A membrane-permeant ester of phosphatidylinositol 3,4,5-trisphosphate (PIP_3) is an activator of human neutrophil migration

Verena Niggli*

Department of Pathology, University of Bern, Murtenstr. 31, P.O. Box 62, CH-3010 Bern, Switzerland

Received 27 January 2000; received in revised form 3 April 2000

Edited by Masayuki Miyasaka

Abstract Activity of phosphatidylinositol (PI) 3-kinase is required for optimal migration of human neutrophils [Niggli and Keller (1999) Eur. J. Pharmacol. 335, 43–52]. We have tested the direct effect of a product of PI 3-kinase, phosphatidylinositol 3,4,5-trisphosphate (PIP_3), on neutrophil migration. To this end, a membrane-permeant ester of PIP_3, dilauroyl phosphatidylinositol 3,4,5-trisphosphate-heptakis-(acetooxymethyl)ester (PIP_3/AM) was used. PIP_3/AM (ED_{50}: 10–17 μM) induced development of polarity and accumulation of F-actin in the leading lamellae in up to 70% of the cells. These cells exhibited stimulated random migration, comparable to that observed in uniform concentrations of chemotactic peptide. Evidence is provided for a role of Rho-kinase and for activation of PI 3-kinase in a positive feedback loop in PIP_3/AM-induced motility.

Key words: Neutrophil; Cell migration; Phosphatidylinositol 3,4,5-trisphosphate; Rho-activated kinase; Phosphatidylinositol 3-kinase

1. Introduction

Directed migration is an indispensable feature of neutrophil granulocytes in fullfilling their physiological role in host defense against pathogens. However, the molecular mechanisms of directed migration are as yet poorly understood. Upon activation with chemotactic stimuli, neutrophils rapidly extend protrusions in all directions, concomitant with a marked increase in total F-actin and cytoskeletal actin [1,2]. After a few minutes of incubation with the stimulus, the cells assume a polarized morphology with a broad front lamella and a contracted tail [3,4]. The newly formed F-actin accumulates in the front lamellae of polarized neutrophils [5]. How localized actin polymerization, development of cell polarity and locomotion are achieved and interconnected, is unclear, although a complex network of chemoattractant-induced signalling pathways has been identified in neutrophils [6]. We have recently provided evidence for a role of the enzyme phosphatidylinositol (PI) 3-kinase in chemotactic peptide-induced development of polarity and migration [7]. Similarly, Knall et al. [8] have shown that PI 3-kinase activity is also required for Il-8-induced neutrophil migration.

Activation of PI 3-kinase in human neutrophils via occupancy of receptors of formylated peptides results in increased formation of phosphatidylinositol 3,4,5-trisphosphate (PIP_3) and phosphatidylinositol 3,4-diphosphate ($PI(3,4)P_2$) [9,10]. The downstream targets of these lipids involved in neutrophil migration have not yet been identified. As shown recently, PI 3-kinase not only produces PIP_3 and $PI(3,4)P_2$ but could also act by phosphorylating proteins [11].

We now wanted to know whether one of the products of PI 3-kinase, PIP_3, is sufficient to induce neutrophil motility. We have therefore investigated the effect of directly introducing PIP_3 into the neutrophils. This was achieved by using a recently synthesized membrane-permeant ester of PIP_3: dilauroyl phosphatidylinositol 3,4,5-trisphosphate-heptakis-(acetooxymethyl)ester (PIP_3/AM) [12]. This compound crosses the plasma membrane by passive diffusion and is hydrolyzed by intracellular esterases, generating PIP_3 inside the cells. I show here that introduction of a membrane-permeable ester of PIP_3 into the cells is sufficient to induce development of polarity, polarized accumulation of F-actin and migration in human neutrophils.

2. Materials and methods

2.1. Reagents and suppliers

Reagents and suppliers were: *N*-formyl-L-norleucyl-L-leucyl-L-phenylalanyl-L-norleucyl-L-tyrosyl-L-lysine (fNLPNTL), Bachem, Bubendorf, Switzerland; human serum albumin (HSA), Behringwerke, Marburg, Germany; LY294002, Alexis Corporation, Läufelfingen, Switzerland; wortmannin, Sigma, St Louis, MO, USA.

