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KINETICS OF *N*-FORMYL PEPTIDE RECEPTOR UP-REGULATION DURING STIMULATION IN HUMAN NEUTROPHILS[1]

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The kinetics of receptor up-regulation was examined in isolated neutrophils and in whole blood by flow cytometry during cell activation. Stimulation of neutrophils prepared without exposure to LPS with chemoattractants induced fast up-regulation of *N*-formyl peptide receptors and C receptor type 3 (CR3). Biphasic *N*-formyl peptide binding curves were detected for saturating concentrations of *N*-formyl peptide at 37°C. The bulk of the rapid binding during the first 30 to 60 s is attributed to already expressed binding sites whereas the slow binding over the next 3 to 4 min represents a time course of receptor up-regulation. Support for this interpretation comes from conditions under which the number of binding sites and the progress of the binding curves were affected. Cells treated with LPS, which caused expression of internal *N*-formyl peptide receptors, exhibited rapid, monophasic binding curves with increased total binding. In LPS-untreated, calcium-depleted cells, *N*-formyl peptide receptor up-regulation was inhibited and rapid, monophasic binding to a smaller total number of expressed sites was observed. Cytochalasin B enhanced the total number of available *N*-formyl peptide receptors in LPS-untreated but not LPS-treated cells. In both cases binding was rapid and monophasic suggesting that receptors were either fully or rapidly up-regulated. Although not studied in real-time, C receptor type 3 up-regulation was similar to *N*-formyl peptide receptor up-regulation in response to LPS, or stimulation by *N*-formyl peptide, C product C5a, leukotriene B_4, and platelet-activating factor in isolated cells and in whole blood. After stimulation with formyl peptide, LPS, or C product 5a, the release of vitamin B12-binding protein paralleled up-regulation of receptors. These data indicate that untreated cells up-regulate *N*-formyl peptide receptors during cell response at a rate of ~10,000/min in a calcium-dependent manner whereas LPS-treated cells already express the bulk of their receptors. In cytochalasin B-treated, degranulating cells 30,000 to 50,000 receptors were up-regulated within a minute.

Binding of *N*-formyl peptide to its receptor induces dynamic changes in receptor affinity as well as receptor endocytosis over periods of seconds to minutes (1). Specific aspects of these dynamic interactions have been suggested to reflect receptor-G protein interactions (seconds) and desensitization but not obligatory receptor-cytoskeletal interactions (tens of seconds). Zigmond et al. (2) suggested, based on *N*-formyl peptide binding, uptake and recovery measurements over periods of tens of minutes to hours that *N*-formyl peptide also stimulates the appearance or the reappearance of new binding sites for the *N*-formyl peptide to the cell surface and that such cycles are necessary to sustain chemotaxis over long periods of time. CR3,[3] which is involved in aggregation and adherence responses, is also up-regulated during several types of physiologic responses (3). Data from animal models as well as in vitro experiments suggest that when neutrophils are exposed to LPS the numbers of receptors are elevated and the responses to subsequent stimuli are enhanced (4–8). Although there are reports of the dynamics of receptor up-regulation over minutes to hours during priming, there is relatively little information about up-regulation during stimulation of isolated neutrophils and in whole blood.

There are conflicting reports about the source of receptors and the mechanism for their up-regulation. Several reports localize internal pools of *N*-formyl peptide-binding sites and CR3 to secondary granules (9–13) whereas a novel compartment distinct from secondary granules (14–16) has been proposed to be the source of these and other cell surface components. Contrary reports in which investigators studied the effect of intracellular Ca^{2+} chelators disagree on the role of intracellular Ca^{2+} in the regulation of the *N*-formyl peptide receptor. Andersson et al. (17) reported, for example, that binding of *N*-formyl peptide to neutrophils is reduced in Ca^{2+}-depleted neutrophils whereas Perez et al. (15) suggested that intracellular Ca^{2+} is not required for *N*-formyl peptide binding and receptor up-regulation or recycling. In addition, there is also conflicting interpretation of the role in ligand-receptor interactions of the F-actin disrupting agents cytochalasin or botulinum C2 toxin, which also permit granule enzyme release (18, 19). These contrary publications

