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OPPOSITE EFFECT OF CYTOCHALASIN B ON AGONIST-INDUCED RESPIRATORY BURST IN NEUTROPHILS AND MONOCYTES

MIGUEL LUCAS* and FRANCISCA SOLANO

Dpto. de Bioquímica, Hospital Universitario V. Macarena, Facultad de Medicina, Avda. Sánchez Pizjuán 4, 41009 Sevilla, Spain

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Abstract—The effects of cytochalasin B on the respiratory burst, calcium transients and cell shape change of neutrophils and monocytes has been studied. Cytochalasin B enhanced fMLP-induced respiratory burst in neutrophils whereas the opposite effect, i.e. an inhibition close to 50%, was elicited in fMLP-stimulated monocytes. The differences did not depend on calcium homeostasis. On the basis of cell shape changes, and the well known effect of cytochalasin B on actin polymerization, the opposite effects of cytochalasin B could stem from differences between both cell types concerning the pattern of cytoskeleton-membrane interaction which could affect the recruitment and assembly of membrane-bound or cytosolic components of NADPH-oxidase.

In neutrophils, onset of the respiratory burst following agonist stimulation relies on signal transduction sequences leading to the activation of NADPH-oxidase, a membrane enzyme system activatable by a number of agonists with a tight control that depends on the recruitment and assembly of soluble cytosolic components within the plasma membrane [1]. The triggering of Ca^{2+}-dependent and Ca^{2+}-independent sequences and a functional interaction of the NADPH-oxidase with the cytoskeleton have been described on the basis that respiratory burst occurs in parallel with oscillations in apparent cell shape which in turn appears to be correlated with periodic fluctuations in the cellular content of filamentous actin [2, 3].

Changes in cell shape, that are associated with a number of cellular processes including polymerization of actin and movements of cytoskeleton, are blocked by cytochalasin B which inhibits the polymerization of actin filaments [4, 5]. The following points are of particular interest since: (1) cytochalasin B enhances the production of reactive oxygen metabolites by fMLP-stimulated neutrophils and, in contrast, inhibits the response to zymosan, a well known phagocytic stimulus [6]; (2) chemotactic peptide induces changes in actin polymerization and neutrophils' shape [7, 8] in a process which does not require a rise in cytoplasmic Ca^{2+} [9–11]; (3) incomplete actin depolymerization may contribute to the deactivation of neutrophils [12] and it has been described that clamping actin in polymerized form inhibits oxidase activation [13]; (4) the cytosolic components of the NADPH-oxidase of neutrophils and monocytes are different [14]. Therefore, cytochalasins are suitable tools to study the possible interaction between cytoskeleton and membrane receptors in signal transduction mechanisms. Bearing in mind these considerations, we decided to study the effect of cytochalasin B on early events triggered by activation in neutrophils and monocytes. We present here results comprising the effect of cytochalasin B on cell shape, cytosolic calcium transients and reactive oxygen production in granulocyte and monocyte. The comparative analysis of the responses in both cell types indicates that the different pattern of NADPH-oxidase activation do not rely on $[Ca^{2+}]_i$, rather, it points to an involvement of the interaction of cytoskeleton and plasma membrane.

MATERIALS AND METHODS

Preparation of cells. Human neutrophils were prepared from freshly venesected blood by dextran sedimentation, Ficoll-hypaque separation and hypotonic lysis of remaining erythrocytes [15]. Cells were resuspended in Hepes-buffered salt solution (HBSS) consisting of: 25 mmol/L Hepes, 120 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgSO_4$, 1.3 mmol/L $CaCl_2$, 15 mmol/L glucose, pH 7.4. Cells, were stored on ice and assayed after 30 min and within 2 hr. Cell viability, checked with either 0.25% Trypan blue or 50 μmol/L ethidium bromide, was over 95%. Human monocytes were prepared from leucocyte-rich plasma obtained by dextran sedimentation of freshly venesected blood, according to the method described by the manufacturer of Nycodenz Solution. In brief, leucocyte-rich plasma, 6–7 mL, was layered over 3 mL Nycodenz Monocytes solution. The tube was centrifuged for 15 min at approximately 500 *g*. The band of monocyte at the interphase was collected and washed in Hank's balanced salt solution (HBSS).