PIP_3/AM was a kind gift from Dr. R.Y. Tsien (Howard Hughes Medical Institute, University of California, San Diego, CA, USA). PIP_3/AM was prepared as a stock solution of 25 mM in DMSO and stored at −20°C. Addition of PIP_3/AM to the cells resulted in a final concentration of maximally 0.4% DMSO. DMSO alone, up to 0.4%, had no effects in control experiments. Y-27632 was kindly provided by Yoshitomi Pharmaceutical Industries, Ltd., Japan. Stock solutions of Y-27632 (10 mM) were prepared in H_2O and aliquots were stored at −20°C.

2.2. Isolation of human neutrophils

Neutrophils were isolated from heparinized human blood (10 units/ml) as detailed previously [13]. Neutrophils were resuspended in Gey's medium containing 138 mM NaCl, 6 mM KCl, 1 mM Na_2HPO_4, 5 mM $NaHCO_3$, 5.5 mM glucose, 20 mM HEPES, pH 7.3, without or with divalent cations and HSA, depending on the assays (see Section 3). Especially for the experiments with wortmannin, in order to minimize variability of findings possibly due to donor variability, neutrophils prepared from the blood of at least three donors were combined.

2.3. Analysis of effects of PIP_3/AM and of PIP_3 on neutrophil shape

Neutrophils (3×10^6 cells/ml) were incubated in medium in a recip-

*Fax: (41)-31-318 34 12.
E-mail: niggli@patho.unibe.ch

Abbreviations: fNLPNTL, *N*-formyl-L-norleucyl-L-leucyl-L-phenylalanyl-L-norleucyl-L-tyrosyl-L-lysine; ERK, extracellular signal-regulated kinase; HSA, human serum albumin; MAPK, mitogen-activated protein kinase; PI 3-kinase, phosphatidylinositol 3-kinase; $PI(3,4)P_2$, phosphatidylinositol 3,4-diphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PIP_3/AM, dilauroyl phosphatidylinositol 3,4,5-trisphosphate-heptakis-(acetooxymethyl)ester

PII: S0014-5793(00)01534-9

rocating waterbath at 37°C without or with inhibitors and DMSO or PIP_3/AM as indicated in Section 3. The reaction was stopped by fixing the cells in 1% glutaraldehyde (final concentration) for 30 min. Cell shape was determined as described [14].

As a control, neutrophils (3×10^6 cells/ml) were also incubated for 45 min at 37°C in medium lacking divalent cations and HSA with 5 or 10 μM PIP_3, obtained from Matreya Inc. (Pleasant Gap, PA, USA), followed by analysis of morphology. A PIP_3 stock solution (0.4 mM) was prepared by dissolving the lipid in a buffer containing 20 mM HEPES, pH 7.4. This stock solution was sonicated for 2 min in a bath sonicator.

2.4. Locomotion

For locomotion assays, a discontinuous Ficoll-metrizoate gradient instead of NIM was used for removal of mononuclear cells [7]. Neutrophils (6×10^6/ml) were incubated in Gey's medium without divalent cations, containing 1% HSA, with 0.4% DMSO or 25 μM PIP_3/AM for 45 min at 37°C. The cells were centrifuged at $300\times g$ for 5 min and resuspended in Gey's medium containing 2.5% HSA, 1.1 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1 mM EGTA. Subsequently, the percentage of migrating cells and the mean speed of migrating cells was determined as described previously [7].

2.5. Localization of F-actin

The intracellular localization of F-actin was determined by NBD-phallacidin-staining of paraformaldehyde-fixed cells as described [15], with modifications [16]. Neutrophils were incubated without or with PIP_3/AM as described in Section 3. The cells were then fixed in 4% paraformaldehyde and stained with NBD-phallacidin [16].

