Single-Cell Analysis of Macrophage Chemotactic Protein-1–Regulated Cytosolic Ca\(^{2+}\) Increase in Human Adherent Monocytes

By Cinzia Bizzarri, Riccardo Bertini, Paola Bossù, Silvano Sozzani, Alberto Mantovani, Jo Van Damme, Aldo Tagliabue, and Diana Boraschi

The increase in intracellular free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) associated with interaction of monocyte chemotactic protein-1 (MCP-1) and related chemokines \(\beta\) with adherent human blood monocytes was investigated at the single-cell level. We used f-MLP as reference chemotactic agent. MCP-1 caused an increase in [Ca\(^{2+}\)]\(_i\) in individual adherent monocytes, with 95\% of cells responding to the chemokine at 20 ng/mL. Response to MCP-1 was already detectable at 1 pg/mL, whereas at least 5 ng/mL were required for significant chemotactic response. The kinetics of the increase in [Ca\(^{2+}\)]\(_i\) were considerably different for MCP-1 compared with f-MLP. MCP-1 produced a slow increase of [Ca\(^{2+}\)]\(_i\), that reached a plateau in 5 to 7 minutes. On the other hand, the increase of [Ca\(^{2+}\)]\(_i\) induced by f-MLP appeared to be biphasic, with a fast phase peaking after 5 to 40 seconds followed by a slower wave. Blocking of Ca\(^{2+}\) channels by thapsigargin, and/or chelation of extracellular free Ca\(^{2+}\), considerably reduced but did not abolish response to MCP-1, had no effect on the first wave of [Ca\(^{2+}\)]\(_i\) induced by f-MLP, and completely abrogated the second, slower wave. Thapsigargin, which empties intracellular plastic Ca\(^{2+}\) stores, inhibited f-MLP–induced [Ca\(^{2+}\)]\(_i\) increase but fully blocked the action of MCP-1 only when combined with Ni\(^{2+}\). Thus, increase of [Ca\(^{2+}\)]\(_i\) induced by MCP-1 is apparently due to independent opening of a channel and mobilization from intracellular stores, whereas f-MLP–induced mobilization of Ca\(^{2+}\) from stores causes subsequent opening of a channel. At variance with MCP-1, the related chemokine MCP-2 induced only a low increase of [Ca\(^{2+}\)]\(_i\) in about 40\% of adherent monocytes. Inhibition of chemokine–induced increase of [Ca\(^{2+}\)]\(_i\), by cholera or pertussis toxin indicated that MCP-1 and MCP-2 activate monocytes through different intracellular pathways. These results demonstrate at the single-cell level that the mechanisms and dynamics of increase of [Ca\(^{2+}\)]\(_i\), are considerably different for f-MLP and chemokines \(\beta\). In addition, the [Ca\(^{2+}\)]\(_i\) increase induced by the two related chemokines MCP-1 and MCP-2 appears to be differently regulated, suggesting interaction with distinct receptors.

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HEMOKINES ARE A superfamily of soluble proteins involved in a wide range of acute and chronic inflammatory processes, as well as in neoplastic and immune-related reactions.\(^1\)\(^2\) Monocyte chemotactic protein-1 (MCP-1), a member of the chemokine \(\beta\) (or C-C chemokine) family, is a 76-amino acid-long polypeptide produced by monocytes and many other cells in response to inflammatory stimuli.\(^3\) Receptors for MCP-1 were found on monocytes but not on resting lymphocytes and neutrophils.\(^4\) MCP-1 is chemotactic for monocytes in vitro and in vivo,\(^5\) \(\beta\) induces the expression of CD11b and CD11c integrins on monocytes,\(^6\) and activates monocyte functions such as cytostatic activity, superoxide anion and lysosomal enzyme release.\(^7\) Recently, two monocyte chemotactic factors, MCP-2 and MCP-3, have been identified that are highly homologous to MCP-1.\(^8\) MCP-2, the product of an interferon \(\gamma\)-inducible gene,\(^9\) differs considerably from MCP-1 and MCP-3 in terms of sensitivity to cholera and pertussis toxin, arachidonic acid mobilization, and capacity to compete for labeled MCP-1.\(^1\)\(^1\) The molecular bases for monocyte activation by MCP-1 and MCP-2 are still unknown. The rise in intracellular free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)), one of the early events that occurs after cell stimulation, plays an important role in phagocyte functions such as locomotion, degranulation, and activation of the oxidative burst.\(^1\)\(^2\)\(^3\) Recent studies have demonstrated an increase of [Ca\(^{2+}\)]\(_i\), in suspensions of human monocytes stimulated with MCP-1 but not with MCP-2.\(^1\)\(^4\)\(^1\)\(^5\) This increase is rapid and transient, and it is dependent on the opening of second messenger-operated channels (SMOC). The cloning of two receptors for chemokines \(\beta\) (one promiscuous receptor that binds MCP-1 and other chemokines \(\beta\), and another one specific for MCP-1) has shown that these receptors belong to the G protein-linked heptahelical receptor family.\(^1\)\(^5\)\(^1\)\(^6\) This family includes heptahelical receptors for neurotransmitters, hormones, and chemotactic molecules that, after activation, bring about increased [Ca\(^{2+}\)]\(_i\), through the opening of a Ca\(^{2+}\) channel and/or by the release of Ca\(^{2+}\) from intracellular stores, mostly dependent on inositol 1,4,5-trisphosphate (IP\(_3\)).\(^1\)\(^7\)\(^1\)\(^8\)

