

# CHEMOTACTIC PEPTIDE RECEPTOR MODULATION IN POLYMORPHONUCLEAR LEUKOCYTES

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## ABSTRACT

The binding of the chemotactic peptide *N*-formylnorleucylleucylphenylalanine (FNLLP) to its receptor on rabbit polymorphonuclear leukocytes (PMNs) modulates the number of available peptide receptors. Incubation with FNLLP decreases subsequent binding capacity, a phenomenon that has been termed receptor down regulation. Down regulation of the chemotactic peptide receptor is concentration dependent in both the rate and extent of receptor loss. The dose response parallels that of FNLLP binding to the receptor. The time-course is rapid; even at concentrations of FNLLP as low as  $3 \times 10^{-9}$  M, the new equilibrium concentration of receptors is reached within 15 min. Down regulation is temperature dependent, but does occur even at 4°C.

Concomitant with down regulation, some of the peptide becomes irreversibly cell associated. At 4°C, there is a small accumulation of nondissociable peptide that rapidly reaches a plateau. At higher temperatures, accumulation of nondissociable peptide continues after the receptor number has reached equilibrium, and the amount accumulated can exceed the initial number of receptors by as much as 300%. The dose response of peptide uptake at 37°C reflects that of binding, suggesting that it is receptor mediated. This uptake may occur via a pinocytosis mechanism. Although PMNs have not been considered to be pinocytic, the addition of FNLLP causes a fourfold stimulation of the rate of pinocytosis as measured by the uptake of [<sup>3</sup>H]sucrose.

Polymorphonuclear leukocytes (PMNs) are phagocytic cells that are able to exhibit chemotaxis to a variety of agents, including denatured proteins (34), bacterial products (14), oxidized lipids (29), cell-derived factors (27, 32), serum components, particularly C5a (13, 25, 31), and small formylated peptides (23, 26). The ability of PMNs to orient and move in chemotactic gradients provides a system in which two key biological problems can be studied: the sensory detection of chemical substances, and cell movement. We have used the chemotactic peptide, *N*-formylnorleucylleucylphenylalanine (FNLLP), to study the first step in

the chemotactic response, namely, the interaction of FNLLP with its receptor.

Several studies have demonstrated the presence of specific, saturable receptors for formylated peptides on PMNs (1, 22, 36). These peptides have made it possible to study the behavior of PMNs in specific gradients. Characteristics of the binding can be predicted from the behavior. The orientation of the cells in a chemical gradient is rapid and readily reversible; the rates of binding and dissociation are also rapid. Cells in a gradient have been shown to orient optimally at concentrations close to the dissociation constant,  $K_d$ , for the bind-

ing. In addition, it has been shown that PMNs can orient in this optimal range when the concentration difference across their dimensions is only ~1% (37).

We have recently presented evidence which suggests that the sensitivity of PMNs to concentration changes may be increased by their ability to adapt to the concentration of peptide present (38). PMNs treated with chemotactic factors undergo transient changes in morphology (38), adhesion (7, 20), and ion fluxes (3, 18). After ~3 min the cells adapt to this chemotactic stimulation and revert, at least partially, to their prestimulated state. Cells adapted to a given concentration of chemotactic factor will respond to changes in concentration. It has been known for many years that incubation with chemotactic factors can result in a loss of chemotactic responsiveness. This phenomenon has been termed deactivation (30). In this report, we demonstrate that one of the responses of PMNs to the binding of FNLLP is a modulation of the number of receptors that are subsequently available for binding. This receptor modulation may be one mechanism of adaptation and/or deactivation.

## MATERIALS AND METHODS

### Cells

Rabbit peritoneal exudate cells were collected four h after injection of 0.1 g of shellfish glycogen (Schwartz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) in 250 ml of 0.9% saline. Contaminating erythrocytes were lysed by brief (60 s) treatment with hypotonic (0.18%) saline. The cells were washed twice with 0.9% saline and then resuspended in Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.) buffered with 2.4 mg/ml of HEPES (Sigma Chemical Co.) at a concentration of  $3.3 \times 10^6$  cells/ml. 3 ml of the cell suspension was added to 60 × 15 mm petri dishes (Falcon Labware, Div., Becton, Dickinson & Co., Oxnard, Calif.), and the cells were allowed to settle for 15 min at 23°C. Dishes were checked with an inverted microscope (Olympus Corp. of America, New Hyde Park, N. Y.) to insure the cells had formed an even monolayer.