Measurement of NADPH-oxidase activity. Oxygen radical production was monitored by measuring luminol-dependent chemiluminescence by means of a Berthold LB 9500 C luminometer, attached to a chart-recorder, conveniently modified to enable the injection of reagents via a microsyringe through a light-tight septum. All experiments were carried out

* To whom correspondence should be addressed.

at 37°. Samples for neutrophil chemiluminescence determination were prepared by adding aliquots of the neutrophil suspension to HBSS supplemented with 10 μM luminol, 4 units/mL horseradish-peroxidase and 100 μM sodium azide. Azide, which inhibits myeloperoxidase, and peroxidase, which provides the catalytic component for luminol oxidation, were used to analyse the chemiluminescence independently of myeloperoxidase release. Further details are described in the legends to figures.

Measurement of $[Ca^{2+}]_i$. Fluo-3, the calcium-sensitive fluorescein-derived chromophore [16], was used to determine $[Ca^{2+}]_i$ by flow cytometry which allows the analysis of simultaneous changes in cell shape by determination of forward-angle and side-angle light scattering of cells. Cells, 1–5 × 10^6 cells/mL HBSS, were loaded for 40 min with 4 μM fluo-3, from a stock of 2 mM in dimethyl-sulphoxide. After washing out the medium, following centrifugation at 500 *g*, the cells were resuspended in HBSS. Aliquots of the cell suspension were diluted in HBSS just before starting the experiment. Acquisition and analysis of the cells were made in a FACScan flow cytometer (Becton Dickinson, San Jose, CA, U.S.A.). Excitation was from an argon laser at 488 nm. Emission at 525 nm was measured on a linear scale. In some experiments, the fluorescence units were converted into $[Ca^{2+}]_i$ as described in [17]. The calibration procedure includes the obtention of F_{max} and F_{min}, which were achieved with 4 μM Br-A23187 and 2 mM $MnCl_2$ respectively. Intracellular free calcium, $[Ca^{2+}]_i$ was calculated by the equation:

$$[Ca^{2+}]_i = K_d\,(F - F_{min})/(F_{max} - F)$$

where K_d, the dissociation constant for Ca^{2+}-bound fluo-3 is 400 nM and F represents the experimental fluorescence.

The calibration of $[Ca^{2+}]_i$ was performed only in representative experiments. The results obtained by fluo-3 and flow cytometry agree with those calculated [18] from batch analysis of neutrophils loaded with Fura-2 (not shown).

Cytometry. Flow cytometry measurements were performed on a FACScan (Becton Dickinson). Forward and orthogonal light scattering, FITC, fluorescein, and PE, phycoerythrin, fluorescence signals were collected in list mode. Acquisition and analysis were performed with the FACScan and Chronys Research software. Additional details are described in legends to the figures.

Chemicals. Cytochalasin B, the chemotactic peptide formyl-Met-Leu-Phe (fMLP), 4*b*-phorbol-12*b*-myristate-13*a*-acetate (PMA) and, luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Fluorescein- and phycoerythrin-labelled monoclonal antibodies were from Becton Dickinson. Nycodenz Monocytes solution was from Nycomed AS (Oslo).

RESULTS

Changes in $[Ca^{2+}]_i$ *and cell shape in neutrophils*

The time course of changes in $[Ca^{2+}]_i$ and cell shape in neutrophils and monocytes was studied by flow cytometry. The chemoattractant peptide fMLP produced a rapid increase of $[Ca^{2+}]_i$, up to 760 nM, and a simultaneous decrease of forward light scattering (see Fig. 1) and orthogonal light scattering (see Fig. 2), with a half time close to 5 ± 0.03 sec. Afterwards, $[Ca^{2+}]_i$ declined back to basal values and the recovery of cell volume was initiated. The non-fluorescent Ca^{2+}-ionophore bromo-A23187 produced an additional increasing of $[Ca^{2+}]_i$ and blocked the recovering of cell volume, i.e. forward light scattering (see Fig. 1).

