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Disruption of β_2-Integrin–Cytoskeleton Coupling Abolishes the Signaling Capacity of These Integrins on Granulocytes

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Integrin-dependent adhesion and dynamic modulations of the actin network are prerequisites for normal cell locomotion. To investigate whether the actin microfilamentous system does play a role in regulation of β_2-integrin-induced signalling, we pretreated granulocytes with staurosporine, a well-known protein kinase inhibitor that has also been shown to disrupt the cytoskeleton of intact cells. Pretreatment with staurosporine completely inhibited the β_2-integrin-induced Ca^{2+} signal and also its ability to trigger actin polymerisation. This inhibition was not related to phosphorylation of the CD18-chain of the β_2-integrin, nor to inhibition of protein kinases. Instead, association of β_2-integrins with the cortical cytoskeleton, which was observed in untreated cells, was abolished after exposure to staurosporine, indicating that β_2-integrin signalling depends on integrin–cytoskeleton interaction. These results suggest not only that the actin network provides an adhesive link to the extracellular matrix and a driving force for the locomotory response, but also that it participates in regulation of β_2-integrin signalling during granulocyte locomotion.

Cell migration is a phenomenon that requires dynamic adhesive interactions between the internal motile machinery, more specifically the actin-rich cytoskeleton, and the external substratum, and adhesion receptors such as integrins serving as transmembrane links in these interactions. Numerous studies have focused on how integrins are linked to the cytoskeleton and how this linkage is accomplished. Consequently, it is known that the association between integrins and the cytoskeleton is essential for the ability of a cell to establish firm contact with other cells and with extracellular matrix proteins. It is also clear that in addition to physically mediating adhesion, integrins participate in the regulation of cell migration, because of their ability to generate intracellular signals. These outside-in signals can be triggered by ligand occupation and/or clustering of the integrins (1–3). However, to date little is known about the influence of integrin-cytoskeleton interactions on the outside-in signalling capacity of integrins, including β_2-integrins on granulocytes.

The most abundant integrins on human granulocytes belong to the β_2-integrin family (4). β_2-integrins are non-covalently associated heterodimers consisting of a common β chain, termed CD18, and one of four unique α chains, designated CD11a, CD11b, CD11c and CD11d (5). CD11b/CD18 is the dominating β_2-integrin on granulocytes. Over the past few years, the body of knowledge about the signalling mechanism of β_2-integrins on human granulocytes has grown considerably. Apparently there is an initial activation of several types of src-related tyrosine kinases (6–7), most likely responsible for tyrosine phosphorylation of PLCγ2 (8) and a subsequent localised Ca^{2+} transient (9) In addition, clustering of β_2-integrins activates phospholipase D, PtdIns 3-kinase and the small monomeric G-protein p21ras (10–12). Furthermore, inactivation of rho blocks chemoattractant-induced β_2-integrin-mediated adhesion of granulocytes (13). These observations are particularly interesting in relation to the ability of β_2-integrins to induce actin reorganisation (11, 14) and to mediate phagocytosis (10). Although it has been established that integrin-dependent adhesions is dynamically modulated, little is known about the mechanisms that regulate the signalling capacity of β_2-integrins. It has been suggested that cAMP

Abbreviations used: ECL, enhanced chemiluminescence; PKC, protein kinase C; PLC, phospholipase C; RAM, rabbit anti-mouse antibodies; Tris-HCl, tris[hydroxymethyl]-aminomethane hydrochloride.

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and protein kinase C can mediate receptor cross-talk between chemotactic receptors and β_2-integrins (11, 15–16, 17). Such an co-ordination of intracellular signals is most likely crucial for an efficient regulation of granulocyte locomotion.

Both the receptor for the chemotactic N-formyl peptide and the EGF receptor appear to associate with the cytoskeleton upon activation (18, 19), whereas β_2-integrins are constitutively linked to the cytoskeleton in resting leukocytes (20). Moreover, it has been suggested that in human granulocytes interaction between the chemotactic N-formyl peptide receptor and the cytoskeleton leads to inactivation of that receptor (21), a process that seems to be induced by activation of a heterotrimeric Gi2-protein (22–23). Such cytoskeleton-mediated interaction does not seem to be restricted to cell-surface receptors, because there is evidence that a similar mechanism may be involved in the regulation of PLCγ1 (24). However, it is important to stress that these observations do not necessarily imply that all transmembrane signalling elements found to be connected with the cytoskeleton are directly modulated by that intracellular framework. In fact, the cytoskeleton also functions as a matrix that is responsible for localising different signalling elements to distinct sites (25).