### Binding Studies

Each dish containing  $10^7$  cells was incubated in 0.5 ml of Hanks' solution with the appropriate concentration of tritiated (12.5 Ci/mM) FNLLP. After incubation, this medium was aspirated and the dish was washed quickly (6 s) and vigorously in two baths of 4°C saline. The reversibility of binding was measured by varying the time of the wash in chilled saline. Cell-associated radioactivity was measured by adding 0.5 ml of 0.1 N NaOH to each dish. The cells were scraped into this fluid with a rubber policeman, and then each dish was washed with 0.5 ml of distilled water. This 1 ml of cell suspension was counted in 15 ml of scintillation fluid Formula 963 (New England Nuclear, Boston, Mass.) with an efficiency of ~40%. In preliminary experiments, Lowry assays were done (16) to confirm that cells were not lost

from any of the dishes during the treatment. In all experiments the dishes were monitored visually for cell loss.

### Down Regulation

Cells were preincubated with unlabeled FNLLP for various lengths of time at 37° or 4°C. At the end of the incubation, the medium was aspirated and the cells were washed for 5 min at 4°C with chilled saline (5 × 2 ml washes). The rebinding capacity was measured by adding 0.5 ml of Hanks' solution with tritiated FNLLP for 15 min at 4°C. At the end of this incubation, the cells were washed quickly as outlined above. For receptor recovery experiments, the cells were incubated for 20 min at 37°C and washed for 5 min. Cells were then incubated in Hanks' solution for various times at 37°C before being assayed for rebinding. Control cells were preincubated without peptide but were subjected to the same washing procedure.

### Pinocytosis

Fluid uptake was measured by the uptake of tritiated sucrose (11.2 Ci/mmol, New England Nuclear) from the medium. Cells were incubated with  $10^{-6}$  M [<sup>3</sup>H]sucrose in the presence or absence of unlabeled FNLLP. At the end of the incubation, the medium was aspirated and the cells were washed for 5 min at 4°C. Cell-associated activity was assayed as described previously.

### Drugs

2-Deoxy-D-glucose (Sigma Chemical Co.) was used in binding studies at a final concentration of 0.5 mM in Hanks' medium containing  $1 \times 10^{-5}$  M glucose. Cells were preincubated without glucose for 90 min at 37°C before deoxyglucose was added. Cycloheximide (Sigma Chemical Co.) was used at a final concentration of 1 μg/ml. Cells were preincubated with cycloheximide for 30 min at 37°C before being tested. This was shown to decrease the incorporation of mixed tritiated amino acids (New England Nuclear) by 92%. Carbobenzoxy-phenylalanyl-methionine (CBZ-Phe-Met) (Vega-Fox Biochemicals Div., Newbery Energy Corp., Tuscon, Ariz.) was used at a final concentration of  $10^{-4}$  M in 0.2% dimethyl sulfoxide (DMSO) (Sigma Chemical Co.). This concentration of DMSO had no effect on binding or cell viability. Preliminary experiments indicated that the  $K_d$  of CBZ-Phe-Met binding to the peptide receptor is approximately  $5 \times 10^{-6}$  M.

## RESULTS

### Analysis of Binding

PMNs are able to respond to peptide stimulation by forming a pseudopod within 30 s. If the peptide is removed, the pseudopods are rapidly withdrawn. Consequently, the binding and dissociation of FNLLP would be expected to be rapid. The initial rates of binding were determined at four different concentrations of peptide. The  $k_{on}$  can be calculated from these initial rates according to the equation  $v_i = k_{on} (R)_0 (P)_0$ , where  $(R)_0$  and  $(P)_0$  are the concentrations of receptor and peptide initially present. The number of receptors per cell is estimated from Scatchard analysis to be  $5 \times 10^4$  (38). The  $k_{on}$  is calculated to be  $2.1 \pm 0.1 \times 10^7$

$M^{-1} \text{ min}^{-1}$  (mean  $\pm$  SD). The off rate is calculated from the half times of reversal after washing or competition with excess unlabeled peptide (Fig. 1). The amount of reversal is similar with these two methods. The  $k_{off}$  is  $\sim 0.6 \text{ min}^{-1}$ . However, not all of the bound peptide is reversible. The irreversible binding will be discussed in a later section.

The rate of approach to equilibrium binding,  $k_{obs}$ , can also be used to derive the on and off rates (24). The half time for the approach to equilibrium is determined for various concentrations of peptide by plotting the fraction of equilibrium binding as a function of time,  $k_{obs} = \ln 2/t_{1/2}$  (Fig. 2). The rate of approach to equilibrium is described by the equation:

$$\frac{d(RP)}{dt} = k_{on}(R)(P) - k_{off}(RP).$$

Because the concentration of free peptide remains essentially constant, the reaction kinetics are pseudo first order. Thus, integration of this equation can be used to determine the  $k_{obs}$  at different concentrations of peptide:

$$k_{obs} = k_{on}(P) + k_{off}.$$

A plot of  $k_{obs}$  as a function of peptide concentration is also presented in Fig. 2. The on rate derived from the slope is  $2 \times 10^7 M^{-1} \text{ min}^{-1}$  which agrees with the value determined from the initial binding rates. The off rate obtained from the y intercept is  $0.4 \text{ min}^{-1}$  which is also in good agreement with that determined from the reversal of binding. The  $K_d$  obtained from this kinetic analysis is  $1.8 \times 10^{-8}$