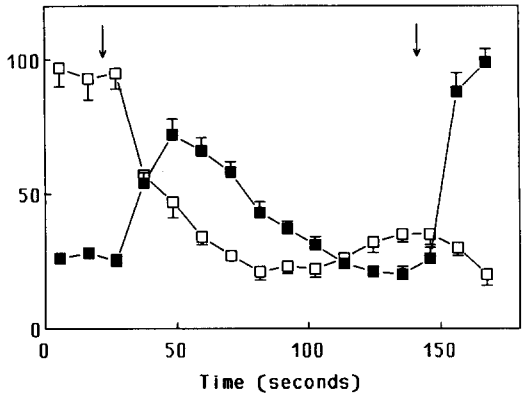

Fig. 1. Kinetics of $[Ca^{2+}]_i$ and cellular shape changes in neutrophils upon fMLP stimulation. Human neutrophils were loaded with fluo-3/AM as indicated in Materials and Methods. Experiments were performed by acquisition, about 10,000 events over a 3 min period, and analysis of neutrophils with the Chronys software of a Facscan flow cytometer. Arrows indicate the addition of 1 μM fMLP and 4 μM Br-A23187 at the 2nd and 13th interval respectively; one interval refers to 1/16 of the time scale. Cellular volume (unfilled squares) and $[Ca^{2+}]_i$ (filled squares) were analysed in seven separate experiments and refer to forward light scattering and fluo-3 fluorescence respectively. Each point represents the mean and SEM of the per cent of maximal change of fluorescence obtained in the channel scale. Maximal change refers to the change of the mean value of the channel scale in the Chronys software obtained by addition of 4 μM Br-A23187 to the cell suspension. The observed change was 191 ± 12. In practice, a value of 200 was used to calculate the data. The 100% value for cell refers to forward scattering of cells at resting conditions.

Cytochalasin B produced a sustained raise of $[Ca^{2+}]_i$, close to 700 ± 82 nM, slowly decreased the cell volume (Fig. 3) and, by contrast to fMLP, induced a slight but significant increasing of orthogonal light scattering (Fig. 2). Thereafter, fMLP produced a slow but significant decreasing of $[Ca^{2+}]_i$ (Fig. 3). Under these conditions the addition of bromo-A23187 (not shown) further raised $[Ca^{2+}]_i$. A striking result obtained in these experiments was the high degree of inhibition produced by cytochalasin B on fMLP-induced decreasing of orthogonal light scattering (see Fig. 2).

Changes in $[Ca^{2+}]_i$ *and cell shape in monocytes*

The stimulation with fMLP produced in monocytes an increasing of $[Ca^{2+}]_i$ which peaked after 20 sec

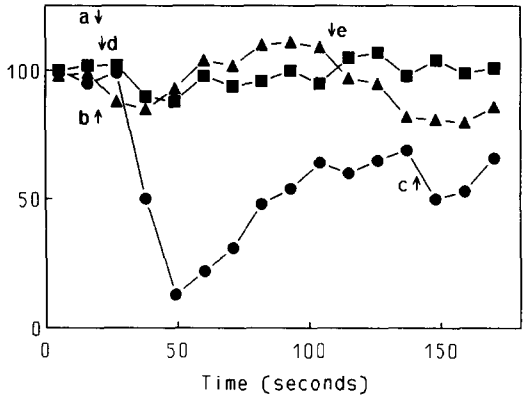

Fig. 2. Effect of cytochalasin B and fMLP on orthogonal light scattering in human neutrophils. Human neutrophils, loaded with fluo-3/AM, were acquired and analysed with the Chronys research software (Becton Dickinson). Symbols refer to the mean values obtained from data of orthogonal light scattering from experiments shown in Fig. 1 (circles) and 3 (triangles). Squares refer to a single separate experiment. For comparative purpose with the changes in forward scattering, a value of 200 (see legend to Fig. 1) in the channel scale, was assigned for a change of 100%. Arrows refer to the addition of: (i) control (squares), 0.1% dimethyl-sulfoxide, a; (ii) experiment 1 (circles), 1 μM fMLP and 4 μM Br-A23187, b and c, respectively; (iii) experiment 3 (triangles), 5 μg/mL cytochalasin B and 1 μM fMLP, d and e, respectively. The number of separate experiments and additional details are described in the legend to Figs 1 and 3. SEM, was inferior to 12% of the mean.