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[3] Abbreviations used in this paper: CR3, C receptor type 3; C5a, activated fifth component of C; LTB_4, leukotriene B_4; MAPTAM, (bis-(2-amino-5-methylphenoxy)-ethane-*N,N,N',N'*-tetraacetic acid tetraacetoxymethyl ester); PAF, platelet-activating factor.

and the lack of detailed kinetic analysis prompted us to examine the kinetics of *N*-formyl peptide binding. We compared untreated and LPS-treated neutrophils by using real time flow cytometric methods in isolated neutrophils and whole blood to reduce the complications associated with cell isolation and binding assays that require wash steps. In this report, we relate these kinetics to receptor up-regulation under conditions modulating cytoskeleton, intracellular calcium, and secretion.

MATERIALS AND METHODS

Neutrophils. Anticoagulated blood was sedimentated for 45 min with 6% dextran 70 (Pharmacia, Piscataway, NJ). The white cell enriched plasma of the sedimentation step was layered onto a Percoll gradient (Pharmacia, 55% Percoll over 74% of Percoll) and centrifuged for 45 min with 400 × *g* at 4°C. The cells were characterized in flow cytometry as a single population by forward and side scatter, which was at least 95% neutrophils. Neutrophils were washed and resuspended in incubation buffer. The status of the neutrophil was routinely determined with *N*-formyl peptide-stimulated superoxide production. Studies were performed with neutrophils producing less than 0.5 nmol superoxide radicals/10^6 cells after stimulation with saturating, 4 nM fluoresceinated *N*-formyl peptide. Responsiveness was checked with actin- and degranulation-associated light scatter responses (19).

Incubation buffer. The incubation buffer contained 137 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.5 mM glucose, 1.5 mM $CaCl_2$, and 10 mM HEPES, pH 7.45. For limiting contamination of neutrophils with traces of LPS the incubation buffer was purified by using a polymyxin B-Sepharose column (20).

Reagents. Fluorescein-labeled *N*-formyl-norleu-leu-phe-norleu-tyr-lys was prepared as described previously (21). LPS from *Salmonella minnesota* RE 595 was prepared as described (7). The *N*-formyl peptide receptor blocker *t*-butoxycarbonyl-phe-leu-phe-leu-phe was obtained from Vega Biotechnologies (Tucson, AZ).

Cell responses. Oxidant production was determined by using the chromophore parahydroxyphenylacetic assay as described by Hyslop and Sklar (22). Right angle light scatter measurements were performed on aliquots of cells by using an SLM 8000 photon counting spectrofluorometer with 340 nm for excitation and emission (19). Release of vitamin B12-binding protein and elastase were measured as previously described (23, 24). Because Clark and Borregaard (25) demonstrated autoinactivation of vitamin B12-binding protein by myeloperoxidase-catalyzed oxidation and Hibbs and Bainton suggested that the conventional test for specific granule release, the vitamin B12-binding assay, might be complicated by oxidized reagents (26), the vitamin B12-binding protein assay was performed in the presence of catalase, azide, and superoxide dismutase. Intracellular Ca^{2+} was determined by using Indo 1 (27).