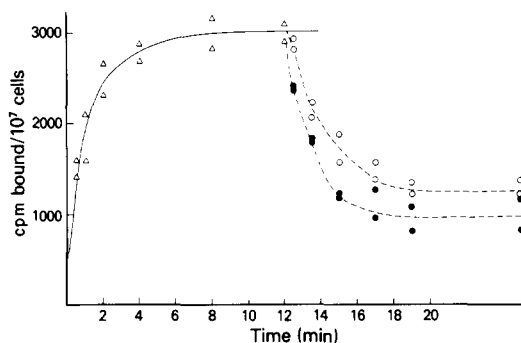


FIGURE 1 FNLLP binding and reversal at  $4^\circ\text{C}$ . ( $\Delta$ ) Time-course of binding of  $2 \times 10^{-8} \text{ M}$  FNLLP plotted as cell-associated cpm after a 6-s wash. ( $\circ$ ) Reversal of binding plotted as a function of time after the addition of  $2 \times 10^{-6} \text{ M}$  unlabeled FNLLP. ( $\bullet$ ) Reversal of binding as a function of time of washing in  $4^\circ\text{C}$  saline.

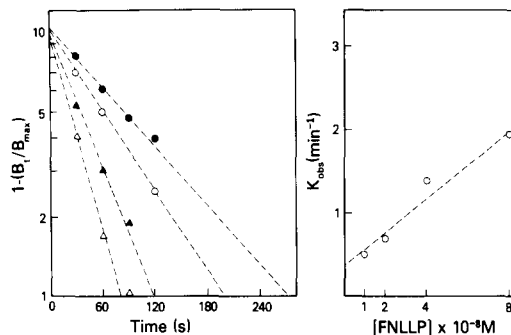


FIGURE 2 Left panel, saturable binding plotted semi-logarithmically as a function of time.  $B_t$  = saturable binding at time  $t$ ;  $B_{max}$  = saturable binding at equilibrium. Saturable binding is the difference in the amount of  $[^3\text{H}]$ FNLLP bound in the absence and presence of 100-fold excess unlabeled FNLLP. ( $\Delta$ )  $8 \times 10^{-8} \text{ M}$   $[^3\text{H}]$ FNLLP, ( $\blacktriangle$ )  $4 \times 10^{-8} \text{ M}$   $[^3\text{H}]$ FNLLP, ( $\circ$ )  $2 \times 10^{-8} \text{ M}$   $[^3\text{H}]$ FNLLP, and ( $\bullet$ )  $1 \times 10^{-8} \text{ M}$   $[^3\text{H}]$ FNLLP. Right panel,  $k_{obs}$  calculated from the half time to equilibrium binding plotted as a function of peptide concentration. The rate constants determined from the slope and intercept are  $k_{on} = 2 \times 10^7 M^{-1} \text{ min}^{-1}$  and  $k_{off} = 0.36 \text{ min}^{-1}$ , respectively.

$M$  ( $K_d = k_{off}/k_{on}$ ). This confirms the  $K_d$  obtained previously from Scatchard analysis (38).

The nonformylated analog of FNLLP, HNLLP, was shown by Aswanikumar et al. (1) not to be chemotactic or to compete for binding to the chemotactic peptide receptor. In these binding studies, HNLLP added in concentrations of  $5 \times 10^{-8}$  to  $5 \times 10^{-4} \text{ M}$  also did not compete for FNLLP binding.

#### Evidence for Receptor Modulation

We have reported that PMNs are able to adapt to chemotactic stimulation (38). Experiments were designed to determine whether this adaptation involves a change in receptor number or affinity. Cells are incubated at  $37^\circ\text{C}$  with unlabeled concentration, washed thoroughly, and then incubated with tritiated peptide at  $4^\circ\text{C}$ . The binding capacity of cells preincubated with peptide is significantly less than that of cells preincubated without peptide. To rule out the possibility that the reduced binding was caused by competition by released unlabeled peptide, cells were incubated with tritiated peptide and the time-course of loss of label was examined. Essentially all of the peptide that can be removed by washing is removed during the 5-min wash. There is no further release of labeled peptide into the medium during a 15-

min incubation at 4°C with unlabeled peptide.