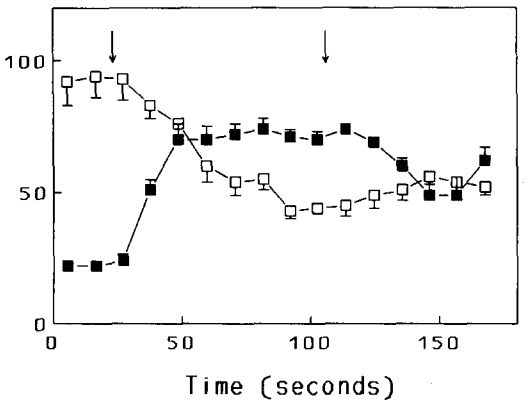

Fig. 3. Effect of cytochalasin B on the kinetics of $[Ca^{2+}]_i$ and cellular shape changes in human neutrophils. Human neutrophils, loaded with fluo-3/AM, were analysed as described in the legend to Fig. 1. Arrows indicate the addition of 5 μg/mL cytochalasin B and 1 μM fMLP and at 2nd and 10th interval, respectively. Cellular volume (unfilled squares) and $[Ca^{2+}]_i$ (filled squares) were analysed in five separate experiments and refer to forward light scattering and fluo-3 fluorescence respectively. The addition of 4 μM Br-A23187 to the cell suspension gave a response similar to Fig. 1 (not shown).

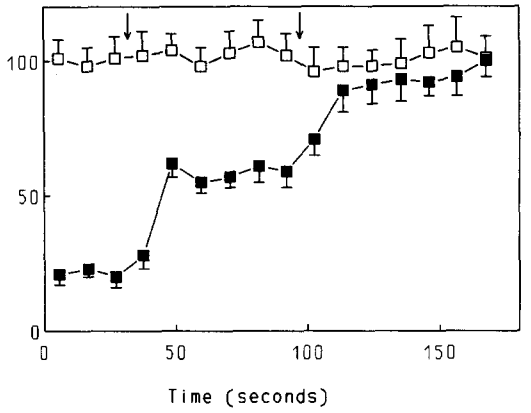

Fig. 4. Kinetics of $[Ca^{2+}]_i$ and cellular shape changes after stimulation of human monocytes with fMLP. Human monocytes were obtained as described in Materials and Methods. Events were acquired, 10,000 in 3 min, and analysed with the Chronys software. Monocytes were gated by forward and side scattering leading to a selection of nearly 75% of the total cell population and almost 98% were CD14+ as judged by the binding of Anti-Leu-M3. Arrows indicate the addition of 1 μM fMLP and 4 μM Br-A23187 at 3rd and 9th interval respectively. Each point is the mean and SEM of five separate experiments. Further details are described in the legend to Fig. 1.