Binding assays. Analysis of *N*-formyl peptide binding was performed with cytometric methods described by Sklar et al. (21) by using as calibration standard the calibrated fluorescent microbeads from Flow Cytometry Standards Corporation (Research Triangle Park, NC). Buffers are derived from binding studies of Sklar et al. (21) and Norgauer et al. (19). For stabilizing the fluorescence of the ligand we used HEPES buffer, pH 7.45, with 1 mg/ml BSA, 0.08 mg/ml superoxide dismutase, 0.08 catalase, 4 mM azide, and 15 mM NH_4Cl. NH_4Cl was present for preventing acidification of intracellular endocytotic compartments. In pilot experiments, the saturation of the formyl peptide receptors was verified as a function of ligand concentration: 0.4 nM ligand, 24,690 receptors/cell ± 2,187(SEM); 1 nM, 30,715 ± 2,387; 4 nM 39,762 ± 1,675; 10 nM, 38,495 ± 1,378. In these experiments, neutrophils (previously incubated with 100 ng/ml LPS for 60 min at 37°C) were incubated with different concentrations of *N*-formyl peptide for 90 min at 4°C.

Analysis of expressed CR3 was performed with phycoerythrin-conjugated anti-human CR3 (CD 11b: Leu-15, Becton Dickinson, San Jose, CA). Neutrophils were incubated for 40 min on ice with Leu-15 and analyzed with the Facscan cytometer (Becton Dickinson, Sunnyvale, CA). Specific binding of Leu-15 was calculated by subtracting the mean channel number of fluorescence in the presence of 10-fold excess of unlabeled Leu-15 from the mean channel number in the absence of unlabeled Leu-15.

Whole blood-binding experiments were performed with anticoagulated blood in the presence of 15 nM NH_4Cl. The blood was diluted 1 to 10 and analyzed at low flow rate (12 μl/min) with the Facscan as described by Lemke and Sklar (footnote 4 in *FASEB J. 3:A1086*, 1989). Red cells were excluded by live-gating on fluorescence channel 1 (fluorescein). The population of neutrophils was gated by forward and side-scatter as described by Miller et al. (28) and bound fluorescence was analyzed as in the experiments of isolated neutrophils.

RESULTS

Earlier studies of the binding of *N*-formyl peptide to neutrophils at 4°C suggested that fluoresceinated *N*-formyl peptide binding to neutrophils plateaus in 90 min of incubation (21). In order to confirm that the expressed *N*-formyl peptide receptors were saturated under these conditions, LPS-treated neutrophils were incubated for 90 min with different concentrations of *N*-formyl peptide at 4°C. At the highest concentrations of ligand (4 and 10 nM peptide) similar numbers of receptors were occupied, indicating saturation of high affinity binding sites (*Materials and Methods*). When LPS was omitted the number of receptors in this cell preparation remained low and was barely up-regulated by incubation at 37°C (Table I). The temperature-dependent up-regulation of receptors (*N*-formyl peptide and CR3) is slow (occurs within minutes to hours) and was previously described by several authors as a general phenomenon in isolated neutrophils (6, 29). Its extent may depend on isolation procedures and stress during incubation and isolation in vitro (6, 29).

In order to exclude alteration of neutrophils during the isolation procedure, binding experiments were performed in whole blood under physiologic conditions and were compared with isolated neutrophils. About 15,000 expressed *N*-formyl peptide receptors were detected in neutrophils in whole blood (Table II). Stimulation of neutrophils with the chemoattractants *N*-formyl peptide, C5a, PAF and LTB_4 induced up-regulation of about 10,000 to 25,000 receptors within 5 min. Similar numbers of expressed *N*-formyl peptide receptors and similar up-regulation were detected in isolated neutrophils. Studies of CR3 performed in parallel by whole blood studies and isolated neutrophils revealed up-regulation similar in character to the *N*-formyl peptide receptor. Exploratory kinetic studies were performed by incubating blood with PAF for 30 s to 5 min at 37°C and then reducing the temperature to 4°C (H. D. Lemke and L. A. Sklar, unpublished observations). Maximal formyl peptide up-regulation was detected within 2 to 3 min.