The rate and extent of receptor loss are dependent on the concentration of FNLLP present during the preincubation. Down regulation is rapid. Most of the receptor loss occurs during the first 5 min, even before equilibrium binding is reached (Fig. 3). A plateau level of receptors is also rapidly achieved (within 15 min even at  $3 \times 10^{-9}$  M). The rapidity of the down regulation caused us to miss the phenomenon in our original experiments (38).

Down regulation is mediated by peptide binding to the specific, saturable receptor. The dose response correlates with the binding of FNLLP to its receptor, with the half-maximal loss of receptors occurring at a concentration near the  $K_d$  of binding (Fig. 4). The analogue HNLLP, which does not bind to the receptor, does not cause any receptor loss. The competitive inhibitor CBZ-Phe-Met also does not result in any down regulation. CBZ-Phe-Met has been shown to compete for the peptide-binding site without inducing any of the chemotactic responses (21). CBZ-Phe-Met does inhibit FNLLP-induced down regulation.

The affinity of the receptors which remain after down regulation was determined by Scatchard

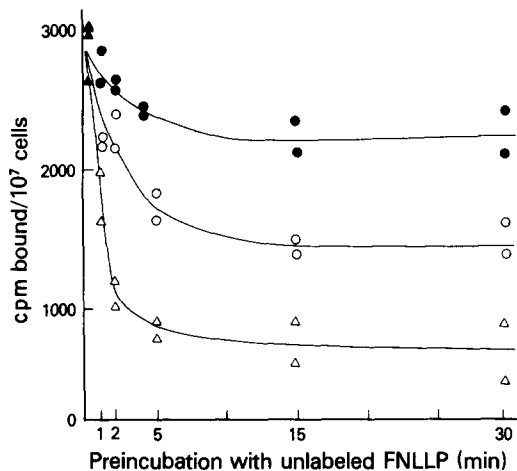


FIGURE 3 Rate of receptor loss at different peptide concentrations. Cells were preincubated at 37°C with (●)  $3 \times 10^{-9}$  M, (○)  $3 \times 10^{-8}$  M, and (Δ)  $3 \times 10^{-7}$  M unlabeled FNLLP for various times. At the end of this incubation, the cells are washed for 5 min at 4°C and then incubated at 4°C for 15 min with  $2 \times 10^{-8}$  M [ $^3$ H]FNLLP. The binding capacity is plotted as cpm bound/ $10^7$  cells as a function of preincubation time with unlabeled FNLLP. (▲) Cells preincubated without peptide. Nonsaturable binding at this concentration of [ $^3$ H]FNLLP is  $\sim 500$  cpm.

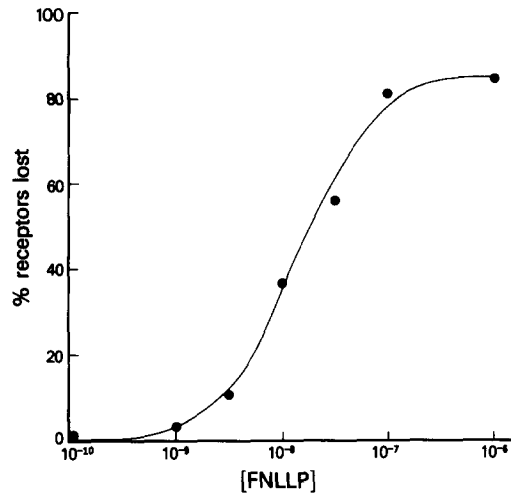


FIGURE 4 Down regulation dose response. The percent saturable receptor loss as a result of incubation with unlabeled peptide for 30 min at 37°C is plotted semilogarithmically as a function of peptide concentration. Receptor loss is calculated as a percent of the saturable binding of  $2 \times 10^{-8}$  M [ $^3$ H]FNLLP by cells preincubated without peptide.

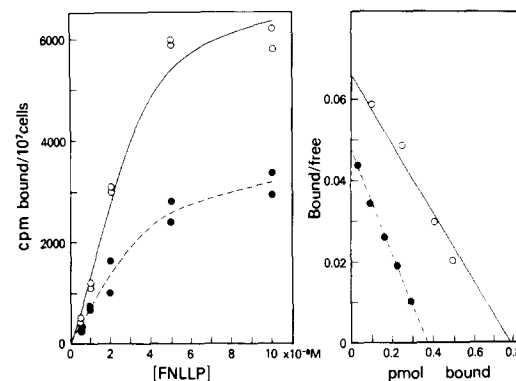


FIGURE 5 Dose response of FNLLP binding before and after down regulation. Left panel, cells were incubated in the presence (●) or absence (○) of  $10^{-7}$  M unlabeled FNLLP at 37°C for 30 min and washed for 5 min at 4°C. Binding capacity was measured after incubation with various concentrations of [ $^3$ H]FNLLP for 15 min at 4°C. Right panel, data plotted according to the method of Scatchard.