2.6. Analysis of phosphorylation of p42/44 mitogen-activated protein kinase (MAPK) (extracellular signal-regulated kinase, ERK) in neutrophils

Neutrophils (10^7/ml) were exposed to diisopropylfluorophosphate as described [7] and resuspended in Gey's medium lacking divalent cations and HSA. Aliquots (0.45 ml) were exposed to stimuli and inhibitors as described in Section 3. The reaction was stopped by centrifugation. The pelleted cells (2.5×10^6 cells per lane) were solubilized in a buffer containing 1% SDS, 50 mM DTT, 15% (v/v) glycerol, 62.5 mM Tris, pH 6.8, 0.001% bromophenol blue, followed by separation of proteins on a 5–10% SDS–polyacrylamide gradient gel and immunoblotting using a polyclonal antibody specific for p42/44 MAPK phosphorylated on threonine and tyrosine, diluted 1:1000 (New England Biolabs, Beverly, MA, USA).

2.7. Statistical analysis of data

Differences between data were analyzed with the Student's *t*-test for paired data, with a P value of <0.05 considered significant. Data correspond to the mean ± S.E.M. of n independent experiments.

3. Results

3.1. PIP_3/AM induces development of polarity and migration in human neutrophils

We studied the effect of incubating human neutrophils for 45 min at 37°C with PIP_3/AM on the shape of these cells. As shown in Fig. 1, this treatment caused a dose-dependent marked increase in the percentage of polarized cells. The majority (80–98%) of untreated cells were spherical with a smooth surface, 0–11% showing spontaneous polarization (Fig. 2A). This morphology was markedly changed by incubation with PIP_3/AM, resulting in 30–70% of polarized cells with contracted tail and anterior lamellae (Fig. 2C), comparable to the morphology of cells incubated with chemotactic peptide (Fig. 2B; [7]). This response was maximal at 25–50 μM of PIP_3/AM, and half-maximal effects were obtained at 10–17 μM (Fig. 1). For comparison, incubation of neutrophils with 1 nM fNLPNTL for 30 min results in induction of polarity in 80–90% of the cells [7]. The effects of PIP_3/AM were obtained in buffers without $CaCl_2$ and $MgCl_2$. In Gey's medium containing 1.1 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1 mM EGTA, 25 μM PIP_3/AM induced polarity in only 11–16% of the cells. Divalent cations are known to promote fusion of PIP_2 micelles into insoluble aggregates [17], and probably similarly affect PIP_3/AM, preventing optimal activation of cells.

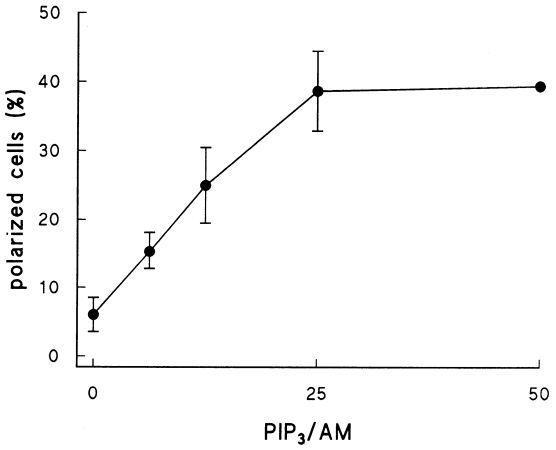

Fig. 1. Concentration-dependent effect of PIP_3/AM on the shape of human neutrophils. Neutrophils (3×10^6 cells/ml) were incubated for 45 min at 37°C in Gey's medium lacking divalent cations and HSA in the absence or presence of increasing concentrations of PIP_3/AM, as indicated. Cells were subsequently fixed with glutaraldehyde, and the percentage of polarized cells was determined (100 cells per sample inspected). Mean ± S.E.M. of three independent experiments (50 μM PIP_3/AM: $n=2$).

Incubation of neutrophils for 45 min at 37°C with a commercially available non-membrane-permeant PIP_3 analogue (15 μM) did not induce a significant increase in the percentage of polarized cells (14 ± 6% polarized cells in controls versus 15 ± 6% polarized cells after incubation with the PIP_3 analogue, $n=3$). Higher concentrations of the lipid induced cell aggregation. These data suggest that PIP_3/AM acts intracellularly.

