Interleukin 8 (Monocyte-derived Neutrophil Chemotactic Factor) Dynamically Regulates Its Own Receptor Expression on Human Neutrophils

Ajoy K. Samanta†, Joost J. Oppenheim, and Kouji Matsushima§

From the Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, Frederick, Maryland 21701-1013

The regulation of monocyte-derived neutrophil chemotactic factor (MDNCF)/interleukin 8 (IL 8) receptor expression by the MDNCF/IL 8 ligand was examined using freshly isolated human peripheral blood neutrophils. MDNCF/IL 8 down-regulated >90% of its own receptor expression within 10 min at 37°C. This down-regulation was associated with internalization of the ligand. The radiolabeled MDNCF/IL 8 molecules after internalization were proteolytically degraded, and trichloroacetic acid-soluble molecules were released into the culture medium starting at 60 min. Lysosomotropic agents could inhibit this degradation of ligand suggesting the involvement of lysosomal enzymes in this proteolytic digestion. MDNCF/IL 8 receptors reappeared on the cell surface within 10 min after removal of free ligands from the culture medium. Cycloheximide did not alter the reappearance of the receptor suggesting that de novo protein synthesis of MDNCF/IL 8 receptors is not involved in this event and that receptors probably recycled. The addition of lysosomotropic agents partially inhibited the reappearance/recycling of the receptors, although none of these agents inhibited the binding of ligand to the surface receptors or ligand internalization. Ammonium chloride reduced the MDNCF/IL 8-induced neutrophil chemotactic response in a dose-dependent fashion. These data suggest that MDNCF/IL 8 receptor expression is dynamically regulated by MDNCF/IL 8 and that the rapid recycling of MDNCF/IL 8 receptors may be essential for the chemotactic response of neutrophils.

Directed migration of leukocytes from the blood stream to inflammatory sites may play a vital role in host defense. Polymorphonuclear neutrophils are active participants in acute inflammatory reactions. Monocyte-derived neutrophil chemotactic factor (MDNCF)1 is a recently purified and molecularly cloned cytokine that exhibits a potent neutrophil chemoattractant activity (1, 2). The mature form of MDNCF consists of 72 amino acids (2), and the fully active form has been chemically synthesized (3) and also expressed in Escherichia coli (4). IL 1 and tumor necrosis factor stimulate peripheral blood mononuclear cells, fibroblasts, endothelial cells, and keratinocytes to produce MDNCF (2, 5, 6). Recently we showed that our purified T lymphocyte chemotactic factor from phytohemagglutinin A-stimulated peripheral blood mononuclear cell-conditioned media is identical to MDNCF (7). In vitro, this MDNCF polypeptide induces neutrophil respiratory burst (8), augments lysosomal enzyme release in the presence of cytochalasin B (9), increases Mac-1 expression on neutrophils (10), and enhances neutrophil Candida albicans killing (11). Intraperitoneal or intravascular injection of MDNCF caused rapid neutrophil accumulation or neutrophilia, respectively (4, 12), and intradermal injection of the MDNCF caused a dose-dependent accumulation of neutrophils and lymphocytes at the site of injection (5, 7). Since this molecule is produced by multiple types of cells and acts on different target cells, this polypeptide has been proposed to be renamed interleukin 8 (IL 8) (7, 13).

Using radioiodinated recombinant MDNCF/IL 8, we have recently demonstrated the presence of MDNCF/IL 8-specific receptors on human neutrophils which are different from the receptors for a number of other neutrophil chemotactic agents (14). About 20,000 high affinity binding sites (Kd = 8 × 10^-10 m) for IL 8/MDNCF have been detected on peripheral blood neutrophils (14). In the present study, we report that MDNCF/IL 8 very rapidly down-regulates its own receptor expression associated with ligand internalization and that the down-regulated receptor can be rapidly recycled/reappeared to the surface of neutrophils. The rapid reappearance of the MDNCF/IL 8 receptor seems to be important for the neutrophil chemotactic response to MDNCF/IL 8.