This study was undertaken to characterize the signaling pathway(s) regulating changes in [Ca\(^{2+}\)]\(_i\), by MCP-1 and f-MLP, a prototypic chemotactic peptide that also binds to a heptahelical receptor. This is the first report of single-cell analysis of the interaction of chemokines with target leukocytes. Results obtained indicate that the mechanisms and dynamics of [Ca\(^{2+}\)]\(_i\) increase in human adherent monocytes are considerably different for MCP-1 and f-MLP. Furthermore, activation by MCP-2 appears to follow a transduction pathway different from that of MCP-1, suggesting the possibility of interaction with a different receptor.

MATERIALS AND METHODS

Reagents and chemicals. Recombinant MCP-1 was from Pepro-Tech Inc (Rocky Hill, NJ). Human synthetic MCP-2 was prepared...
Ca\textsuperscript{2+} fluxes regulated by MCP-1

Traces represent levels of three to five cells which spikes in the trace due to exposure to light. The basal [Ca\textsuperscript{2+}] in resting adherent monocytes was 100.6 ± 0.9 mmol/L (on 318 tested cells). Thapsigargin (which empties intracellular Ca\textsuperscript{2+} stores and inhibits re-uptake; 2 μmol/L) induced a rapid and transient increase in [Ca\textsuperscript{2+}], after addition to cells (200% ± 7% of basal; n = 24), due to opening of intracellular stores. MCP-1 or f-MLP was added 2 to 3 minutes later, when [Ca\textsuperscript{2+}], levels had decreased to baseline. Treatment with 3,4,5-trimethoxy benzoic acid, 8-(diethylamino) octyl ester (TMB-8; 500 μmol/L; Sigma) was performed for 30 minutes at 37°C together with FURA-2AM. Cells in Ca\textsuperscript{2+}-free buffer (ie, after addition of 10 mmol/L EGTA) showed, as expected, decreased [Ca\textsuperscript{2+}], to 55 ± 3 mmol/L (n = 12). Cells were pretreated with PTox or CTox (at the optimal dose of 1 μg/mL) for 90 minutes at 37°C and washed before loading with FURA-2AM. Viability of adherent monocytes was always greater than 98%, as assessed by trypan blue dye exclusion after adherence, after loading with FURA-2AM, and at the end of the experiment. No morphologic heterogeneity was ever observed among adherent monocytes on coverslips at any experimental point.

Measurement of cytosolic calcium concentration. Coverslips were then washed three times, mounted on a temperature-controlled holder (Medical System Corp, Greenvile, NY), and covered with 1 mL bathing medium. Chemokines or f-MLP were properly diluted in phosphate-buffered saline (PBS) and added to the bathing medium in a volume of 10 μL. In some experiments, 20 ng of boiled MCP-1 was added to cells to assess possible nonspecific effects. Temperature was maintained at 37°C throughout the experiment. The assembly was positioned on the stage of a Zeiss Axiosvert 135 epifluorescence microscope (Oberkochen, Germany), equipped with fluorescence optics and dichroic mirror (400 nm cut-off wavelength) appropriate for FURA-2 fluorescence. FURA-2 was excited at 350 nm and 380 nm every second by alternating between two xenon light sources, which converge into the microscope through a coaxial fiber optic bundle. FURA-2-emitted fluorescence was filtered between 510 nm and 530 nm by barrier filters and monitored using a CCD camera (CCD72; Dage MTI, Michigan City, IN) and a Georgia Instruments Image Analyzer (Atlanta, GA). Regions of interest cor-