Development of polarity is thought to be a prerequisite for cell migration [3]. We analyzed whether cells polarized upon incubation with PIP_3/AM were also able to migrate. Migration was assessed by preincubating cells with 25 μM PIP_3/AM in medium lacking divalent cations, in order to avoid adverse effects on PIP_3/AM, followed by centrifugation and resuspension in medium with divalent cations. Migration of cells was then assessed by videomicroscopy in the absence of PIP_3/AM in the medium. As shown in Table 1, this pretreatment of cells with 25 μM PIP_3/AM resulted in significantly increased migration in 47–61% of the cells with a mean speed approaching that of cells stimulated with chemotactic peptide (Table 1). No obvious difference could be detected in shape changes observed during migration of cells induced by treatment with PIP_3/AM and chemotactic peptide (data not shown). The direction of migration was random, comparable to migration in uniform concentrations of chemotactic peptide.

3.2. PIP_3/AM induces polarized accumulation of F-actin in neutrophils

As shown in Fig. 2C,C′, neutrophils polarized by incubation with 25 μM PIP_3/AM for 45 min show not only a clearly polarized shape, but also markedly accumulate F-actin in the leading lamellae, comparable to the effect induced by incuba-

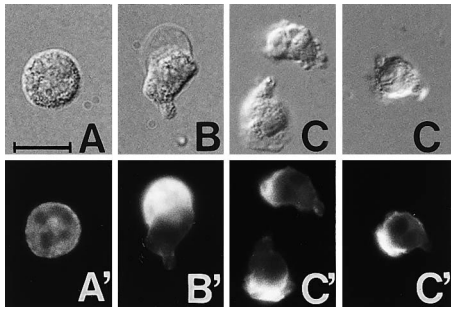

Fig. 2. Morphology and F-actin localization of neutrophils exposed to 25 μM PIP_3/AM. Cells (3×10^6 cells/ml) were incubated for 45 min at 37°C in Gey's medium lacking divalent cations and HSA in the absence (A, A′) or presence of 25 μM PIP_3/AM (C, C′). For comparison, cells are shown which were preincubated for 10 min at 37°C in medium followed by the addition of 1 nM fNLPNTL and a further incubation for 30 min (B, B′). Cells were subsequently fixed and stained with rhodamine phalloidin to visualize F-actin. Photographs were obtained with Nomarski optics (A–C) and in fluorescent light (A′–C′). For cells incubated with PIP_3/AM, several typical examples of cells are shown. Bar: 10 μm.

tion with fNLPNTL (Fig. 2B,B′). In contrast, resting cells show a diffuse cytoplasmic distribution of F-actin (Fig. 2A,A′). In 81 out of 100 cells polarized by addition of 25 μM PIP_3/AM we observed this polar accumulation of F-actin as compared to 83 out of 100 cells polarized in the presence of 1 nM fNLPNTL. PIP_3/AM thus induces F-actin reorganization comparable to chemotactic peptide.

3.3. *Identification of signalling pathways located downstream of PIP_3*

We have previously shown that activity of the Rho-associated coiled coil kinase appears to be required for chemotactic peptide-induced development of polarity and migration in neutrophils [18]. We therefore studied a possible role of activation of Rho-kinase in PIP_3/AM-induced neutrophil shape changes. Preincubation with 10 μM of Y-27632, a selective inhibitor of Rho-kinase [19], significantly inhibited development of polarity induced by PIP_3/AM by $82\pm5\%$ ($n=3$), as shown in Fig. 3. Cells became spherical with unifocal surface projections, comparable to cells preincubated with Y-27632 prior to addition of chemotactic peptide (Fig. 3A, panel c). PIP_3 may thus act by stimulating Rho which activates Rho-kinase. However, Y-27632 could not suppress membrane protrusion, suggesting that a target of PIP_3/AM other than Rho-kinase is essential for this process.

Table 1
PIP_3/AM stimulates neutrophil migration

Additions	Migrating cells (%)	Speed of migrating cells (μm/min)
Medium	5 ± 3	0.7 ± 0.4
fNLPNTL (1 nM)	48	9.8
PIP_3/AM (25 μM)	57 ± 3	6.2 ± 1.2

Neutrophils (6×10^6/ml) were either incubated with 0.4% DMSO or with PIP_3/AM (25 μM) for 45 min, or, after a preincubation with 0.4% DMSO for 15 min, with 1 nM fNLPNTL for 30 min at 37°C in Gey's medium containing 1% HSA, without divalent cations, followed by centrifugation and resuspension in Gey's medium containing 2.5% HSA and divalent cations. In case of fNLPNTL, 1 nM fNLPNTL was added after centrifugation. Subsequently the percentage of migrating cells and the mean speed of migrating cells was determined in slide coverslip preparations at 37°C using videomicroscopy. Mean ± S.E.M. of four independent experiments (+fNLPNTL: $n=2$).

