2. Effect of KCN on (apparent) ^{3}H-FMLP receptor affinity. Neutrophils were incubated with or without 1 m*M* KCN for 15 min at 37°C, followed by ^{3}H-FMLP binding analysis at the concentrations indicated. One of four experiments.

Table 2. Effect of KCN on ^{3}H-FMLP Binding to Neutrophils

	Control		1m*M* KCN	
	EC_{50}, (n*M*)	R/C	EC_{50}, (n*M*)	R/C
Exp. 1	48	14,400	18	12,200
Exp. 2	33	18,480	18	20,400
Exp. 3	35	17,280	15	15,360
Exp. 4	40	18,000	16	19,500
Mean ± SE	39 ± 3	17,040 ± 910	17 ± 1*	16,820 ± 1,940

Neutrophils (10^7 PMN/ml) were incubated with saline (control) or 1m*M* KCN for 5 min at 37°C, followed by ^{3}H-FMLP binding analysis (5–100 n*M* ^{3}H-FMLP, see Materials and Methods). EC_{50} (n*M*) refers to the concentration of ^{3}H-FMLP required to half-saturate FMLP receptors. R/C refers to total FMLP receptors/PMN at saturation.

*$p < 0.01$ compared to the control.

2). In four such experiments (Table 2), 1 m*M* KCN consistently enhanced apparent receptor affinity, reducing the concentration of FMLP required to half-saturate receptors (ED_{50}) from 39 n*M* (control) to 17 n*M* FMLP ($p < 0.01$). Similar enhancement of apparent receptor affinity without change in total receptor numbers was observed after incubation of PMN with 10 m*M* aminotriazole.

The effect of KCN was most pronounced at 37°C and was almost negligible at 4°C (Fig. 3). Also, incubation of neutrophils with 1 m*M* KCN altered neither the cleavage of ^{3}H-FMLP (control—18% ± 4% cleaved, KCN—15% ± 7% cleaved; 3 experiments) nor the ability of the cells to down-regulate chemotactic receptors[12] in the presence of 10 $^{-4}$*M* FMLP (control—92% down-regulated, KCN—96% down-regulated; 2 experiments). In addition, in 6 experiments, the binding of ^{3}H-FMLP by PMN under anaerobic conditions was 34% ± 8% ($p < 0.01$) greater than PMN under aerobic conditions (room air). Anaerobiosis was confirmed by greater than 90% inhibition of cytochrome-c reduction by phagocytosing neutrophils, as previously described.[11]

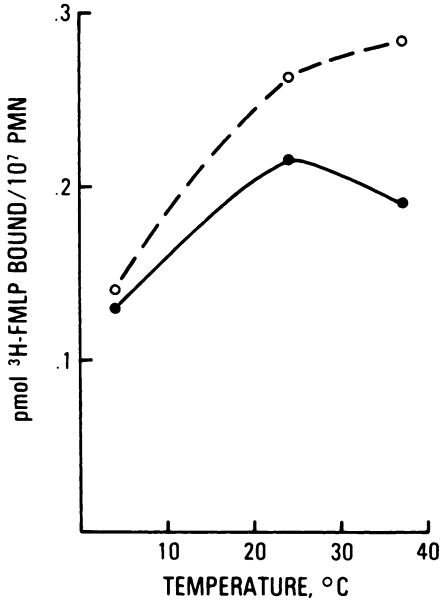

Fig. 3. Temperature dependence of the effect of KCN on ^{3}H-FMLP binding to neutrophils. Neutrophils were incubated with or without 1 m*M* KCN for 5 min at 37°C, followed by binding analysis of ^{3}H-FMLP at 4°C, at room temperature, or at 37°C. One of three experiments. Closed dots—control; open dots—KCN.

Table 3. Effect of Myeloperoxidase Inhibitors on ^{3}H-FMLP and ^{125}I-FNLPNTL Binding to Neutrophils

	Percent ^{3}H-FMLP Bound ± SE	*n*	Percent ^{125}I-FNLPNTL Bound ± SE	*n*
Control	100*	6	100†	6
KCN, 1 m*M*	145 ± 20‡	6	92 ± 16	6
Azide, 0.1 m*M*	150 ± 31	3	96 ± 31	3

Neutrophils (10^7 PMN/ml) were incubated with saline (control) or the indicated agent for 5 min at 37°C, followed by binding analysis (see Materials and Methods).

n: Refers to number of experiments.