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**Fig 1.** MCP-1 increases [Ca\textsuperscript{2+}] in single adherent monocytes. Traces represent levels of [Ca\textsuperscript{2+}] in single adherent human monocytes in response to MCP-1 alone (20 ng/mL; A) or in the presence of thapsigargin (TG, 2 μmol/L; B), Ni\textsuperscript{2+} (10 mmol/L; trace C), or EGTA (10 mmol/L; D). Traces are representative of 6 to 20 cell recordings (six donors, three to five cells per coverslip). Arrows indicate the addition of agents to the bathing medium, which causes a spike in the trace due to exposure to light.
responding to individual cells were identified in each experiment, and average fluorescence at 350 nm and 380 nm in each region was recorded and stored as individual data files. Pixels corresponding to areas without cells were used for background subtraction at each wavelength. Fluorescence intensities for the two wavelengths were ratioed and converted into [Ca^{2+}], by applying the following formula, proposed by Grynkiewicz et al.\textsuperscript{12}

\[
[\text{Ca}^{2+}] = \frac{R - R_{\text{min}}}{R_{\text{max}} - R}
\]

where R is the measured ratio, Rmin and Rmax are the values of R at minimal and saturating concentrations of [Ca^{2+}] (i.e., in the presence of 10 mmol/L EGTA and of 5 μmol/L ionomycin, respectively), and β is the ratio of emission intensities at 380 nm excitation in these two sets of conditions. The dissociation constant (kd) for FURA-2 + Ca^{2+} was assumed to be 224 nmoVL.\textsuperscript{2} In these experiments, the following were the average values of calibration parameters for monocytes: Rmin = 0.42 ± 0.01 (n = 127); Rmax = 3.59 ± 0.24 (n = 63); and β = 3.71 ± 0.15 (n = 98).

Statistical analysis. Data are reported as percent of the [Ca^{2+}] value at minimal and saturating concentrations of [Ca^{2+}] (ie, in the presence of 10 mmol/L EGTA and of 5 μmol/L ionomycin, respectively), and β is the ratio of emission intensities at 380 nm excitation in these two sets of conditions. The dissociation constant (kd) for FURA-2 + Ca^{2+} was assumed to be 224 nmoVL.\textsuperscript{2} In these experiments, the following were the average values of calibration parameters for monocytes: Rmin = 0.42 ± 0.01 (n = 127); Rmax = 3.59 ± 0.24 (n = 63); and β = 3.71 ± 0.15 (n = 98).

Statistical significance was assessed by one-way analysis of variance (ANOVA), Student’s multiple comparisons t-test, and Dunnett’s multiple range test.

RESULTS

Cytosolic [Ca^{2+}]. MCP-1 and the related chemokine MCP-2 induced migration of human blood monocytes in a dose-dependent manner. Maximal migration was observed at 10 to 20 ng/mL, with at least 5 ng/mL necessary for significant migration (data not shown). The increase of [Ca^{2+}], one of the first events that occurs after interaction of MCP-1 with monocytes, has been studied at the single-cell level in human blood monocytes isolated by adherence. The trace in Fig 1A represents the recording of intracellular free Ca^{2+} in a single adherent monocyte in response to optimal MCP-1 stimulation (20 ng/mL). MCP-1 produced a slow increase of [Ca^{2+}], in 95% of monocytes, which reached a plateau after 5 to 7 minutes and remained elevated for at least 4 to 5 minutes (Fig 1A). A latency time of 2 to 4 minutes between agonist addition and increase in [Ca^{2+}],
Table 1. Effect of PTox and CTox on Chemokine-Induced Increase of [Ca\(^2+\)]\(_i\) in Single Monocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Responsive Cells</th>
<th>Response</th>
<th>Responsive Cells</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>90 (10)</td>
<td>203 ± 8 (9)</td>
<td>40 (35)</td>
<td>157 ± 4 (14)</td>
</tr>
<tr>
<td>CTox</td>
<td>100 (8)</td>
<td>189 ± 3 (8)</td>
<td>[NS]</td>
<td>0 (17)</td>
</tr>
<tr>
<td>PTox</td>
<td>44 (9)</td>
<td>148 ± 5 (4)*</td>
<td>36 (14)</td>
<td>161 ± 12 (5)</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