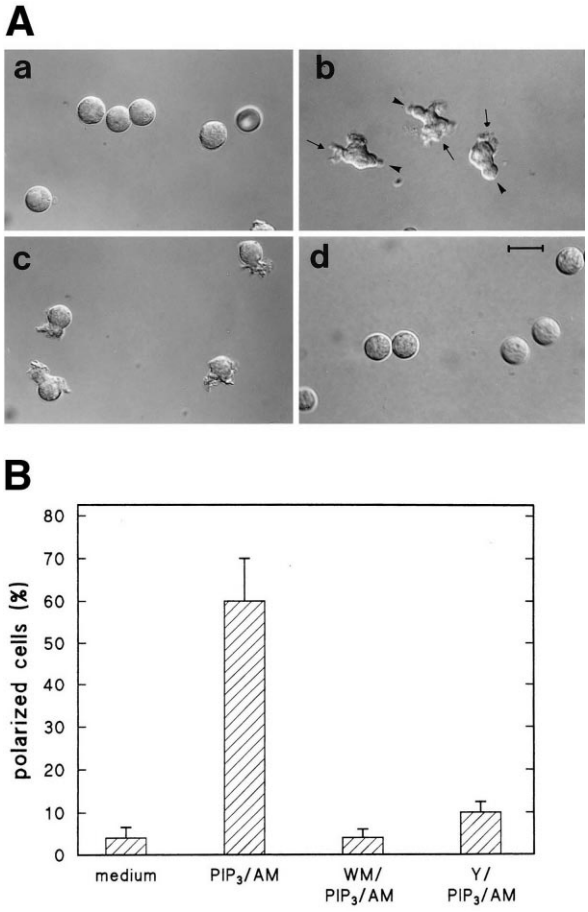

Fig. 3. Effects of inhibitors of Rho-kinase and PI 3-kinase on PIP_3/AM-induced shape changes in neutrophils. A: Neutrophils (3×10^6 cells/ml) were preincubated in Gey's medium lacking divalent cations and HSA in the absence (a, b) or in the presence of 10 μM Y-27632 (c) or 50 nM wortmannin (d) for 10 min at 37°C, followed by the addition of DMSO, 0.4% (a) or 25 μM PIP_3/AM (b, c, d) and a further incubation for 40 min. Subsequently cells were fixed with glutaraldehyde and photographed using Normarski optics. Bar, 10 μm. In panel b, arrows indicate the leading lamella and arrowheads the contracted tail. B: Quantitative evaluation of the experiment shown in A. Neutrophils (3×10^6 cells/ml) were preincubated in Gey's medium lacking divalent cations and HSA in the absence or in the presence of 50 nM wortmannin (WM) or 10 μM Y-27632 (Y) for 10 min at 37°C as indicated, followed by a further incubation in the absence or presence of 25 μM PIP_3/AM for 40 min. Subsequently cells were fixed with glutaraldehyde and the percentage of polarized cells was determined. Mean ± S.E.M. of 3–4 independent experiments.

We have shown previously that wortmannin, a potent PI 3-kinase inhibitor [20], markedly reduces fNLPNTL-induced development of polarity and migration in neutrophils, suggesting that products of PI 3-kinase, possibly PIP_3, are important signalling substances for migration [7]. Shape changes induced by PIP_3/AM should thus not anymore be susceptible to wortmannin. Unexpectedly, 50 nM wortmannin inhibited these shape changes almost completely, by $95\pm6\%$ (Fig. 3). It cannot be excluded that even low, nanomolar concentrations of wortmannin act also on other enzymes [21]. We therefore tested the effect of a structurally different PI 3-kinase inhib-

itor, LY294002 [22]. Preincubation of cells with 75 μM of LY294002 suppressed PIP_3/AM-induced polarity by 42 ± 11% ($n = 6$). LY294002 was thus clearly less effective than wortmannin, suggesting that PI 3-kinase, activated downstream of PIP_3, contributes to migration, but that part of the effect of wortmannin may be explained by inhibition of other enzymes.