MATERIALS AND METHODS

Reagents—Fully biologically active human recombinant MDNCF/IL 8 (2 × 10^6 units/mg) was expressed in E. coli and purified to homogeneity (4). Chloroquine, methylamine, sodium azide, 2,4-dinitrophenol, monooxynal cadaverine, monensin, and bovine serum albumin (BSA) were purchased from Sigma. Formyl-methionyl-leucyl-phenylalanine (fMLP) was obtained from Peninsula Laboratories, Inc., Belmont, CA.

Preparation of Human Neutrophils—A granulocyte-enriched fraction was obtained by leukapheresis of normal donors at the NIH Clinical Center Transfusion Medicine Department, Bethesda, MD. The method of separation was followed as described previously (14). Finally the cells were suspended in HEPES buffer, pH 7.2, and 10 mg/ml BSA. Slides were prepared using a Shandon Cytospin II centrifuge and stained with Wright's stain. Differential counts of neutrophils and mononuclear cells were performed by counting at least 200 cells under a high power (10 × 40) light microscope, and 95–98% of cells were identified as

† Guest researcher and recipient of the Biotechnology Overseas Associateship from the Department of Biotechnology, Government of India.
§ To whom all correspondence should be addressed.

1 The abbreviations used are: MDNCF, monocyte-derived neutrophil chemotactic factor; IL, interleukin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; dansyl, N,N-dimethylaminonaphthalene-1-sulfonfonyl; fMLP, formyl-methionyl-leucyl-phenylalanine; BSA, bovine serum albumin.
polymorphonuclear neutrophils and 2-5% cells were mononuclear cells. The viability of the cells was determined using the trypan blue exclusion method, and the cells thus obtained were 99% viable.

Radiolabeling of IL 8—Human recombinant IL 8 was labeled with Bolton-Hunter reagent (15) as described in our previous report (14). The specific radioactivity of 125I-labeled MDNCF/IL 8 (125I-MDNCF/IL 8) was 107 cpm/μg. After labeling the biological potency of the radiolabeled material was not diminished, as assessed by myeloperoxidase release from neutrophils (9, 14).

Binding Assay—For the standard binding assay, 2 × 106 cells were taken in Eppendorf tubes containing 200 μl of RPMI 1640 medium, 10 mM HEPES buffer, pH 7.2, 10 mg/ml BSA, and different amounts of radiolabeled MDNCF/IL 8. Non-specific binding, determined in the presence of a 100-fold excess of unlabeled MDNCF/IL 8, was subtracted from total binding to give specific binding.

MDNCF/IL 8 bound to cell surface receptors was eluted by brief exposure (1 min) to 0.05 M glycine HCl buffer, pH 3.0, with 0.1 M NaCl. The radioactivity present in the acid-eluted and acid-resistant fractions was measured by a γ counter.

Chemotaxis Assay—Neutrophil chemotaxis was assayed with a Boyden multiwell chemotaxis chamber (Neuro Probe, Inc., Bethesda, MD) and 10 μm thick 3-μm pore polivinylpyrrolidone-free polycarbonate filters (Nucleopore Corp. Pleasanton, CA) as described earlier (1).

RESULTS

Effect of MDNCF/IL 8 on the Cell Surface MDNCF/IL 8 Receptor Expression—In order to examine the effect of MDNCF/IL 8 on the expression of MDNCF/IL 8 receptors on human neutrophils, neutrophils were first incubated for 60 min at 37 °C in the presence of various doses of unlabeled MDNCF/IL 8. After washing the cells twice with cold medium, the neutrophils were treated with 0.05 M glycine HCl, pH 3.0, with 0.1 M NaCl. The radioactivity present in the acid-eluted and acid-resistant fractions was measured by a γ counter.