Role of intracellular Ca\(^{2+}\) stores. The possibility that part of the MCP-1-induced increase in [Ca\(^{2+}\)]\(_i\) could be due to release of ions from intracellular stores has been investigated. The effect of MCP-1 has been measured in the presence of thapsigargin, a sesquiterpene lactone that empties intracellular Ca\(^{2+}\) stores and inhibits re-uptake by blocking the Ca\(^{2+}\)-adenosine triphosphatase (ATPase). As shown in Figs 1B and 4, the increase in [Ca\(^{2+}\)]\(_i\) induced by MCP-1 was only partially inhibited by thapsigargin. However, in monocytes treated with thapsigargin in the presence of Ni\(^{2+}\) (to inhibit Ca\(^{2+}\) channels), the MCP-1-induced increase in intracellular Ca\(^{2+}\) was completely blocked (Fig 4). Ni\(^{2+}\) alone had partial effect (Figs 1C and 4). At variance with MCP-1, thapsigargin could completely inhibit the f-MLP–induced increase in [Ca\(^{2+}\)]\(_i\) (Figs 3B and 4), suggesting that the first, fast [Ca\(^{2+}\)]\(_i\) increase is due to release from intracellular stores and necessary for the subsequent opening of the Ca\(^{2+}\) channel (second, slow phase). Fully superimposable results were obtained with TMB-8, an agent often used to inhibit the release of Ca\(^{2+}\) from intracellular stores.

Sensitivity to PTox and CTox. The role of G proteins in the [Ca\(^{2+}\)]\(_i\) increase induced by MCP-1 has been investigated. It is known that stimulation of monocyte migration by MCP-1 is sensitive to PTox. Table 1 shows that the MCP-1–induced increase in [Ca\(^{2+}\)]\(_i\) was inhibited by PTox, whereas CTox could not significantly affect the response to the chemokine. On the other hand, it has been shown that chemotaxis of monocytes in response to the related chemokine MCP-2 was selectively sensitive to CTox. In agreement with this observation, increased [Ca\(^{2+}\)]\(_i\) in adherent monocytes in response to MCP-2 was not affected by PTox, but it was significantly inhibited by CTox (Table 1).

Discussion

We investigated at the single-cell level the mechanisms of Ca\(^{2+}\) elevation in human monocytes stimulated with MCP-1, a member of the chemokine \(\beta\) family. Single-cell analysis allows a more in-depth and accurate definition of the interaction of ligands with target cells. The results reported in this study demonstrate how the dynamics and mechanisms of Ca\(^{2+}\) fluxes induced by MCP-1 differ from those of the prototypic classical chemoattractant f-MLP. This finding may underlie the different capacity of chemokines \(\beta\), as compared...
with f-MLP, to activate monocyte functions other than chemotaxis.