Because of the flow rates involved it is not practical to examine up-regulation in real time in blood. Therefore the kinetics of ligand binding and receptor up-regulation were analyzed in isolated neutrophils. Binding studies at 37°C in untreated neutrophils with saturating (4 to 10 nM) fluoresceinated *N*-formyl peptide resulted in a biphasic binding curve. Binding was rapid during the first 30 to 60 s, followed by slower binding that lasted about 3 to 4 min and then leveled off (Fig. 1*A*). In parallel with the binding kinetics at 37°C, the number of expressed *N*-

TABLE I

The influence of LPS on the N-formyl peptide receptor up-regulation

No. of occupied *N*-formyl peptide receptors	16,720 (±1,267)	20,875 (±1,340)	43,280 (±1,590)
Incubation of neutrophils[a]	4°C	37°C	37° + LPS

[a] Neutrophils were incubated with and without 100 ng/ml LPS for 60 min at 37°C or kept on ice. The number of expressed *N*-formyl peptide receptors were analyzed by incubating neutrophils with 4 nM *N*-formyl peptide for 90 min on ice. Data is SEM of three experiments performed in duplicates.

TABLE II

Up-regulation of N-formyl peptide receptors and CR3 by isolated neutrophils and in whole blood[a]

Incubation condition	4°C	37°C	Pep	LTB_4	PAF	C5a
Whole blood						
Formyl peptide receptor number	13,709 (±475)	13,290 (±898)	39,760 (±1,092)	26,280 (±877)	31,970 (±1,188)	32,672 (±992)
Mean channel number Leu-15	11.92 (±1.96)	14.72 (±2.32)	97.85 (±9.75)	62.35 (±4.87)	75.16 (±5.34)	71.98 (±6.34)
Isolated Neutrophils						
Formyl peptide receptor number	15,225 (±1,756)	16,450 (±2,103)	41,728 (±3,290)	30,875 (±1,879)	29,128 (±2,390)	36,799 (±1,922)
Mean channel number Leu-15	14.62 (±2.75)	17.92 (±2.96)	102.92 (±4.76)	81.07 (±6.98)	72.46 (±4.75)	84.19 (±6.88)

[a] Isolated neutrophils and whole blood were stimulated with 4 nM FLPEP, 10 nM C5a, 10 nM LTB_4 and 10 nM PAF for 5 min at 37°C. Reaction was stopped diluting the sample with ice cold binding buffer and the number of expressed N-formyl peptide receptors and CR3 were determined at 4°C. Data is error of the mean of at least two experiments performed in duplicate.

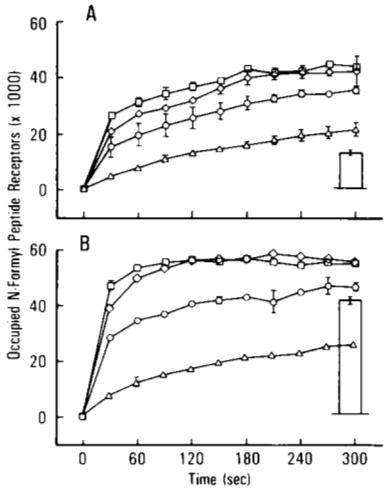

Figure 1. Real-time binding kinetics at 37°C to unprimed (Fig. 1*A*) and primed neutrophils incubated for 60 min with 100 ng/ml LPS (Fig. 1*B*). Cells were stimulated with different concentrations of *N*-formyl peptide (□, 10nM, ◇, 4nM, ○, 1nM and △, 0.2 nM). The bars in both panels indicate the number of expressed *N*-formyl peptide receptors before exposure of neutrophils to *N*-formyl peptide at 37°C for 60 min. These measurements were performed with an aliquot of neutrophils incubated with 4 nM *N*-formyl peptide for 90 min at 4°C. Data are means of duplicates with SD of one representative experiment repeated two times. Averages of data from multiple experiments produce comparable results.