analysis. The  $K_d$  of control cells without preincubation in peptide is  $2.3 \times 10^{-8}$  M. The  $K_d$  of cells preincubated in  $10^{-7}$  M FNLLP which reduced the number of binding sites by 54% is  $1.9 \times 10^{-8}$  (Fig. 5). Both are within the range of error of our estimate for the  $K_d$  which is  $2.2 \pm 0.3 \times 10^{-8}$  M (mean  $\pm$  SD). This supports the contention that

the decrease in binding capacity is not because of competition by unlabeled FNLLP. Competition would alter the affinity constant rather than the number of binding sites (15).

Down regulation also occurs at 4°C, although to a lesser extent. Incubation with  $10^{-7}$  M FNLLP results in a 68% loss in receptors at 37°C compared to 21% at 4°C (Fig. 6). The plateau level of receptor concentration is still reached within 15 min. Examination of the binding of tritiated FNLLP indicates that there is an increase in irreversibly cell-associated peptide with this same time-course. At this temperature, the amount of irreversibly cell-associated peptide is approximately equal to the number of receptors lost. The number of irreversibly bound peptide molecules at  $10^{-7}$  M FNLLP is  $1.8 \pm 0.2 \times 10^4$ . The number of receptors that are lost after down regulation at this concentration and temperature is  $1.9 \times 10^4$ .

#### Recovery from Down Regulation

The recovery from down regulation also occurs rapidly. After down regulation, cells were washed at 4°C and then incubated at 37°C without peptide for various periods of time and then tested for the ability to bind tritiated peptide. Cells regain 80–100% of initial binding capacity within 20 min (Fig. 7). Both down regulation and recovery are independent of protein synthesis. The addition of cycloheximide (1 µg/ml) which inhibits the incor-

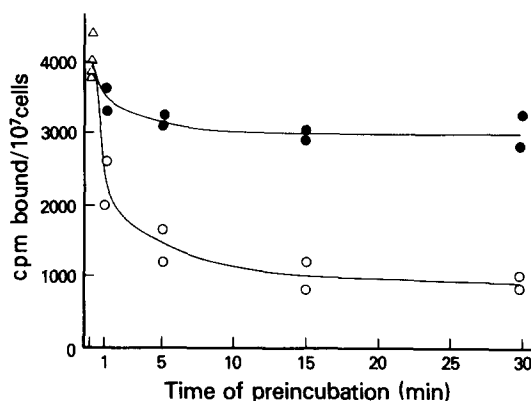


FIGURE 6 Temperature dependence of down regulation. Cells were incubated at 37°C (○) or 4°C (●) for various lengths of time with  $10^{-7}$  M unlabeled FNLLP. After a 5-min wash at 4°C,  $2 \times 10^{-8}$  M [ $^3$ H]FNLLP was added for 15 min at 4°C. Binding capacity is plotted as a function of the length of preincubation with unlabeled FNLLP at each temperature. (Δ) Cells preincubated without peptide.

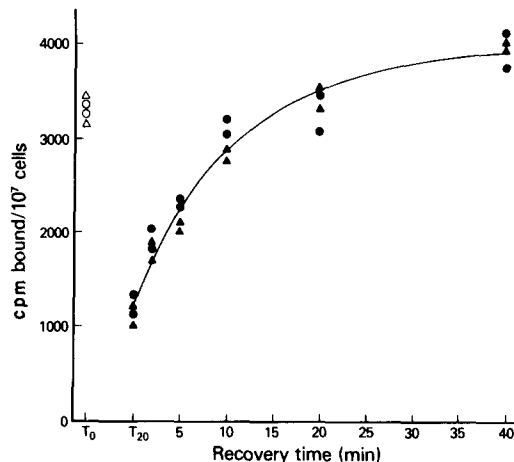


FIGURE 7 Recovery of receptors after down regulation. Cells were incubated for 20 min at 37°C with Hanks' solution (Δ, ○) or  $10^{-7}$  M unlabeled FNLLP (▲, ●) and washed for 5 min at 4°C. The ability to bind  $2 \times 10^{-8}$  M [ $^3$ H]FNLLP was then tested immediately ( $T_0$ ,  $T_{20}$ ) or after incubation in Hanks' solution at 37°C for various times. Cells incubated with 1 µg/ml cycloheximide were subjected to the same protocol (○, ●).

poration of tritiated amino acids by 92% had no effect.