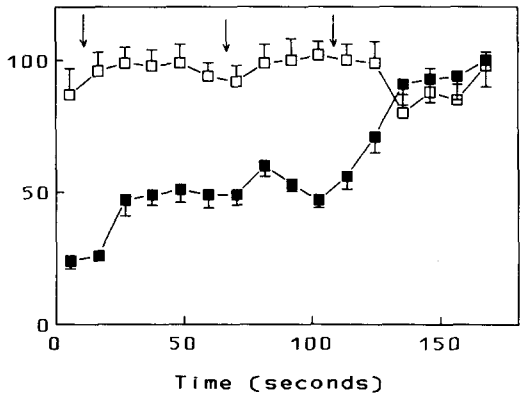

Fig. 5. Effect of cytochalasin B on the kinetics of $[Ca^{2+}]_i$ and cellular shape changes in human monocytes. Human monocytes loaded with fluo-3/AM were acquired and analysed as described in the legend to Fig. 1. Arrows indicate the addition of 5 μg/mL cytochalasin B, 1 μM fMLP and 4 μM Br-A23187 at 1st, 6th and 10th interval, respectively. Cellular volume (unfilled squares) and $[Ca^{2+}]_i$ (filled squares) were analysed in four separate experiments and refer to forward scattering and fluo-3 fluorescence, respectively. Further details are described in the legend to Fig. 1.

and was maintained for at least 2 min. Intracellular free calcium was further increased following the addition of calcium ionophore bromo-A23187. By contrast to neutrophils, these stimuli failed to modify the shape of monocytes (Fig. 4). Cytochalasin B also raised $[Ca^{2+}]_i$ in human monocytes and the subsequent addition of fMLP produced a slight but significant increasing of $[Ca^{2+}]_i$. A23187 did strongly increase cytosolic free calcium and appeared to produce a small but significant decrease in cell volume (Fig. 5).

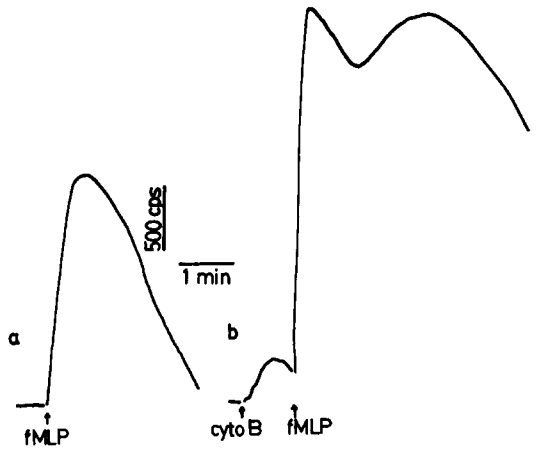

Fig. 6. Stimulation by cytocholasin B of fMLP-induced ROM production in neutrophils. ROM production was determined by luminol-enhanced chemiluminescence (see Methods). The response of 5×10^3 neutrophils to 1 μM fMLP was analysed in the absence (trace a) and in the presence of 5 μg/mL cytochalasin B (trace b). Bars indicate chemiluminescence rate in cps and time in min. Data is from one experiment which is representative of at least six others.

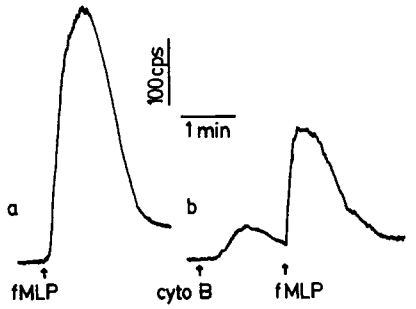

Fig. 7. Inhibition by cytochalasin B of fMLP-induced ROM production in monocytes. Conditions were as described in Materials and Methods and Fig. 6. Note the lower value of the bar of the chemiluminescence rate as compared to Fig. 6. Similar results were obtained in at least six other monocyte preparations.

Reactive oxygen metabolites production

The effect of the above indicated stimuli on ROM production by neutrophils was characterized by a very rapid response to fMLP which after about 1 min declined back to resting values. Cytochalasin B slightly stimulated ROM production in neutrophils but strongly enhanced the effect of fMLP leading to a biphasic pattern which was not blocked by 100 μM sodium azide (Fig. 6). The response of monocytes to fMLP was similar to that of neutrophils although to a lower degree on the basis of cell number. The most significant feature was that, by contrast to neutrophils, cytochalasin B inhibited by nearly 50% fMLP-induced ROM production in monocytes and failed to induce a biphasic response (Fig. 7).