formyl peptide receptors before stimulation was analyzed by incubating neutrophils for 90 min with 4 nM *N*-formyl peptide on ice. The higher number of occupied receptors after 5-min exposure of neutrophils to 4 nM *N*-formyl peptide at 37°C compared with occupied receptors at 4°C indicates an agonist-induced up-regulation of the *N*-formyl peptide receptor of at least 150%. Approximately 20,000 new binding sites were up-regulated and occupied during the first 2 min of stimulation. In contrast, after neutrophils were incubated with LPS (Fig. 1*B*), monophasic binding curves by saturating concentrations of ligand with elevated binding rates during the first minute of stimulation were obtained. The agonist-induced up-regulation of the receptor under these conditions was limited to no more than 30%. At low ligand concentrations, the similarity of the binding curves in treated and untreated cells suggests that the ligand binding kinetics are now limited by the association of the ligand and receptor rather than the up-regulation kinetics.

In subsequent experiments we studied the influence of intracellular Ca^{2+} on *N*-formyl peptide receptor up-regulation and *N*-formyl peptide binding. Incubation of untreated neutrophils with MAPTAM depleted the intracellular Ca^{2+} stores to less than 50 nM and blocked the stimulated Ca^{2+} rise (as described in Reference 25) (data not shown). *N*-Formyl peptide binding studies were performed at 37°C. In parallel the expressed *N*-formyl peptide receptor number before stimulation was determined. In Ca^{2+}-depleted neutrophils, as compared with control neutrophils, receptor up-regulation was minimal and only the rapid phase of ligand binding was detected (Fig. 2*A*).

Cytoskeletal activation during cell stimulation influences the fusion of intracellular granules with the plasma membrane. Cytochalasin B, which blocks the chemoattractant induced F-actin polymerization in human neutrophils (30) permits fast granule release in neutrophils and enhances the total amount of occupied *N*-formyl peptide receptor (Fig. 2*B*). Binding in this situation is roughly monophasic. Analysis of the number of expressed *N*-formyl peptide receptors at 4°C in cytochalasin

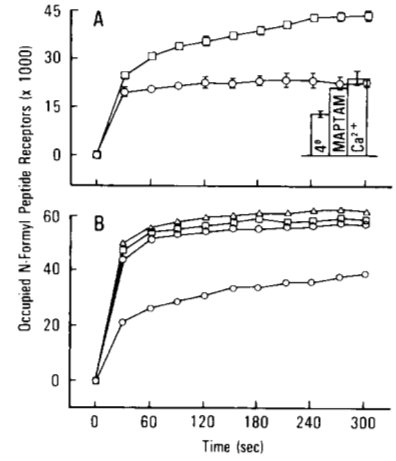

Figure 2. Role of intracellular calcium (*A*) and degranulation (*B*) on kinetics of receptor up-regulation. In *A* cells were incubated with (○) and without (□) 100 μM MAPTAM for 45 min. Neutrophils were resuspended with and without 1.53 mM Ca^{2+} for 10 min at 37°C before stimulation with 4 nM *N*-formylpeptide. The *bars* indicate the number of *N*-formyl peptide receptors before stimulation with *N*-formyl peptide at 37°C. The numbers were evaluated by incubating neutrophils with 4 nM *N*-formyl peptide for 90 min at 4°C. Data are means of duplicates with SD of one experiment repeated two times. In *B*, the influence of LPS and cytochalasin B on *N*-formyl peptide receptor kinetics was studied. Neutrophils were incubated with (□) and without (○) 100 ng/ml LPS for 60 min. Unprimed (○) and primed (△) neutrophils (LPS incubated neutrophils) were additionally incubated with 2μg/ml cytochalasin B for 2 min. Binding course of 4 nM *N*-formyl peptide stimulation is shown. Data are means of duplicates of one experiment performed five times. Data averaged from multiple experiments produce comparable results.

B-treated and untreated neutrophils revealed that cytochalasin B by itself did not stimulate the expression of *N*-formyl peptide receptors (data not shown). In LPS-treated neutrophils no new high affinity binding sites were detected after cytochalasin B treatment.