Data reported here suggest that MCP-1 increases [Ca\(^{2+}\)]\(_{i}\), in human monocytes via a mechanism involving both extracellular Ca\(^{2+}\) influx through a membrane channel and release of Ca\(^{2+}\) from intracellular stores. Increase of [Ca\(^{2+}\)]\(_{i}\) has been already reported in human monocytes in suspension in response to MCP-1 and other chemokines \(\beta\), \(\gamma\), and it was suggested that it could be entirely due to the opening of a channel. By single-cell analysis, however, and in conditions of inhibition of extracellular Ca\(^{2+}\) influx (Ni\(^{2+}\), Cd\(^{2+}\), Ba\(^{2+}\), EGTA), the rise in cytosolic Ca\(^{2+}\) induced by MCP-1 was fully blocked only in a fraction of the observed monocytes (0 to 40%), whereas it was inhibited, but not abolished, in the majority of cells (60% to 100%). Thus, the MCP-1-induced [Ca\(^{2+}\)]\(_{i}\) increase could not be completely accounted for by Ca\(^{2+}\) influx from the extracellular space. Such influx is likely to occur through a second messenger-operated Ca\(^{2+}\) channel (SMOC), which could explain the slow kinetics of [Ca\(^{2+}\)]\(_{i}\) increase in response to MCP-1. The observation that MCP-1-induced [Ca\(^{2+}\)]\(_{i}\) increase was not abolished in the majority of adherent cells in conditions of inhibition of external Ca\(^{2+}\) influx suggests that, in a large fraction of cells, MCP-1 could cause the release of Ca\(^{2+}\) from intracellular stores, in addition to extracellular Ca\(^{2+}\) entrance through a channel. The presence of a double mechanism for [Ca\(^{2+}\)]\(_{i}\) increase was confirmed with the use of thapsigargin (which blocks Ca\(^{2+}\) availability from internal pools). Thapsigargin could inhibit but not abolish the effect of MCP-1 on [Ca\(^{2+}\)]\(_{i}\) increase, suggesting that, indeed, a significant part of the MCP-1-induced increase in [Ca\(^{2+}\)]\(_{i}\), could be attributed to release from intracellular stores. Concomitant block of both membrane channels (with Ni\(^{2+}\)) and depletion of intracellular stores (with thapsigargin) abolished the increase of [Ca\(^{2+}\)]\(_{i}\) in response to MCP-1. Thus, Ca\(^{2+}\) from intracellular stores apparently provides an essential contribution to the total increase of intracellular Ca\(^{2+}\) level induced by MCP-1.

Results obtained by single-cell analysis of intracellular Ca\(^{2+}\) levels indicate heterogeneity of responsiveness to MCP-1 in the adherent monocyte population that could not be accounted for by morphologic heterogeneity of cells or by differences in cell viability. Indeed, monocytes possess at least two distinct receptors for MCP-1, whose distribution on monocyte subpopulations and mechanism of signal transduction are still to be elucidated. In this study, MCP-1 induced a dose-dependent increase in [Ca\(^{2+}\)]\(_{i}\) in single adherent monocytes, with the detection of a significant response with previous reports on the heterogeneity of human monocyte response to chemotactic stimuli, adherent monocytes may represent a subpopulation enriched for responsiveness to chemokine stimulation. However, in preliminary comparative experiments, no difference was evident in the fluorimetric measurement of [Ca\(^{2+}\)]\(_{i}\) increase induced by MCP-1 between monocytes in suspension and adherent cells (data

### Table 2. Role of Ca\(^{2+}\) Channels in MCP-1- and f-MLP-Induced Increase in [Ca\(^{2+}\)]\(_{i}\), in Single Adherent Monocytes

<table>
<thead>
<tr>
<th>Treatment(^{*})</th>
<th>% Responsive Cells</th>
<th>% Responsive Cells</th>
<th>% Responsive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 (27)</td>
<td>93 (41)</td>
<td>88 (41)</td>
</tr>
<tr>
<td>Ni(^{2+})</td>
<td>60 (15)</td>
<td>169 ± 11 (NS)</td>
<td>0 (6)</td>
</tr>
<tr>
<td>Cd(^{2+})</td>
<td>70 (10)</td>
<td>152 ± 6 (NS)</td>
<td>0 (6)</td>
</tr>
<tr>
<td>EGTA</td>
<td>100 (6)</td>
<td>177 ± 19 (NS)</td>
<td>0 (6)</td>
</tr>
</tbody>
</table>

Data are cumulative of eight experiments (eight donors, three to five coverslips). Percentage of responsive cells are calculated on the total number of cells analyzed (shown in parentheses). Monocytes were considered responsive to the agonist when ICa\(^{2+}\), was increased by more than 34% over baseline (normalized to 100%), with this value corresponding to 2 SD over the mean of baseline values. Data are expressed as percent of basal [Ca\(^{2+}\)], and represent the mean of values of responsive cells ± SEM.

\(^{*}\) MCP-1, 20 ng/mL; f-MLP, 100 nmol/L; Ni\(^{2+}\), 10 mmol/L; Cd\(^{2+}\), 500 μmol/L; EGTA, 10 mmol/L.

\(\dagger\) P < .05 v control cells.
not shown). This suggests that selection of adherent monocytes does not account for the observed differences of response to MCP-1 in the single-cell measurement, but that the single-cell analysis allows a more precise and sensitive determination of Ca²⁺ fluxes by detecting even small variations in a limited percentage of cells within the population examined. Many of these data could be lost in the fluorimetric analysis, which only gives the mean response of the whole population.