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FIG. 1. Effects of various doses of MDNCF/IL 8 on the down-regulation of MDNCF/IL 8-specific receptors on human neutrophils. Human polymorphonuclear neutrophils (2 × 106 cells) suspended in RPMI 1640 medium containing 10 mg/ml BSA, 20 mM HEPES, pH 7.2, were incubated with various concentrations of unlabeled MDNCF/IL 8 at 37 °C for 1 h. The cells were washed with cold medium and then treated with 200 μl of the acidic glycine solution, as described under “Materials and Methods” to remove any surface-bound MDNCF/IL 8. The cells were centrifuged in a microcentrifuge at 10,000 rpm for 90 s. The pellet was suspended in medium and kept on ice. Then to each tube 5 ng of 125I-MDNCF/IL 8 was added and the tubes were incubated at 4°C for 1 h. Cell-bound labeled material was measured by a γ counter. The mean counts/min with ± S.D. of duplicate samples are shown in this representative experiment.

FIG. 2. Rate of down-regulation of MDNCF/IL 8 receptors in human neutrophils at 37 °C. Human neutrophils (2 × 106 cells/200 μl of medium) were incubated for different periods at 37 °C in RPMI 1640 medium, 10 mg/ml BSA, 20 mM HEPES, pH 7.2, and 200 ng/ml MDNCF/IL 8. In control cells no MDNCF/IL 8 was added. After washing with cold medium the cells were treated with the acidic solution, centrifuged, and then resuspended in medium. The surface binding of 125I-MDNCF/IL 8 was determined as above, and the means with ± S.D. of duplicate counts/min are shown in this representative experiment.
the cell surface, because most of the radioactive counts were recovered (>95% of the total bound \(^{125}\)I MDNCF/IL 8) in the supernatant fraction after centrifugation of the acid-treated cells. Acid-washed cell pellets consisting of neutrophils showed <5% radioactive count. Therefore, this indicates that at 4 °C MDNCF/IL 8 was bound on the surface of neutrophils and that a brief treatment with an acidic glycine/NaCl mixture provides a method for the removal of surface-bound MDNCF/IL 8 from the neutrophils.

We have examined that the binding of MDNCF/IL 8 to the surface of neutrophils is very stable. The rate of dissociation at 4 °C after 1 h was 4.8% and after 2 h it was about 15% of the total binding of \(^{125}\)I-MDNCF/IL 8 to the neutrophils. To examine the effects of temperature on internalization, in another set of experiments \(^{125}\)I-MDNCF/IL 8-bound neutrophils were incubated at different temperatures for 20 min. After incubation, acid wash treatment was carried out to elute the surface-bound \(^{125}\)I-MDNCF/IL 8 from the neutrophils. We observed that at 10 °C we obtained only 60% of \(^{125}\)I-MDNCF/IL 8 from the surface, and the rest of the count was recovered with the acid-washed cells. After incubation at 25 and 37 °C, most of the counts (96%) were obtained in the acid-washed cell pellet, and the supernatant fraction showed only about 4% of the total binding of \(^{125}\)I-MDNCF/IL 8. Consequently, this suggests that the down-regulation of the receptors was due to a temperature-dependent internalization of the receptor.

Internalization of MDNCF/IL 8 by the neutrophil was confirmed by failure to recover \(^{125}\)I-MDNCF/IL 8 by the treatment of neutrophils with Pronase. After binding of \(^{125}\)I-MDNCF/IL 8 to cells at 4 °C the surface-bound \(^{125}\)I-MDNCF/IL 8 was incubated for 20 min at 37 °C. Then Pronase, a proteolytic enzyme which was previously determined to be catalytically active, was mixed with the incubation mixture and incubated for another 45 min at 37 °C. A control set without Pronase was also incubated at 37 °C. After centrifugation, we measured the count recovered in the supernatant and pellet. We observed them to be exactly equal to the same fractions of the (acid-washed) control counts. This suggests that at 37 °C the \(^{125}\)I-MDNCF/IL 8 was no longer accessible to Pronase.