#### Evidence for FNLLP Uptake

As seen in Fig. 1, the amount of peptide bound at 4°C reaches a plateau within 15 min. In contrast, at 37°C the binding does not reach a plateau but continues to increase at a reduced rate. However, the increase is caused by irreversibly bound peptide. The amount of reversibly bound peptide does indeed reach a plateau at 37°C as at 4°C. The number of reversibly bound receptors at  $3 \times 10^{-8}$  M FNLLP is  $2.45 \pm 0.3 \times 10^4$  (Fig. 8). This corresponds well with the number of receptors available for binding after down regulation at this concentration,  $2.58 \pm 0.4 \times 10^4$ . This increase in cell-associated peptide appears to be energy dependent because it is inhibited by 2-deoxy-D-glucose as well as by a temperature of 4°C. The accumulation of irreversibly cell-associated peptide decreases by 74% when the cells are incubated at 37°C with 0.5 mM 2-deoxy-D-glucose. Down regulation at 37°C is not affected by the addition of 2-deoxy-D-glucose.

The dose-response curve for the accumulation of irreversibly cell-associated FNLLP at 37°C is shown in Fig. 9. The dose-response curve reflects the binding of FNLLP to its receptor, suggesting

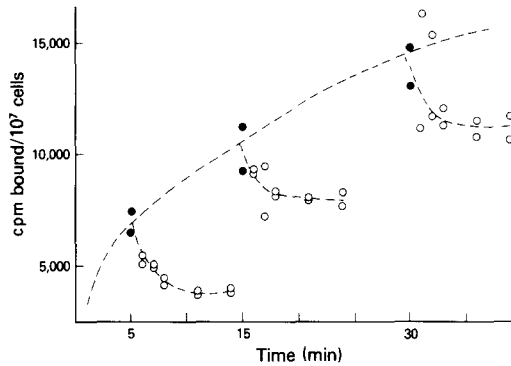


FIGURE 8 Reversibility of binding at 37°C. (●) Cells were incubated for various lengths of time at 37°C with  $3 \times 10^{-8}$  M [ $^3$ H]FNLLP and washed quickly (6 s) at the end of the incubation. (○) After incubation for 5, 15, and 30 min, cells were washed at 4°C for various lengths of time.

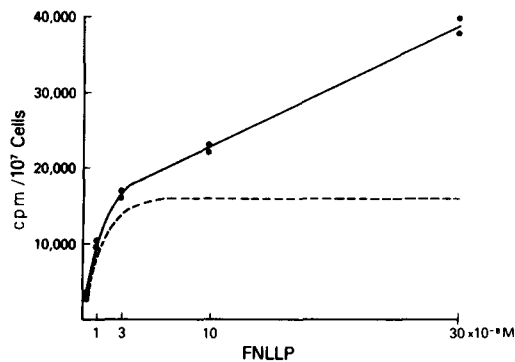


FIGURE 9 FNLLP uptake. (●) FNLLP remaining cell associated after a 30-min incubation at 37°C followed by a 5-min wash at 4°C as a function of FNLLP concentration. The dotted line represents saturable uptake (total-nonsaturable). Nonsaturable uptake is calculated from the uptake of  $10^{-8}$  M [ $^3$ H]FNLLP in the presence of  $10^{-5}$  M unlabeled FNLLP. This data is not corrected for peptide release.

that the uptake is receptor mediated. However, uptake continues to increase at concentrations above those that would saturate all of the receptors. This is presumably because of pinocytotic uptake of increasing amounts of FNLLP in the fluid and nonspecifically associated with the cell surface. The amount of nonsaturable uptake was measured as the irreversible accumulation of  $10^{-8}$  M [ $^3$ H]FNLLP in the presence of  $10^{-5}$  M unlabeled FNLLP. The nonsaturable uptake appears to be linear, so the degree of nonsaturable uptake at each concentration was calculated and subtracted from total to give saturable uptake. The calculated

curve for saturable uptake (Fig. 9) is very similar to that of FNLLP binding to the receptor. CBZ-Phe-Met competition for uptake provides additional support for the hypothesis that this uptake is receptor mediated. The addition of  $10^{-4}$  M CBZ-Phe-Met decreases the rate of  $10^{-8}$  M FNLLP uptake by  $\sim 70\%$  (Fig. 10). This correlates with the degree of competition for receptor binding seen at these concentrations of antagonist and peptide. A study of peptide release indicates  $\sim 20\%$  of the "irreversibly" associated counts are released into the medium during a 30-min incubation at 37°C in Hanks' solution.