DISCUSSION

The present studies were performed to compare the initial steps in the activation response of human neutrophils and monocytes by a number of experiments capable of assessing individual cell responses. This is of particular interest in cases such as monocyte preparations where contaminating cell subpopulations can be excluded during the aquisition and analysis steps of flow cytometry (see Fig. 8). The time course of shape change in resting and stimulated cells, and cytosolic calcium transients were measured by cell forward light scatter, orthogonal light scatter and fluo-3 fluorescence. In addition, the production of reactive oxygen metabolites by both cell types was compared taking in mind the effect of cytochalasin B. The more salient points can be discussed as follows:

Calcium transients

fMLP Increased $[Ca^{2+}]_i$ in both neutrophils and monocytes, but the rate of recovery of resting $[Ca^{2+}]_i$ was significantly lower in monocytes. It could be ascribed to differences in either calcium-pumping ATPase of plasma membrane, which fits quite well with the pattern of calcium efflux in human neutrophils [19], or calcium transport into internal store sites. Cytochalasin B produced very similar calcium transients in both neutrophils and monocytes but cytochalasin B-stimulated neutrophils failed to recover resting $[Ca^{2+}]_i$, in contrast to the pattern of fMLP-induced calcium transients in neutrophils. These results indicated that cytochalasin B blocked some component of the calcium-pumping ATPase in plasma membrane of neutrophils or hindered calcium transport through intracellular membranes.

Shape changes

The more salient features of the coupled cell shape changes and cellular activation were the striking differences between neutrophils and monocytes. In fact, the shape of monocytes was almost unchanged whereas cell volume decreased in fMLP- and cytochalasin B-treated neutrophils. The decrease in cell volume in the immediate response of neutrophils to fMLP described in the present work contrasts with a recent report indicating an increasing in volume of neutrophils, but the differences can be explained since, in the latter [20], cell volume was discontinuously analysed after 5 min of neutrophils stimulation. The results in the present work agree quite well the described stimulation by fMLP of volume contraction in rabbit neutrophils [21].

fMLP decreased orthogonal light scattering and this could be expected from granule secretion, however, this may not be the case in the present experiments since cytochalasin B, which is a potent agonist for the chemotactic stimulus [6, 15, 21], inhibited significantly the decrease of orthogonal light scattering induced by fMLP. The effect of fMLP alone on orthogonal light scattering can be explained on the basis of actin polymerization, in agreement with previous reports [7–12]. In addition, the inhibition produced by cytochalasin B on fMLP-induced changes of orthogonal light scattering could be explained by the inhibitory effect of cytochalasin