Then we checked the rapidity of internalization of \(^{125}\)I-MDNCF/IL 8 at 37 °C. The neutrophils were first incubated at 4 °C with \(^{125}\)I-MDNCF/IL 8 for 1 h, unbound ligand was removed by repeated washing of the cells with cold binding medium, and the cells were then incubated at 37 °C for different time periods as shown in Fig. 3. \(^{125}\)I-MDNCF/IL 8 molecule bound to the cell surface receptors, as measured in the acid-eluted fraction, was rapidly internalized and became resistant to acid elution. The rate of ligand internalization (Fig. 3) correlated well with the rate of loss of MDNCF/IL 8 binding capacity (down-regulation) of neutrophils as has been shown in Fig. 2. After various incubation periods, the culture media were also collected and the proteins were precipitated with trichloroacetic acid at 4 °C. Prior to 60 min, no trichloroacetic acid-soluble radioactivity could be detected in the culture media (Fig. 3). However, after 120 min of incubation, about 20% of the total internalized count was no longer recovered in cell-associated form but could now be recovered in the trichloroacetic acid-soluble fraction of the culture medium. Presumably by 120 min 20% of the internalized \(^{125}\)I-MDNCF/IL 8 has been proteolytically digested and released.

In order to further confirm whether internalized MDNCF/IL 8 is proteolytically digested, the effect of an inhibitor of proteolytic digestion, namely ammonium chloride, was tested. In the next experiment, neutrophils were first incubated at 4 °C with \(^{125}\)I-MDNCF/IL 8 for 1 h, unbound ligand was removed by repeated washing with cold binding medium, and the cells were then cultured at 37 °C in the presence of 20 mM ammonium chloride. The radioactivity present in the trichloroacetic acid-soluble fraction of ammonium chloride-untreated cells was markedly decreased in comparison with ammonium chloride-treated cells (Fig. 4). For example, when the cells were cultured in the presence of 20 mM ammonium chloride for 180 min, the radioactivity in the trichloroacetic acid-soluble fraction was 53% less than that in the control. The counts/min of the trichloroacetic acid-insoluble fractions of ammonium chloride-treated culture medium and the control remained almost the same (Fig. 4). This suggests that at 180 min the ammonium chloride inhibited proteolytic digestion of MDNCF/IL 8 in the cells.

**Blocking of the Internalization**—We examined the effects of several agents on the rapid internalization of the ligand-receptor complex. Metabolic blockers, e.g. 0.1% sodium azide and 2,4-dinitrophenol, lysosomalotropic agents, e.g. 20 mM ammonium chloride, 50 μM chloroquine, 5 mM methylamine, failed to inhibit receptor-mediated IL 8 internalization at 37 °C (Table I). Bacitracin, a peptide antibiotic, and monodansyl cadaverine are potent inhibitors of transglutaminase essential for clustering of the receptor before endocytosis of
ligand-receptor complexes in the case of many polypeptide hormones including α1 macroglobulin (16). Bactracin at a concentration of 1.5 mM could not inhibit the internalization of 125I-MDNCF/IL 8. The same concentration of dansyl cadaverine could apparently block the internalization of the ligand. Further investigation confirmed that the inhibitory effects of dansyl cadaverine on internalization were due to its inhibition of binding of 125I-MDNCF/IL 8 to the neutrophils at 4°C. For example, 300 μM dansyl cadaverine could inhibit 95% of the binding of MDNCF/IL 8 to neutrophils, whereas 1.5 mM could block 89% of the binding. Interestingly, the bound molecule could not be protected from internalization at 37°C by dansyl cadaverine. Most of the counts were recovered in the acid-washed cell pellet indicating that it did not block internalization.

The other metabolic blockers and lysosomotrophic agents have no significant effect on binding of MDNCF/IL 8 at 4°C. Therefore, only at a low temperature (4°C) could the bound cytokine be inhibited from being internalized along with the receptor.

Recycling of the Receptor—Saturation of binding of MDNCF/IL 8 at 4°C demonstrated that freshly prepared human neutrophils express approximately 20,000 surface receptors/cell (14). When a similar experiment was carried out at 37°C, several times greater total binding of 125I-MDNCF/IL 8 was obtained than at 4°C and the binding at 37°C was not saturable. This suggested that the MDNCF/IL 8 receptor is in a state of rapid dynamic turnover. To examine this possibility, the neutrophils were first incubated with 200 ng/ml unlabeled MDNCF/IL 8 at 37°C for 20 min to down-regulate the MDNCF/IL 8 receptor. The cells were then washed with the medium to remove free (unbound) ligand and further incubated at 37°C to evaluate the rate of reappearance of down-regulated MDNCF/IL 8 receptors. At different time intervals, cells were collected, cooled to 4°C, and binding studies were performed using radioiodinated MDNCF/IL 8. The binding of 125I-MDNCF/IL 8 gradually increased with time of incubation at 37°C. At 20 min the binding was maximal and remained so for up to 40 min. Subsequently, this level gradually decreased to one-half by 120 min (Fig. 5). These data support the view that MDNCF/IL 8 receptors are quickly being recycled and reappear on the surface from the intracellular pool.