*0.164 ± 0.022 pmole/10^7 PMN; 20 n*M* ^{3}H-FMLP.

†0.629 ± 0.083 pmole/10^7 PMN; 2 n*M* ^{125}I-FNLPNTL.

‡$p < 0.01$.

The possibility that the effect of KCN, azide, and aminotriazole might be mediated by inhibition of myeloperoxidase[23] was examined by incubating PMN with or without KCN or azide, followed by binding analysis of ^{3}H-FMLP or a non-methionine-containing peptide, ^{125}I-FNLPNTL. As shown on Table 3, KCN and azide did not enhance the binding of ^{125}I-FNLPNTL to PMN. Also, KCN did not enhance ^{3}H-FMLP binding to MPO-deficient PMN (Table 4).

Myeloperoxidase-sufficient PMN oxidized FMLP to a greater extent than MPO-deficient PMN, and FMLP oxidation by control PMN was partially inhibitible by 1 m*M* KCN (Table 5). A baseline level of 10%–17% FMLP oxidation using either MPO-deficient PMN or control PMN in the presence of KCN has been previously noted.[8]

Table 4. Effect of KCN on ^{3}H-FMLP Binding to Myeloperoxidase-Deficient Neutrophils

	Percent FMLP Bound ± SE	*n*
Normal PMN		
Control	100*	4
KCN, 1m*M*	162 ± 29†	4
MPO-deficient PMN		
Control	100‡	4
KCN, 1m*M*	88 ± 9	4

Neutrophils (10^7 PMN/ml) were incubated for 5 min at 37°C with saline (control) or 1 m*M* KCN, followed by ^{3}H-FMLP binding analysis.

n: Refers to number of experiments.

*0.108 ± 0.038 pmole/10^7 PMN (20 n*M* ^{3}H-FMLP).

†$p < 0.05$.

‡0.322 ± 0.163 pmole/10^7 PMN (20 n*M* ^{3}H-FMLP).

Table 5. Effect of KCN on ^{3}H-FMLP Oxidation by Neutrophils

	Percent FM_sLP			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Control PMN	39	12	20	30
PMN + KCN, 1 m*M*	12	5	14	—
MPO-deficient PMN	17	—	—	15

Neutrophils (10^7 PMN) were incubated with saline (control) or 1 m*M* KCN for 5 min at 37°C followed by addition of 20 n*M* ^{3}H-FMLP and incubation for 30 min at 37°C, and determination of ^{3}H-FMLP oxidation.

Percent FM_sLP: fraction of oxidized ^{3}H-FMLP recovered from plate, as percent of total recovered ^{3}H-FMLP (see Materials and Methods).

Finally, aminotriazole decreased the concentration of FMLP required to simulate half-maximal chemotaxis (EC_{50}) to a mean of 42% of the control value ($p < 0.01$), concomitantly increasing the chemotactic index by an average of 26% ($0.05 < p < 0.1$) (Table 6). The effect of KCN on chemotaxis was variable. The chemotactic index was unchanged by KCN, but KCN inhibited both random migration and chemotaxis by 20%–30%.

DISCUSSION

Neutrophil migration toward the F-met peptides is mediated by interaction of the peptides with specific membrane receptors.[24-26] Several lines of evidence suggest that the extent of chemotaxis is influenced by physiologic modifications in specific receptor numbers and function. Both chemotaxis and total receptor numbers are decreased by exposure of the cells to the chemotactic peptide[12,27] (down-regulation), phagocytosis,[11] and exposure to certain chemicals,[14-16] while chemotaxis and receptor affinity are decreased by partial neutrophil degranulation[9] and granulocyte storage.[10] Chemotaxis may also be regulated by control of the chemotactic stimulus. Clark et al.[6,7] and Tsan et al.[8] have demonstrated that myeloperoxidase may inactivate the methionine-containing F-met peptides.

Table 6. Effect of Aminotriazole and KCN on Chemotaxis of Neutrophils Toward FMLP

	EC_{50} (%)	CI (%)	*n*
Control	100*	100†	4
ATZ, 10m*M*	42 ± 7‡	126 ± 21	4
Control	100§	100‖	4
KCN, 1m*M*	126 ± 60	106 ± 4	4

*15 ± 14n*M* FMLP.