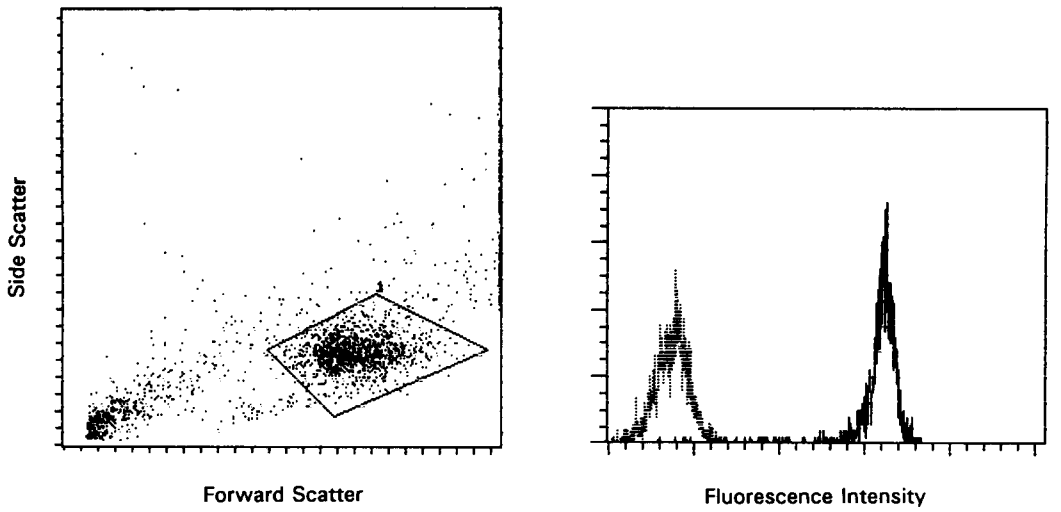

Fig. 8. Flow cytometer analysis of monocytes. Monocytes were prepared as described in Materials and Methods. Cells were acquired and analysed with the Facscan software. The left hand side figure shows a dot-plot display of forward (volume) and side (granularity) light scatterning and the gate to select monocytes for subsequent analysis. The figure on the right hand side is a profile of fluorescence intensity of the gated cells which had been previously incubated with: (a) non-specific fluorescein-labeled IgG control antibodies, shaded trace; (b) Fluorescein-labeled Anti-Leu-M3 (CD14) antibody, bold trace.

B on actin polymerization [4, 5] which in turn can explain that cytochalasin B alone did not decrease orthogonal light scattering. The slow decrease of orthogonal light scattering observed in Fig. 2, i.e. when fMLP was added to cytochalasin B treated neutrophils, could be due to granule secretion. In other words, the described changes in orthogonal light scattering should include two major components: actin polymerization and granule secretion. The lack of effect of the agonist on the shape of monocyte could be expected from a non-granular chemotactic cell when analysed under conditions of no-adherence [22].

Reactive oxygen metabolites

The maximal rate of ROM production in monocytes was almost 4-fold lower than in neutrophils and can be explained, even in a cell-free system on the basis of cell count, NADPH-oxidase activity is significantly lower in monocytes than in neutrophils [14]. These differences could be ascribed to cytosolic components [14] of the NADPH-oxidase which have even been described to be involved in the defective response of neutrophils from patients with autosomal chronic granulomatosis disease [23].

The potentiation by cytochalasin B of fMLP-triggered NADPH-oxidase activity could be explained by incorporation in the plasma membrane of granule-bound cytochrome b_{558} [24]. It should be pointed out that azide was present in our assay conditions, indicating that the biphasic response can not be explained by a cytochalasin B-elicited increase of myeloperoxidase activity [15]. The opposite effect of cytochalasin B on ROM production in monocytes and neutrophils is not easy to explain. The fact that cytochalasin B increased $[Ca^{2+}]_i$ in both neutrophils and monocytes (see Figs 3 and 5) implies that the opposite effect of cytochalasin B relies on a process beyond calcium signal or independent of $[Ca^{2+}]_i$. Taking into account that: (1) actin polymerization takes place in neutrophils by agonist-induced calcium-independent mechanisms [8–11] and (2) cytochalasin B blocks actin in the depolymerized form, the most likely explanation could be an effect of cytochalasin B on the interaction of cytoskeleton and membrane-bound component of the NADPH-oxidase. In other words, cytochalasin B, by blocking actin in depolymerized form, stabilizes a more active form in neutrophils and a less active form in monocytes, depending on whether it promotes or hinders the recruitment and stacking of some cytosolic component to the membrane-bound NADPH-oxidase components.

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REFERENCES

1. Babior BM, The respiratory burst oxidase. *Trends Biochem Sci* **12**: 241–243, 1987.
2. Dewald B, Thelen M and Baggiolini M, Two transduction sequences are necessary for neutrophil activation by receptor agonist. *J Biol Chem* **263**: 16179–16184, 1988.
3. Bagglioni M and Wymann MP, Turning on the respiratory burst. *Trends Biochem Sci* **15**: 69–72, 1990.
4. Fletcher SM and Pollard TD, Mechanism of action of cytochalasin B on actin. *Cell* **20**: 329–341, 1980.
5. Brenner SL and Korn ED, The effects of cytochalasins on actin polymerization and actin ATPase provide insights into the mechanisms of polymerization. *J Biol Chem* **255**: 841–844, 1990.