To determine whether the receptors which reappeared on the cell surface were newly synthesized or performed, cycloheximide, a protein synthesis inhibitor, was added to the cultures of neutrophils at 37°C. As shown in Fig. 5, 10 μg/ml cycloheximide did not alter the recovery of binding by MDNCF/IL 8 receptors. These data suggest that de novo protein synthesis is not involved in the rapid reappearance of down-regulated MDNCF/IL 8 receptors on neutrophils.

Blocking of Receptor Recycling—The effect of several agents on the recycling of MDNCF/IL 8 surface receptors was examined. As shown in Table II, lysosomotrophic agents (chloroquine, ammonium chloride, methylamine), ATP synthesis inhibitors, (sodium azide, 2,4-dinitrophenol), and a carboxylic ionophore (monensin) all could to varying degrees inhibit the reappearance of the receptors on the cell surface. None of these drugs blocked the binding of MDNCF/IL 8 to the surface receptors at 4°C or the internalization of ligand-receptor complexes at 37°C (Table I). Ammonium chloride acted in a dose-dependent manner to block the reappearance of the receptors. Ammonium chloride at a concentration of 5 mM could inhibit 64% of the reappearance/recycling of the receptor whereas at a dose of 20 mM it could inhibit 82%.

**Table I**

<table>
<thead>
<tr>
<th>Name of agent</th>
<th>Dose used</th>
<th>Percentage of inhibition</th>
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<tbody>
<tr>
<td>Sodium azide</td>
<td>0.05%</td>
<td>1.2 ± 0.15</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.1%</td>
<td>2.6 ± 0.22</td>
</tr>
<tr>
<td>9-4 Dinitrophenol</td>
<td>0.05%</td>
<td>3.0 ± 0.17</td>
</tr>
<tr>
<td>2-4 Dinitrophenol</td>
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<td>3.8 ± 0.31</td>
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<tr>
<td>Methylamine hydrochloride</td>
<td>5 mM</td>
<td>1.3 ± 0.12</td>
</tr>
<tr>
<td>Methylamine hydrochloride</td>
<td>10 mM</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Monensin</td>
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</tr>
<tr>
<td>Monensin</td>
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</tr>
<tr>
<td>Ammonium chloride</td>
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</tr>
<tr>
<td>Ammonium chloride</td>
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</tr>
<tr>
<td>Chloroquine</td>
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</tr>
<tr>
<td>Chloroquine</td>
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<td>3.2 ± 0.15</td>
</tr>
<tr>
<td>Monodansyl cadaverine</td>
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<tr>
<td>Monodansyl cadaverine</td>
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<td>4.0 ± 0.25</td>
</tr>
<tr>
<td>Bactracin</td>
<td>1.5 mM</td>
<td>2.2 ± 0.4</td>
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**Fig. 4.** Effects of ammonium chloride on the release of internalized 125I-MDNCF/IL 8 by human neutrophils at 37°C. In controls, 2 × 10⁶ cells/tube were incubated in RPMI 1640 medium, 20 mM HEPES buffer, pH 7.2, 10 mg/ml BSA, and 125I-MDNCF/IL 8 (20 ng) at 4°C for 1 h. Parallel samples were run with added 20 mM ammonium chloride. After incubation the cells were washed with cold "binding" medium and aliquoted into Eppendorf tubes. To each tube 200 μl of prewarmed "binding" medium was added and the tubes were then incubated at 37°C for the time periods indicated in the figure. The tubes were then centrifuged and the supernatants were precipitated with trichloroacetic acid as in Fig. 3. The mean of triplicate counts/min with ±S.D. in the trichloroacetic acid-soluble and insoluble fractions is shown.
Binding to Neutrophils at 37 °C—In order to examine the effect of ammonium chloride on the total binding and uptake of MDNCF/IL 8 by neutrophils at 37 °C, different amounts of radiolabeled MDNCF/IL 8 were added to the cells and incubated at 37 °C for 60 min in the absence or presence of 20 mM ammonium chloride. As predicted, we observed that ammonium chloride could significantly decrease (70%) the binding of MDNCF/IL 8 to cells at 37 °C (Fig. 6A). The MDNCF/IL 8 receptors on the neutrophils which were cultured in the absence of ammonium chloride could not be saturated by any dose of MDNCF/IL 8. On the other hand, ammonium chloride-treated cells showed an apparent saturable binding of MDNCF/IL 8 to the receptors (Fig. 6A). In an identical situation the sodium azide (0.1%) could reduce only 18% of the binding of 125I-MDNCF/IL 8 to the neutrophils, but the overall pattern of binding of different doses of 125I-MDNCF/IL 8 paralleled the binding of the control. Sodium azide did not show saturation binding as did ammonium chloride, suggesting sodium azide has minimal effects on 125I-MDNCF/IL 8 binding to cells at 37 °C (Fig. 6B).