The unexpected inhibitory effect of wortmannin and LY294002 suggests that incubation of cells with PIP_3/AM results in activation of PI 3-kinase by a positive feedback loop. We therefore tested whether PIP_3/AM induces activation of a target of PI 3-kinase, p42/44 MAPK (ERK), by analyzing phosphorylation of MAPK (indicative of activation) using a phosphorylation-specific antibody. It has been shown previously that chemotactic peptides induce an increase in phosphorylation and activation of p42/44 MAPK in human neutrophils and that this process can be suppressed by preincubation of cells with wortmannin [23]. As shown in Fig. 4A (lanes 5, 6), in cells exposed to 1 nM fNLPNTL for 5 min phosphorylation of p42/44 MAPK indeed was increased 16-fold. Similarly, incubation of neutrophils with 25 μM PIP_3/AM induced a very marked increase in phosphorylation of p42/44 MAPK 25 min after addition of the compound (Fig. 4B), and a smaller increase 30 min after its addition (Fig. 4A, lane 2). At 45 min after addition of the compound, phosphorylation of p44 MAPK was decreased almost to control values whereas that of p42 MAPK was still increased about 2-fold (Fig. 4A, lane 4). We then wanted to know whether PIP_3/AM-induced MAPK activation is due to activation of PI 3-kinase by testing the effect of wortmannin. As shown in Fig. 4B, preincubation of cells with 50 nM wortmannin prior to addition of PIP_3/AM did completely prevent increased phosphorylation of p44 MAPK, whereas that of p42 MAPK was also markedly suppressed (44–72% inhibition). This finding suggests indeed that PIP_3/AM induces activation of PI 3-kinase

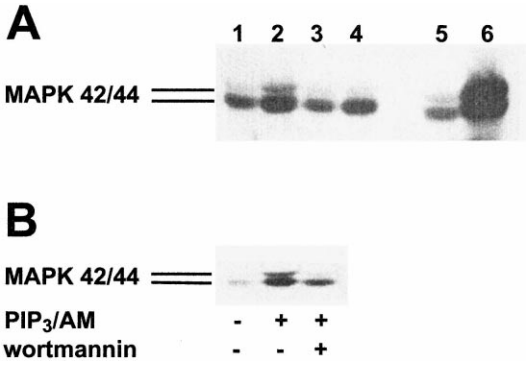

Fig. 4. MAPK phosphorylation in neutrophils exposed to PIP_3/AM or fNLPNTL. A: Neutrophils were preincubated in the absence (lanes 1, 3) or in the presence of PIP_3/AM (25 μM) (lanes 2, 4) for 30 (lanes 1, 2) or 45 (lanes 3, 4) min at 37°C. For comparison, neutrophils were either incubated for 15 min in medium (lane 5) or exposed for 5 min to 1 nM fNLPNTL after 10 min of preincubation at 37°C (lane 6). B: Neutrophils were preincubated for 10 min at 37°C in the absence or presence of 50 nM wortmannin, followed by addition of medium or PIP_3/AM (25 μM) and a further incubation for 25 min, as indicated. Subsequently, for A and B, cells were solubilized and subjected to gel electrophoresis and immunoblotting using an antibody specific for phosphorylated p42/44 MAPK (ERK). Arrows indicate the bands of MAPK of 42 000 and 44 000 Da. The data shown are representatives of two independent experiments with comparable results.

in a positive feedback loop, which then results in increased phosphorylation and activation of p42/44 MAPK.

4. Discussion

Our results show that an intracellular increase of PIP_3 in human neutrophils, resulting from incubation of the cells with a membrane-permeant ester of this lipid, induces development of polarity, accumulation of F-actin in the anterior lamellae and migration. These responses are indistinguishable from those observed in uniform concentrations of chemotactic peptide. Significant effects were obtained at 10–25 μM of the membrane-permeant ester. For comparison, in human neutrophils PIP_3 levels in resting cells amount to 50 pmol per ml of packed cells [24]. This level rises to a peak value of 2 nmol per ml packed cells, corresponding to a concentration of 2 μM [24]. We need approximately 10 times more for a maximal response. This may be due to incomplete entry and hydrolysis of PIP_3/AM into the cell. Moreover, very likely only PIP_3 incorporated into plasma membranes may be active, but part of the hydrolyzed ester may incorporate also into other cellular membranes. PIP_3 will also be rapidly metabolized.