Parallel analysis of expressed *N*-formyl peptide receptors and CR3 was performed after LPS treatment and/or *N*-formyl peptide stimulation in the presence or absence of cytochalasin B (Fig. 3). *N*-formyl peptide stimulated a 2-3-fold upregulation of CR3 (Fig. 3*B*). Additional preincubation of neutrophils with cytochalasin B enhanced the *N*-formyl peptide stimulated CR3 expression in untreated neutrophils by about 80%. LPS induced up-regulation of CR3 and *N*-formyl peptide enhanced the number of expressed CR3 in LPS-treated neutrophils further. However, cytochalasin B preincubation did not enhance the number of expressed CR3 in LPS-treated and stimulated neutrophils.

Vitamin B12-binding protein, a marker for secondary granules, was detected in the supernatant from treated neutrophils (Fig. 3*C*) and, to a small extent, from untreated cells that up-regulated a fraction of their receptors at 37°C. In our hands, LPS and *N*-formyl peptide released vitamin B12-binding protein to a similar but submaximal extent whereas cytochalasin B treatment enhanced the *N*-formyl peptide-stimulated release of vitamin B12-binding protein to maximal levels.

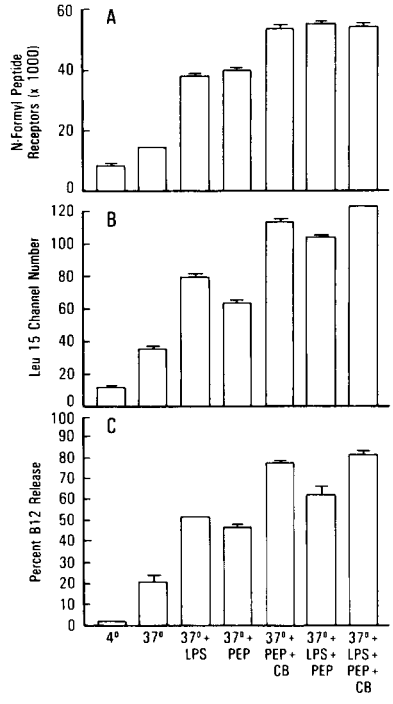

Figure 3. Comparison of expressed *N*-formyl peptide receptors (*A*), CR3 (*B*), and vitamin B12-binding protein release (*C*) after stimulation, priming, secretion, or incubation at 37°C. Data from *left* to *right*: the *first three bars* show cells incubated at 4°C, at 37°C, or at 37°C with LPS. The binding of 4 nM *N*-formyl peptide was examined at 4°C. In the *next four bars* are represented cells incubated at 37°C, at 37°C with CB, at 37°C with LPS, or at 37°C with LPS and CB. The binding of 4 nM *N*-formyl peptide is then reported after 5 min at 37°C. The relative fluorescence of Leu-15 in neutrophils after different treatments is shown in *B*. Neutrophils were treated as previously indicated and then incubated for 40 min on ice with Leu-15 and fluorescence profiles were acquired. The secretion of vitamin B12-binding protein is shown in *C*. In parallel with the receptor measurements, the release of vitamin B12-binding protein was determined as described. The data are means of duplicates with SD of one representative experiment repeated three times.

The sensitivity of up-regulation and enzyme release was tested in neutrophils incubated with Ca^{2+} or with MAPTAM in the absence of Ca^{2+}. Control neutrophils and Ca^{2+}-depleted neutrophils were stimulated with different concentrations of C5a for 5 min and the expressed *N*-formyl peptide receptors were measured at 4°C (data not shown). Although in control neutrophils C5a stimulated the up-regulation of *N*-formyl peptide receptors, in Ca^{2+}-depleted neutrophils no up-regulation could be detected. Additional incubation of control neutrophils with cytochalasin B for 2 min enhanced the C5a-stimulated expression of *N*-formyl peptide receptors. Under all these conditions *N*-formyl peptide receptor and CR3 up-regulation roughly parallels the appearance of vitamin B12-binding protein.