Effect of Ammonium Chloride on Neutrophil Chemotaxis—To establish the biological importance of the rapid recycling of MDNCF/IL 8 receptors after ligand binding, neutrophils were preincubated with or without ammonium chloride and the chemotactic response of neutrophils to MDNCF/IL 8 was examined.

MDNCF/IL 8-induced migration of neutrophils was inhibited by ammonium chloride in a concentration-dependent...
Events that modify the mobility of the cell. The recent identification of neutrophils, which elicits a sequence of intracellular processes, is presumably initiated by the binding of chemoattractant to receptors on the surface of peripheral blood neutrophils supported this concept (14).

We have shown that following the binding of MDNCF/IL 8 to receptors on the surface of human neutrophils, the receptor-bound MDNCF/IL 8 is rapidly internalized at 37 °C. This internalization is an absolutely temperature-dependent process, because at 4 °C the internalization was completely inhibited, as shown by the elution of 95% of the surface-bound 125I-MDNCF/IL 8 by acid washing of neutrophils. Internalization starts at 10 °C and is very rapid at 37 °C. Moreover, MDNCF/IL 8 rapidly down-regulated the cell surface expression of its own receptor. The degree of down-regulation was dose-dependent. There was a good correlation between the rate of cell surface receptor down-regulation and the rate of internalization of 125I-MDNCF/IL 8. Internalization of 125I-MDNCF/IL 8 was confirmed by treatment of cells with Pronase. Since 125I-MDNCF/IL 8 was internalized, the enzyme could not proteolytically digest the radiolabeled MDNCF/IL 8.

The internalized MDNCF/IL 8 was released in the form of partly fragmented molecules. Based on the reports of other receptors, it is established that weak bases such as ammonium chloride, chloroquine, and mephalamine neutralize the intracellular acidic compartments of the organelles and therefore interrupt the lysosomal degradation of the ingested polypeptides. Acidity of lysosomes is a prerequisite criteria for delivery of ingested material to the lysosomes (17-19). Consequently, as expected, neutralization of lysosomes by ammonium chloride inhibited fragmentation of MDNCF/IL 8.

The failure of sodium azide and 2,4-dinitrophenol to inhibit internalization suggests that after the binding of MDNCF/IL 8 to its receptor, the internalization does not depend on the generation of new ATP.

Down-regulation of cell receptor may result in an attenuated response of the cells to the ligand. This attenuation can be avoided by rapid replacement with new receptors. Our data suggested that MDNCF/IL 8 receptors in neutrophils are in fact rapidly replaced on the cell surface. The reexpressed receptors are not synthesized de novo, but it is not clear at present whether the reexpressed MDNCF/IL 8 receptors are translocated from an internal preformed receptor pool or are really recycled receptors. Similar rapid reappearance of down-regulated receptors has been observed in several cases of transporting receptors for e.g. low density lipoprotein (20), aminoglycoprotein (21), and macroglobulin (22).