PMNs have not been considered to be actively pinocytotic cells. Nevertheless, the addition of the chemotactic peptide stimulates the uptake of tritiated sucrose from the medium, presumably through a pinocytosis mechanism. The addition of  $10^{-7}$  M FNLLP increases the rate of fluid uptake, as measured by the amount of  $10^{-6}$  M sucrose that becomes irreversibly cell associated, from 1.7 to 6.6 nl/min per  $10^7$  cells (Fig. 11). This stimulation of fluid uptake is not sufficient to account for the uptake of FNLLP, which again suggests that the

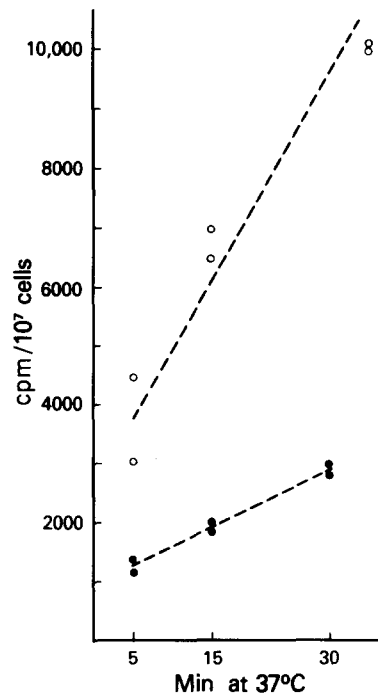


FIGURE 10 CBZ-PHE-MET competition for FNLLP uptake. Accumulation of irreversibly cell-associated  $10^{-8}$  M [ $^3$ H]FNLLP in the presence (●) and absence (○) of  $10^{-4}$  M CBZ-PHE-MET.

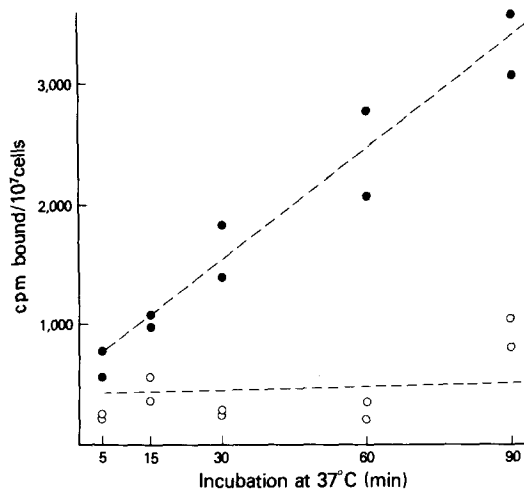


FIGURE 11 Peptide stimulation of pinocytosis. Cells were incubated for various lengths of time at 37°C with  $10^{-6}$  M [ $^3\text{H}$ ]sucrose in the presence (●) and absence (○) of  $10^{-7}$  M unlabeled FNLLP. At the end of the incubation, the cells were washed for 5 min at 4°C.

peptide uptake is receptor mediated. Stimulation of sucrose uptake does not appear to be dependent upon the concentration of FNLLP present. Concentrations ranging from  $10^{-7}$  to  $10^{-9}$  M FNLLP result in the same fourfold increase.

This peptide uptake could be caused by transport or receptor-mediated internalization. If this increase in irreversibly cell-associated counts does represent receptor-mediated internalization, a mechanism for resupplying receptors to the cell surface would be required. At  $10^{-7}$  M FNLLP, the amount of nondissociable peptide accumulated after 90 min at 37°C is equivalent to three times the number of receptors initially present.

There is also some stimulation of pinocytosis even at 4°C. The addition of  $10^{-7}$  M FNLLP results in a rapid, transient increase in sucrose uptake which lasts less than 5 min (Fig. 12). Although this uptake could be entirely separate from the down regulation, it suggests that a pinocytic mechanism is functional.

## DISCUSSION

Many recent studies have shown that the binding of ligands to their cell surface receptors modulates the number of available receptors and, thus, the sensitivity and responsiveness of a cell to that ligand. A decrease in the concentration of accessible receptors as a result of incubation with the ligand has been termed down regulation. Receptor

down regulation has been demonstrated for a number of hormones and neurotransmitters (4-6, 10-12), low density lipoprotein (2), and for the chemotactic agent cyclic AMP in *Dictyostelium discoideum* (15).

Our data indicate that down regulation of the chemotactic peptide receptor is a rapid response of PMNs to incubation with FNLLP. Several lines of evidence support this as being a specific result of FNLLP binding to its saturable receptor. Both the rate and extent of down regulation are concentration dependent, and the dose response parallels that of FNLLP binding. Receptor loss is not induced by HNLLP, a nonchemotactic analogue of FNLLP that does not bind to the receptor. The antagonist CBZ-Phe-Met competes with FNLLP for receptor binding but does not stimulate any of the chemotactic responses, including down regulation. However, CBZ-Phe-Met will inhibit FNLLP-induced down regulation presumably because of competition for the receptor.