The aim of the present study was to explore the possible existence of cytoskeleton-dependent control of β_2-integrin-induced outside-in transmembrane signalling in granulocytes.

MATERIALS AND METHODS

Chemicals. The chemicals and their sources were as follows: lysophosphatidylcholine, phenylmethylsulphonyl fluoride (PMSF) and dimethyl sulphoxide (DMSO), Sigma Chemical Co. (St. Louis, MO); fura2/AM and fluorescein-labelled phalloidin, Molecular Probes Inc. (Eugene, OR); enhanced chemiluminescence kit (ECL) and [^{32}P] orthophosphate, Amersham International (Amersham, Bucks, UK); staurosporine, leupeptin-O, and pepstatin, Boehringer-Mannheim (Mannheim, Germany); H7, Seikagaku Corp. (Tokyo, Japan); protein A-Sepharose, Pharmacia Fine Chemicals (Uppsala, Sweden); SDS-PAGE reagents, Bio-Rad (Richmond, CA); nitrocellulose membranes, Schleicher and Schuell (Dassel, Germany). The mAb IB4 (IgG_{2a}) directed against the CD18 chain of the β_2 integrin originated from Dr. S. Wright (Rockefeller Univ., New York, NY; ref. 26), and the rabbit anti-mouse (RAM) immunoglobulins and the mouse anti-human LFA-1 β chain ($IgG1_\kappa$) were obtained from Dakopatts (Glostrup, Denmark). The cell culture media were obtained from Nordcell (Stockholm, Sweden), and the plastic flasks were from Costar (Cambridge, UK). All other chemicals used were of analytical grade.

Cell culture. HL60 cells were cultured in suspension in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated foetal calf serum, 2 mM L-glutamine, 100 UI/ml penicillin and 100 μg/ml streptomycin. The cells were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Before use, the cells were differentiated into neutrophil-like granulocytes by exposure to 1.3% (v/v) DMSO for 6 days. Cells differentiated in this manner are, like freshly isolated human neutrophils, capable of adhesion, migration towards a chemotactic gradient and phagocytosis (27). Prior to all experiments, cell viability was determined by trypan blue dye exclusion; only batches that contained less than 5% stained cells were used.

Antibody-induced clustering of β_2 integrins. Cells (5×10^6/ml) were suspended in a buffer consisting of 136 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5 mM $NaHCO_3$, 20 mM HEPES, 1.1 mM $CaCl_2$, 0.1 mM EGTA and 5.5 mM glucose (pH 7.4), hereafter referred to as calcium-containing medium, and incubated for 20 min with 10 μg/ml of the mouse mAb IB4 against CD18. After washing, the cells were resuspended in calcium-containing medium and then stimulated by the addition of RAM immunoglobulins (final dilution 1:50). Control experiments were performed using $F(ab')_2$ fragments of both the mAb IB4 and RAM to exclude the possibility that any of the observed responses were due to Fc-receptor clustering. The results show that these $F(ab')_2$ fragments elicited the same type of calcium transient and F-actin response as the intact antibodies did.

Determination of the cytosolic free calcium concentration. Cells were loaded with the calcium indicator fura2 as previously reported (28). Briefly, 5×10^6 cells/ml were incubated for 20 min at 37°C with 2 μM fura2/AM in calcium-containing medium. In experiments involving β_2 integrin clustering, the mAb IB4 was present during the incubation. The cells were then washed and resuspended in a cuvette containing 2 ml of calcium-containing medium. Fluorescence was measured on a SPEX spectrofluorimeter equipped with a thermostated (37°C) cuvette holder with a continuous stirring device. Excitation wavelengths were 340 and 380 nm, and the emission wavelength was 505 nm. The cytosolic free Ca^{2+} concentrations were calculated as described previously (29).