Recycling of the receptor could be selectively inhibited by several agents although their mode of actions is different. Ammonium chloride, chloroquine, and mephalamine raise the pH of acidic endosomes and lysosomes and thus may hamper intracellular degradation of MDNCF/IL 8 as well as the recycling of the receptor. Due to the intracellular effects of ammonium chloride presumably MDNCF/IL 8 receptors were trapped inside the cells. Since the receptors could not return to the cell surface to uptake further labeled MDNCF/IL 8, this would result in a saturation of binding at 37 °C. In contrast, in untreated cells due to continuous recycling of the intracellular receptors, binding of 125I-MDNCF/IL 8 could not be saturated. Monensin, a carbonyl ionophore, which disrupts intracellular traffic and recycling of many receptors including the epidermal growth factor receptor in isolated rat hepatocytes (17, 23, 24), also inhibited the reappearance/recycling rate of MDNCF/IL 8 receptors. Sodium azide and 2,4-dinitrophenol also partly inhibited MDNCF/IL 8 receptor recycling. The specific role of these agents is not clearly understood.

In summary, the effects of ammonium chloride are as follows: (a) ammonium chloride does not interfere with binding of 125I-MDNCF/IL 8 to neutrophils at 4 °C; (b) it has no effects in receptor-mediated internalization of MDNCF/IL 8 at 37 °C; (c) it can reduce the release of digested fragments of...
internalized 125I-MDNCF/IL 8; (d) it can inhibit the recycling/reappearance of MDNCF/IL 8 receptors in a dose dependent manner; therefore (e) it can decrease the overall binding at 37 °C of 125I-MDNCF/IL 8 on the surface of neutrophils; and finally (f) ammonium chloride can reduce MDNCF/IL 8-induced chemotactic response of neutrophils in Boyden chambers. Preincubation of neutrophils of ammonium chloride at 37 °C presumably affected the above cellular processes (a-f) which are presumably essential for a normal cellular response (cell migration) toward a chemoattractant. This suggests that the presence of abundant MDNCF/IL 8 receptors on the surface of neutrophils might be necessary for the initiation of signal transduction inside the cell to elicit an MDNCF/IL 8 induced chemotactic response (cell migration). Since ammonium chloride partially disrupted the process of receptor recycling inside the neutrophils, it is reasonable to speculate that the reduced migration of neutrophils in the chemotactic chamber is somehow related to the interruption of the normal recycling process of MDNCF/IL 8 receptor. Alternatively it is possible that ammonium chloride might have some other effects on the cells and there is no relationship between these observations. We favor the idea that rapid recycling or reappearance of down-regulated MDNCF/IL 8 receptors is essential for normal cellular chemotaxis.

We also unexpectedly observed some reduction in fMLP-induced neutrophil chemotaxis by ammonium chloride. Since ammonium chloride raised the pH of the intracellular compartment, it might also partially interfere with fMLP-induced migration of neutrophils by reducing the recycling process of the fMLP receptor (25).

MDNCF/IL 8 receptor expression on human neutrophils is dynamically regulated by the ligand itself, and the rapid reexpression of MDNCF/IL 8 receptors on the cell surface potentially provides the opportunity for continuous signaling of the cell by MDNCF/IL 8. Although the rate of internalization of MDNCF/IL 8 ligand-receptor complexes and reappearance of down-regulated MDNCF/IL 8 receptors are extremely rapid compared with many other polypeptide receptors, the eventual fate of internalized MDNCF/IL 8 molecules seems to be similar to other ligand-receptor complexes.

The data shown in this paper suggest the importance of rapid reappearance/recycling of MDNCF/IL 8 receptors in signaling of neutrophils. Blocking of receptor reappearance by lysosomotropic agents may provide a way to inhibit the proinflammatory activity of MDNCF/IL 8 in vivo.

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Interleukin 8 (monocyte-derived neutrophil chemotactic factor) dynamically regulates its own receptor expression on human neutrophils.

A K Samanta, J J Oppenheim and K Matsushima