DISCUSSION

The regulation of cell-surface receptors plays a variety of roles in neutrophil function. For example, the exposure of neutrophils with chemoattractants, with LPS, or inadvertantly by isolation methods is associated with elevated numbers of surface receptors and enhanced superoxide responses (4–8). In addition, with respect to chemotaxis, it is thought that receptors are cycled in a scheme in which occupied receptors leave the surface and are returned to the surface unoccupied. Zigmond et al. have proposed kinetic models of some features of these processes (2), but direct measurements of up-regulation kinetics under most physiologic conditions are lacking. Measurements of *N*-formyl peptide receptor binding, uptake, and recovery (2) are also complicated by the need to discriminate between very high affinity receptors on the surface and internalized *N*-formyl peptide receptors. Moreover, the kinetics of reappearance of *N*-formyl peptide receptors in prestimulated or LPS-treated neutrophils has not previously been distinguished from the kinetics of receptor up-regulation in untreated neutrophils.

In order to exclude effects of isolation methods or materials in our experiments and in subsequent calculations, we compared binding experiments in whole blood and isolated neutrophils. The isolated neutrophils exhibited comparable numbers of receptors to the cells in the whole blood. Furthermore, exposure of neutrophils to agonists induced up-regulation of *N*-formyl peptide receptor and CR3 in isolated neutrophils and in whole blood to a qualitatively similar extent. Several previous reports of *N*-formyl peptide-binding kinetics from our lab were performed with neutrophils isolated from LPS-containing media in which the cells were characterized by a high stimulated superoxide production and high number of surface *N*-formyl peptide receptors before stimulation (19, 21). Those cells were prepared by using a gelatin-sedimentation step in which prior exposure to LPS appears to cause maximal receptor up-regulation. In contrast to earlier reports with those neutrophils, exposure of these present bloodlike neutrophils to *N*-formyl peptide caused minimal production of superoxide (<0.5 nM/10^6 cells) and showed a biphasic *N*-formyl peptide-binding curve at 37°C by using saturating concentrations of ligand. The occupancy of already expressed receptors results in rapid binding during the first 30 sec. The subsequent slower binding appears to represent the time course of up-regulation of *N*-formyl peptide receptors

during the stimulation. *N*-formyl peptide stimulated the up-regulation of 10,000 new binding sites/min during the first 2 min of stimulation.

The evidence that the second phase of binding is related to up-regulation of receptors is based on conditions in which the progress of binding and the number of receptors is varied by LPS exposure, degranulation, and regulation of intracellular calcium. Previous reports demonstrated that LPS induces up-regulation of *N*-formyl peptide-binding sites and CR3 (4, 6–8). Essentially monophasic binding to a higher number of sites at saturating concentrations of *N*-formyl peptide was detected in LPS-treated cells compared with untreated neutrophils. Recently we reported binding kinetics similar to those obtained after LPS treatment in a cell preparation with the use of a gelatin sedimentation step in which prior exposure to LPS appears to cause receptor up-regulation during the isolation (7, 19, 21).

Roles for intracellular Ca^{2+} in receptor dynamics have been suggested previously. For example, Andersson et al. (17) reported *N*-formyl peptide binding that was dependent upon intracellular Ca^{2+} in a fashion consistent with our results. Perez et al. (15) suggested that although *N*-formyl peptide-induced chemotaxis and receptor recycling does not have an absolute requirement for intracellular Ca^{2+}, the rate and magnitude of re-expression was clearly sensitive to the calcium concentration. In our hands, depletion of intracellular Ca^{2+} with the intracellular Ca^{2+} chelator, MAPTAM, inhibited the agonist-stimulated *N*-formyl peptide receptor up-regulation. However, binding of the ligand to the expressed receptor was not influenced. In contrast to our studies and those of Andersson et al. (17), Perez et al. (15) detected comparable binding in both normal and calcium-buffered cells possibly because the receptor levels were already up-regulated from their native levels in blood during isolation.