Direct addition of PIP_3 micelles to NIH 3T3 fibroblasts has been shown previously to induce fibroblast motility, presumably by fusion of the micelles with the plasma membrane [25]. In these experiments, the effect of PIP_3 on motility was only about 25% of the response induced by PDGF. In contrast we observed no significant response when micelles of a non-membrane-permeant PIP_3 analogue were added to the neutrophils, suggesting that these cells do not spontaneously incorporate sufficient amounts of this lipid into the inner leaflet of their plasma membrane. PIP_3/AM however induced effects comparable to that of the potent chemoattractant fNLPNTL. Very likely, uptake of membrane-permeant PIP_3/AM is much more efficient than that of PIP_3. Our results also show that an increase in PIP_3 alone can induce a migratory response not only in slow moving fibroblasts but also in neutrophils, which belong to the fastest moving mammalian cells known.

Comparable to the effect of Rho-kinase inhibition on chemotactic peptide-stimulated cells [18], the Rho-kinase inhibitor Y-27632 suppressed tail contraction, but not formation of surface protrusions in PIP_3/AM-activated cells (Fig. 3A, panel c). Our data suggest that PIP_3/AM activates the Rho/Rho-kinase pathway in human neutrophils. Several recent reports, based mainly on in vitro observations, similarly suggest that phosphoinositides can activate Rho-family proteins. The mechanism of this activation could involve both activation of guanosine nucleotide exchange factors, which then stimulate small G-proteins, and direct effects of these lipids on small G-proteins by inducing their membrane association or acting themselves as guanosine nucleotide exchange factors. For example, PIP_3 and phosphatidylinositol 4,5-bisphosphate, but not phosphatidylinositol, appear to induce F-actin assembly in *Xenopus* egg extracts, by a mechanism involving activation of the small GTP-binding protein Cdc42 [26]. In another report, phosphatidylinositol 4,5-bisphosphate has been shown to stimulate GDP release from Cdc42 and RhoA [27]. Our data now suggest that phosphoinositide-induced activation of Rho-family proteins also occurs in intact cells.

The fact that the PIP_3/AM-induced polarity was inhibited by low, nanomolar concentrations of wortmannin was unexpected and may be partly explained by inhibition of enzymes

other than PI 3-kinase by wortmannin. However, LY294002, a structurally different inhibitor of PI 3-kinase, also significantly attenuated PIP_3/AM-induced development of polarity, suggesting that PIP_3 leads to activation of PI 3-kinase. This would imply the interesting possibility of a positive feedback loop. Indeed, we provide evidence for the fact that incubation of cells with PIP_3/AM induces wortmannin-sensitive activation of a target of PI 3-kinase, p42/44 MAPK (Fig. 4). Activation of this enzyme has been shown to result from phosphorylation of MAPK-kinase by PI 3-kinase [11]. Products other than PIP_3, that is, $PI(3,4)P_2$ or the protein kinase activity of PI 3-kinase, may thus play an important role in PIP_3/AM-induced neutrophil migration. The same processes seem to be operative in the chemotactic peptide-induced responses, as PIP_3/AM was only partially able to overcome inhibition of chemotactic peptide-induced polarity by wortmannin (Niggli, unpublished observations).

In summary, we have provided novel evidence that a membrane-permeant ester of PIP_3 can initiate motility in human neutrophils. Activation of Rho-kinase and activation of PI 3-kinase in a positive feedback loop appear to be required for this event.

Acknowledgements: We thank Kathy Mujynya-Ludunge for excellent technical assistance, Dr. E. Sigel for critical reading of the manuscript and Professor H.U. Keller for continuous support. We are grateful to Dr. R.Y. Tsien for generous gifts of PIP_3/AM and to Ms. Akiko Yoshimura (Yoshitomi Pharmaceutical Research Industries, Ltd., Japan) for the generous supply of Y-27632. This work was supported by the Swiss National Foundation for Scientific Research.