At variance with MCP-1, the f-MLP-induced [Ca²⁺]ᵢ increase was detectable in the majority of cells as a biphasic trace, consisting of a first, fast phase followed by a slower increase (Fig 2A). From previous data on human leukocytes, the first phase of [Ca²⁺]ᵢ, increase is apparently due to phospholipase C (PLC)-dependent release of Ca²⁺ from IP₃-sensitive intracellular pools, whereas the second phase is dependent on external Ca²⁺, likely due to Ca²⁺ influx through a channel. These data have been confirmed in single-cell recordings, with complete inhibition of the second [Ca²⁺]ᵢ increase in the presence of EGTA or of Ca²⁺ channel blockers. In contrast, the first phase of f-MLP-induced [Ca²⁺]ᵢ increase is not affected by inhibition of external Ca²⁺ influx, suggesting that, indeed, it depends on the release from intracellular pools. Using thapsigargin, an agent that empties the intracellular stores and inhibits re-uptake by blocking the Ca²⁺-ATPase, it was possible not only to confirm the notion that the first-phase influx is, in fact, due to release from stores but, most notably, to indicate that opening of the channel is a consequence of the emptying of intracellular Ca²⁺ pools, in agreement with previous indications on neutrophils. In fact, Ca²⁺ depletion from intracellular stores with thapsigargin could completely inhibit both the first (store-dependent) and the second (channel-dependent) increases in [Ca²⁺]ᵢ.

The effect of MCP-1 on [Ca²⁺]ᵢ in single adherent monocytes has been compared with that of MCP-2. It was previously shown that MCP-2 may activate monocytes through pathways clearly distinct from those used by MCP-1 and MCP-3, despite their high sequence similarity, and that it was unable to elicit [Ca²⁺]ᵢ increase. By single-cell [Ca²⁺]ᵢ recordings, the relative unresponsiveness of monocytes to an optimal chemotactic concentration of MCP-2 was evident, but it was clear that a consistent number of cells (40%) could react to MCP-2 with a low but significant increase of [Ca²⁺]ᵢ. This increase could be inhibited by CTx but was resistant to PTox, in contrast with the MCP-1–induced increase in [Ca²⁺]ᵢ, which was blocked by PTox and relatively unaffected by CTx. This indicates that different G proteins are involved in the mechanisms of signal transduction initiated by MCP-1 and MCP-2, suggesting the possibility that the two chemokines may bind to distinct receptors.

To our knowledge, the results reported here provide the first analysis of the interaction of chemokines with target leukocytes at the single-cell level. Single-cell analysis revealed that Ca²⁺ fluxes elicited by MCP-1 in human monocytes depend on the opening of a Ca²⁺ channel (possibly SMOC) and on the release from intracellular stores. Although the molecular bases of these mechanisms remain conjectural at present, one can speculate that binding of MCP-1 to its receptor would induce a second messenger, responsible for both the opening of a Ca²⁺ channel and, possibly, for activation of PLC. PLC-generated IP₃ would in turn be responsible for the release of Ca²⁺ from cytosolic stores. MCP-2, a member of the chemokine β family highly homologous to MCP-1, activates adherent monocytes through a clearly distinct mechanism, as judged by its relative inability to induce [Ca²⁺]ᵢ increase and by the involvement of different G proteins. This suggests the existence of specific receptors for MCP-2, distinct from those used by MCP-1 and MCP-3, that initiate cell activation through a different signalling pathway. On the other hand, Ca²⁺ elevation induced by chemokines β (MCP-1, MCP-2) differs considerably in terms of dynamics and mechanisms from that induced by the classic prototypic chemoattractant f-MLP, where initial Ca²⁺ mobilization from intracellular stores is apparently responsible for the subsequent opening of a Ca²⁺ channel. The use of a somewhat different transduction pathway by the endogenous chemoattractants, the chemokines, may underlie a division of the labor whereby these molecules, unlike the bacterial attractants mimicked by f-MLP, mediate mainly attraction and recruitment, while full functional activation is regulated by other mediators.

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Single-cell analysis of macrophage chemotactic protein-1-regulated cytosolic Ca2+ increase in human adherent monocytes

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