Changes of F-actin content have been associated with modulation of the *N*-formyl peptide-stimulated cell responses. Incubation of neutrophils with cytochalasin B inhibits the *N*-formyl peptide-stimulated F-actin polymerization (30). Jesaitis et al. (18) previously reported that dihydrocytochalasin B enhances the total amount of occupied *N*-formyl peptide receptors after exposure to the agonist. However, we could not detect any influence of actin disrupting agents such as cytochalasin B or botulinum C2 toxin on the *N*-formyl peptide-binding kinetics of elutriated neutrophils (19). Elutriated neutrophils prepared as described by Tolley et al. (31) have already been exposed to LPS during the gelatin-sedimentation step. These neutrophils produce high levels of superoxide radicals after stimulation (18, 19, 31); the *N*-formyl peptide binding at saturation is relatively monophasic (19) and the total content of vitamin B12-binding protein is reduced compared with neutrophils prepared under LPS-free conditions (J. Norgauer and L. A. Sklar, unpublished observations). In cytochalasin B-treated but LPS-free neutrophils, the quantity of receptor was elevated during the first minute of stimulation and a higher total of receptors, compared with control cells, was detected. Similar *N*-formyl peptide dynamics were detected after botulinum C2 toxin treatment (J. Norgauer, K. Aktories, and L. A. Sklar; unpublished observations). Additional incubation of LPS-treated neutrophils with cytochalasin B did not further alter the *N*-formyl peptide-binding dynamics, suggesting that a high percentage of the total cellular receptors was already expressed.

Several authors have localized the intracellular pool of *N*-formyl peptide receptors and CR3 to secondary granules (9–13), but the location of *N*-formyl peptide receptor has been questioned (14–16). Our experiments did not attempt to systematically resolve the up-regulation of CR3 and formyl peptide receptor from the release of vitamin B12-binding protein. Although there was a rough parallel between these markers, we cannot eliminate the possibility that they arise from separate compartments with kinetic similarity. LPS, *N*-formyl peptide, C5a, PAF, or LTB_4 triggered the secretion of secondary granules whereas elastase, a marker of primary granules, was only detected during stimulation in the presence of cytochalasin B (data not shown). CR3 up-regulation was also absent in calcium-depleted cells (data not shown). This observation is in agreement with earlier reports of Lew et al. (32) and Berger et al. (33). It is our view that many inconsistencies in the literature concerning neutrophil function (secretion of secondary granules, superoxide production, and receptor dynamics) may depend in part on fusion of granules with the plasma membrane during isolation. LPS contamination of isolation materials has been reported (5, 7) and adherence to glassware seems also to promote the release of secondary granules (34). In addition, chemoattractants such as C5a, LTB_4, and PAF stimulated the rapid up-regulation of the *N*-formyl peptide receptor and CR3. Up-regulation of *N*-formyl peptide receptor with C5a was also inhibited in Ca^{2+}-depleted neutrophils, enhanced after cytochalasin B treatment, and paralleled the release of secondary granules.

In summary, it is generally believed that chemotaxis requires a cycle of receptor expression and internalization of cell-surface receptors. In this report we show that *N*-formyl peptide receptors are rapidly up-regulated in native cells from an intracellular localization via a pathway that appears to be sensitive to intracellular Ca^{2+} at a rate up to ~10,000 receptors/min/cell. Because comparable rates can be elicited by other chemoattractants, it appears that up-regulation does not depend upon occupied formyl peptide receptors, as originally suggested (2), during chemotaxis. In cells exposed to LPS, cytochalasin B-dependent secretion has a minimal impact on the number of high affinity-binding sites because the sites have been already expressed. In contrast, in native cells, LPS and cytochalasin B both permit up-regulation. In the former case, we could not distinguish up-regulation from specific granule release. In the latter, up-regulation and release of specific and azurophilic contents are apparently in concert.

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