Down regulation is clearly temperature dependent although it does occur at 4°C. Examination of the binding at this temperature reveals a component that is not readily dissociable. The amount of irreversibly cell-associated peptide corresponds to the number of receptors down regulated at 4°C. The existence of an irreversible component of binding and down regulation at this temperature is quite surprising. It should be considered in interpreting both Scatchard and kinetic binding

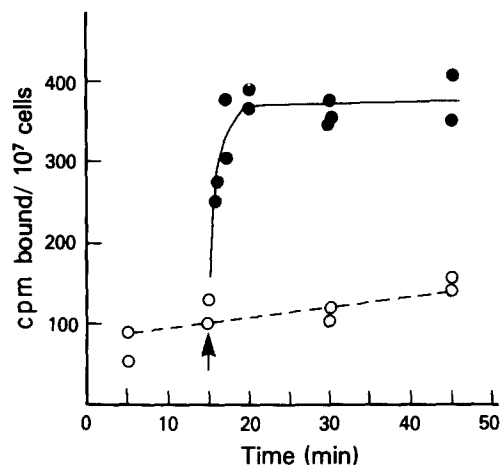


FIGURE 12 Stimulation of pinocytosis at 4°C. (○) Cells were incubated at 4°C with  $10^{-6}$  M [ $^3\text{H}$ ]sucrose for various periods of time. (●) After 15 min at 4°C with [ $^3\text{H}$ ]sucrose,  $10^{-7}$  M unlabeled FNLLP was added for various lengths of time; arrowhead, addition of peptide.

analyses. Although we obtained approximately the same  $K_d$  value using these two independent methods, both may overestimate the binding affinity because each method assumes equilibrium (entirely reversible) binding. The use of labeled plus excess unlabeled peptide as a measure of nonsaturable binding is useful in many instances but also may lead to erroneous estimates because the high concentration of peptide present will induce down regulation. Furthermore, there is a component of nonsaturable binding which is not reversible. Thus we chose to study the reversal of binding through dilution and washing rather than by competition with excess unlabeled peptide.

Several mechanisms could cause the loss of receptors. The binding of peptide could result in a modification of the receptor that leads to much higher affinity binding. A change in receptor affinity has been proposed to account for down regulation in other systems (17, 28, 33, 35). This change could be because of a change in conformation, some other modification such as methylation or phosphorylation, or association with another membrane component. A second possibility is that bound receptors are internalized by the cell. Such a pinocytotic mechanism has been established where it has been possible to follow the receptors directly (5, 8). In other studies, ultrastructural data indicate that receptor-mediated internalization of ligands occurs via coated vesicles (2, 5, 10). There is some evidence for receptor-mediated internalization in our system. We have shown that down regulation is accompanied by a rapid stimulation of [ $^3$ H]sucrose uptake from the medium. Furthermore, Niedel et al. (19) have followed fluorescently labeled chemotactic peptide and presented evidence for its clustering and internalization.

At 37°C, the uptake of FNLLP and the stimulated uptake of sucrose continue, even after the receptor number has equilibrated. The dose-response curve for peptide uptake indicates that it is mediated both by a saturable receptor with approximately the same affinity as the chemotactic receptor and by bulk pinocytosis uptake of FNLLP in the external fluid. Receptor-mediated uptake could be via active transport of peptide or pinocytosis internalization of the receptor ligand complex. If FNLLP uptake is caused by receptor-mediated pinocytosis, there must be a mechanism for resupplying receptors to the cell surface because, after 90 min in  $10^{-7}$  M FNLLP, the amount of peptide internalized exceeds the number of receptors initially present by 300%. This would

require replacement of all the receptors every 30 min from an internal pool or via receptor recycling (9). The relationship between the irreversible binding of peptide, the loss of receptors that occurs rapidly (down regulation), and the increasing accumulation of irreversibly bound peptide that occurs after the receptors are at a steady-state level requires further study.

It is well established that PMNs become deactivated in response to chemotactic stimulation. Deactivation is defined as a decrease in responsiveness to a chemotactic factor as a result of previous exposure to the stimulus (20). We have recently shown that PMNs adapt to the concentration of chemotactic peptide which is present but remain sensitive to changes in concentration. Adaptation is suggested by a number of biochemical and morphological responses to chemotactic factors which are transient even though the chemotactic factor continues to be present and the cells continue to respond to changes in the concentration of the chemotactic factors. Receptor modulation may be involved in one or both of these processes.

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Note added in proof: While this manuscript was in press, two studies appeared which also indicate receptor modulation in PMNs, Vitkausas et al., *Mol. Immunol.* 17: 171-180, and Niedel et al., *J. Biol. Chem.* 254:10700-10706.

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