†2.8 ± 0.8.

‡$p < 0.01$ compared to control.

§25 ± 25n*M* FMLP.

‖2.3 ± 0.6.

CI, Chemotaxis index, calculated as the following ratio: peak migration toward FMLP/random migration. Expressed as percent of control value for each experiment.

EC_{50}, Concentration of FMLP required to stimulate half-maximal migration, expressed as percent of control value for each experiment.

We report that this mechanism may directly modulate FMLP binding to its membrane receptor.

We report that FMLP binding to neutrophils was enhanced by cyanide, that this requires active cell metabolism, and that KCN enhanced apparent receptor affinity but not total receptor numbers. The data suggest that, in normal PMN, myeloperoxidase inactivated FMLP (by oxidation to the sulfoxide), thus lowering local concentrations of the ligand. Since oxidized FMLP (FM_sLP) binds poorly to the F-met peptide receptor,[6,8] the result was a reduction of *apparent* receptor affinity. At saturating FMLP concentrations, sufficient FMLP is presumably available to overcome the effect of MPO. KCN inhibits MPO,[24] thus decreasing FMLP oxidation and enhancing apparent ^{3}H-FMLP binding to PMN.

The evidence suggests that the effect of KCN in these studies was mediated, at least in part, by inhibition of myeloperoxidase. First, other myeloperoxidase inhibitors (such as sodium azide and aminotriazole) had an effect similar to that of KCN. These agents inhibit catalase as well as other heme-containing enzymes;[23] however, we have reported that exogenous catalase does not decrease ^{3}H-FMLP binding to PMN and may, in fact, enhance binding.[11] This may be due to rapid "detoxification" of H_2O_2, thus making it unavailable for myeloperoxidase. Second, a bacterial factor-related chemotactic peptide that does not contain methionine (FNLPNTL) did not exhibit enhanced binding in the presence of MPO inhibitors. The effect of MPO inhibitors on FNLPNTL binding might have been masked by the ability of tyrosine to scavenge oxygen radicals; however, our previous experience with cysteine,[14] a more potent scavenger,[28] suggests that this mechanism would have been insufficient to account for the magnitude of differences in ^{3}H-FMLP binding that we observed using MPO inhibitors. Third, myeloperoxidase-deficient PMN exhibited no enhancement of ^{3}H-FMLP binding in the presence of KCN. Fourth, aminotriazole exerted an effect on chemotaxis toward FMLP that was consistent with its effect on ^{3}H-FMLP binding. In two additional experiments (data not shown), a difference in the EC_{50} for chemotaxis toward FMLP between normal and MPO-deficient PMN could not be ascertained. Finally, the degree to which FMLP was oxidized to FM_sLP (Table 5) corresponded well with the extent to which FMLP binding was altered by MPO inhibitors. The effective FMLP concentration was decreased by 10%–35% in the absence of KCN (i.e., from 20 n*M* down to 13–18 n*M*), and the ^{3}H-FMLP binding data indicate that oxidation of from 25%–40% of added FMLP (i.e., from 20 n*M* down to 12–15 n*M*) would have been sufficient to achieve the observed differences in ^{3}H-FMLP binding with and without KCN.

The effect of MPO inhibition on ^{3}H-FMLP binding might also be partially due to inhibition of oxygen radical-induced oxidation of the membrane receptor itself, which we and others[14,29,30] have reported to be dependent on free thiol groups. The differences in magnitude between the effects of KCN and cysteine[14] on ^{3}H-FMLP binding, the failure of FNLPNTL binding to be enhanced by KCN, and the fact that KCN did not increase total receptor numbers suggest that MPO-induced auto-oxidation of FMLP receptors was not, alone, responsible for the effects of KCN that we observed.

The significance of MPO-mediated modulation of apparent FMLP receptor affinity is not clear. This effect would be expected to decrease cell migration at low concentrations of bacterial peptides; however, at near maximally stimulating concentrations of FMLP, MPO might protect the cell from deactivation induced by excessive concentrations of ligand. The effect of MPO might also be blunted under hypoxic conditions, thus enhancing the relative migration of PMN toward low concentrations of bacterial factor into hypoxic loci. Finally, the absence of MPO-mediated inhibition of FMLP binding might partially offset the potential deleterious effects of MPO deficiency by enhancing relative migration toward low concentrations of bacterial peptides.

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