6. Jenner DE, Holt ME and Campbell AK, Luminol dependent chemiluminescence and thiol group oxidation provoked by neutrophils is attributable to different oxidizing species. *J Biolum Chemilum* **1**: 165–171, 1987.
7. Howard TH and Oresajo CO, The kinetics of chemotactic peptides induced changes in F-actin content, F-actin distribution, and the shape of neutrophils. *J Cell Biol* **101**: 1078–1085, 1985.
8. Wang D, Berry K and Howard TH, Kinetic analysis of chemotactic peptide-induced actin polymerization in neutrophils. *Cell Motil* **16**: 80–87, 1990.
9. Sha'afi RI, Shefcyk J, Yassin R, Molski TFP, Volpi M, Naccache PH, White JR, Feinstein MB and Becker EL, Is a rise in intracellular concentration of free calcium necessary or sufficient for stimulated cytoskeletal-associated actin? *J Cell Biol* **102**: 1459–1463, 1986.
10. Pérez HD, Elfman F, Marder S, Lobo E and Ives HE, Formyl peptide-induced chemotaxis of human polymorphonuclear leukocytes does not require either marked changes in cytosolic calcium or specific granule discharge. *J Clin Invest* **83**: 1963–1970, 1970.
11. Almohanna FA and Hallet MB, Actin polimerization in neutrophils is triggered without a requirement for a rise in cytoplasmic Ca^{2+}. *Biochem J* **266**: 669–674, 1990.
12. Sklar LA, Omann GM and Painter RG, Relationship of actin polymerization and depolymerization to light scattering in human neutrophils: Dependence on receptor occupancy and intracellular Ca^{2+}. *J Cell Biol* **101**: 1161–1166, 1985.
13. Almohanna FA and Hallet MB, Clamping actin in polymerized form in electropermebilized neutrophils inhibits oxidase activation. *Biochem Biophys Res Commun* **169**: 1222–1228, 1990.
14. Thelen M, Baggliolini M, Reconstruction of cell-free NADPH-oxidase from human monocytes and comparison with neutrophils. *Blood* **75**: 2223–2228, 1990.
15. Lucas M and Solano F, Determination of the myeloperoxidase-hydrogen peroxide-halide system activity following the respiratory burst of human neutrophils. *Biomed Biochim Acta* **49**: 45–50, 1990.
16. Minta A, Kao JPY and Tsien RY, Fluorescent indicators for cytosolic calcium based on Rhodamine and Flourescein chromophores. *J Biol Chem* **264**: 8171–8178, 1989.
17. Vandenberghe PA and Ceuppens JL, Flow cytometric measurement of cytoplasmic free calcium in human peripheral blood T lymphocytes with fluo-3, a new fluorescent calcium indicator. *J Immunol Methods* **127**: 197–202, 1990.
18. Grynkiewicz G, Poenie M and Tsien RY, A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* **260**: 3440–3450, 1985.
19. Foder B, Scharff O and Thastrup O, Ca^{2+} transients and Mn^{2+} entry in human neutrophils induced by thapsigargin. *Cell Calcium* **10**: 477–490, 1989.
20. Fletcher MP, Stahl GL and Longhurst JC, *In vivo* and *in vitro* assessment of porcine neutrophil activation responses to chemoattractants: flow cytometric evidences for the selective absence of formyl peptide receptors. *J Leuk Biol* **47**: 355–365, 1990.
21. Koza EP, Wright TE and Becker EL, Lysosomal enzyme secretion and volume contraction induced in neutrophils by cytochalasin B, chemotactic factor and A23187. *Proc Soc Exp Biol* **149**: 476–479, 1975.
22. Marks PW and Maxfield FR, Transient increases in cytosolic free calcium appear to be required for the migration of adherent human neutrophils. *J Cell Biol* **110**: 43–52, 1990.
23. Curnutte JT, Scott PJ and Babior BM, Functional defect in neutrophil cytosols from two patients with autosomal recessive cytochrome-positive chronic granulomatous disease. *J Clin Invest* **83**: 1236–1240, 1989.
24. Borregaard N, Heiple JM, Simons ER and Clark RA. Subcellular localization of the *b*-cytochrome component of the human neutrophil microbicidal oxidase. Translocation during activation. *J Cell Biol* **95**: 52–61, 1983.