References

[1] Rao, K.M.K. and Varani, J. (1982) J. Immunol. 129, 1605–1607.
[2] White, J.R., Naccache, P.H. and Sha'afi, R.I. (1983) J. Biol. Chem. 258, 14041–14047.
[3] Devreotes, P.N. and Zigmond, S.H. (1988) Annu. Rev. Cell Biol. 4, 649–686.
[4] Parent, C.A. and Devreotes, P.N. (1999) Science 284, 765–770.
[5] Zigmond, S.H. (1993) Cell Motil. Cytoskelet. 25, 309–316.
[6] Bokoch, G.M. (1995) Blood 86, 1649–1660.
[7] Niggli, V. and Keller, H.U. (1997) Eur. J. Pharmacol. 335, 43–52.
[8] Knall, C., Worthen, G.S. and Johnson, G.L. (1997) Proc. Natl. Acad. Sci. USA 94, 3052–3057.
[9] Traynor-Kaplan, A.E., Thompson, B.L., Harris, A.L., Taylor, P., Omann, G.M. and Sklar, L.A. (1989) J. Biol. Chem. 264, 15668–15673.
[10] Eberle, M., Traynor-Kaplan, A.E., Sklar, L.A. and Norgauer, J. (1990) J. Biol. Chem. 265, 16725–16728.
[11] Bondeva, T., Pirola, L., Bulgarelli-Leva, G., Rubio, I., Wetzker, R. and Wymann, M.P.C. (1998) Science 282, 293–296.
[12] Jiang, T., Sweeney, G., Rudolf, M.T., Klip, A., Traynor-Kaplan, A. and Tsien, R.Y. (1998) J. Biol. Chem. 273, 11017–11024.
[13] Niggli, V. and Keller, H.U. (1999) Exp. Cell Res. 250, 558–568.
[14] Keller, H.U. and Niggli, V. (1994) J. Cell Physiol. 161, 526–536.
[15] Howard, T.H. and Meyer, W.H. (1984) J. Cell Biol. 98, 1265–1271.
[16] Keller, H.U. and Niggli, V. (1995) Eur. J. Cell Biol. 66, 157–164.
[17] Janmey, P.A., Iida, K., Yin, H.L. and Stossel, T.P. (1987) J. Biol. Chem. 262, 12228–12235.
[18] Niggli, V. (1999) FEBS Lett. 445, 69–72.
[19] Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M. and Narumiya, S. (1997) Nature 389, 990–994.
[20] Arcaro, A. and Wymann, M.P.C. (1993) Biochem. J. 296, 297–301.
[21] Cross, M.J., Stewart, A., Hodkin, M.N., Kerr, D.J. and Wakelam, M.J.O. (1995) J. Biol. Chem. 270, 25352–25355.
[22] Vlahos, C.J., Matter, W.F., Brown, R.F., Traynor-Kaplan, A.E., Heyworth, P.G., Prossnitz, E.R., Ye, R.D., Marder, P., Schelm, J.A., Rothfuss, K.J., Serlin, B.S. and Simpson, P.J. (1995) J. Immunol. 154, 2413–2422.
[23] Downey, G.P., Butler, J.R., Brumell, J., Borregaard, N., Kjeldsen, L., Sue-A-Quan, A.K. and Grinstein, S. (1996) J. Biol. Chem. 271, 21005–21011.
[24] Stephens, L.R., Hughes, K.T. and Irvine, R.F. (1991) Nature 351, 33–39.
[25] Derman, M.P., Toker, A., Hartwig, J.H., Spokes, K., Falck, J.R., Chen, C.-S., Cantley, L.C. and Cantley, L.G. (1997) J. Biol. Chem. 272, 6465–6470.
[26] Ma, L., Cantley, L.C., Janmey, P.A. and Kirschner, M.W. (1998) J. Cell Biol. 140, 1125–1136.
[27] Zheng, Y., Glaven, J.A., Wu, W.J. and Cerione, R.A. (1996) J. Biol. Chem. 271, 23815–23819.