Determination of the cellular content of F-actin. The cellular content of F-actin was analysed by staining with fluorescein-phalloidin, essentially as previously described (30–32). After washing, the samples were resuspended, and the fluorescence of each sample was measured in a SPEX spectrofluorimeter (excitation set at 488 nm and emission at 522 nm).

^{32}P-labelling and immunoprecipitation of β_2 integrins. Cells were suspended at a concentration of 5×10^7/ml in phosphate-free RPMI 1640 medium supplemented with 1% (w/v) BSA and 0.5 mCi/ml [^{32}P] orthophosphate, and then incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C for 3 h. After labelling, the cells were washed once and resuspended in calcium-containing medium at 37°C and immediately stimulated as indicated in the figure legends. The phosphorylation reactions were stopped by placing the samples on ice. The cells were immediately lysed at 4°C for 20 min in a buffer consisting of 50 mM NaCl, 10 mM HEPES, 300 mM sucrose, 2.5 mM $MgCl_2$, 1% (v/v) Nonident P-40, 0.3 mM PMSF, 5 μg/ml leupeptin-O, 2 μg/ml pepstatin, and 0.4 mM Na_3VO_4. This lysis buffer was designed to inhibit protease and phosphatase activity, as well as to disrupt intact cytoskeletal structures with which the membrane proteins might be associated (33–34). Furthermore, the use of Nonidet P-40 as detergent does not dissociate the α and β chain of the integrins, hence it is possible to immunoprecipitate both chains by using an antibody directed toward either chain (35). Following lysis, insoluble material was removed by centrifugation at 4°C for 10 min at 12,000 × g. The lysates were incubated under continuous rotation for 1 h at 4°C with 10 μg of the mAb IB4 and 50 μl of a 50% (v/v) slurry of protein A-Sepharose beads. The beads were washed four times in the lysis buffer. Precipitated proteins were eluted by boiling in Laemmli sample buffer and separated by electrophoresis as described below.

Preparation of a Triton-insoluble cytoskeleton. The cytoskeletal fractions were prepared essentially as described elsewhere (36–37), using a Triton X-100-containing buffer (22). The cells were incubated with ice-cold Triton X-100 medium for 10 min on ice followed by centrifugation for 10 s at approximately 9,000 × g. The obtained cytoskeletal preparations were washed once, and then dissolved in Laemmli sample buffer and separated by electrophoresis as described below.

Gel electrophoresis, Western blotting, and autoradiography. Prior to electrophoresis, all samples were boiled for 10 min in Laemmli

sample buffer consisting of 125 mM Tris-HCl (pH 6.8), 8% (v/v) glycerol, 2.5% (w/v) SDS, 5% β-mercaptoethanol (v/v), and 0.05% (w/v) bromphenol blue. Electrophoresis was performed in 7.5% polyacrylamide gels as described by Laemmli (38), and the resolved proteins were electrophoretically transferred to nitrocellulose membranes. The β_2 integrins associated with the Triton-insoluble cytoskeleton were detected by using the antibodies indicated in the figure legends and a commercial ECL kit purchased from Amersham. Samples obtained from cells labelled with ^{32}P were subjected to electrophoresis, blotted to nitrocellulose membranes, and subsequently visualised by exposure to a Kodak X-Omatic film in combination with a Kodak X-Omatic regular intensifying screen at −70°C for 1 week.

RESULTS

Antibody clustering of β_2 integrins induces a rapid and transient increase in the cytosolic free Ca^{2+} concentration in non-adherent granulocytes (ref. 16; Fig. 1A). This β_2 integrin-induced Ca^{2+} signal was totally abolished when we pre-incubated granulocytes for 10 minutes with 1 μM staurosporine, a broad spectrum inhibitor of serine-threonine protein kinases (Fig. 1B). This effect was not detected when the cells were pre-incubated with 50 μM H7 (Fig. 1C), another broad-spectrum inhibitor of serine-threonine protein kinases (39). Not even if the concentration of H7 was raised to 200 μM were we able to detect any effect on the Ca^{2+} signal (data not shown). The effect of staurosporine, was to a certain extent, receptor selective in the sense that it did not affect the Ca^{2+} signal triggered by the chemotactic peptide receptor (Figs. 1D and 1E).

β_2 integrin clustering caused a significant increase in the cellular content of F-actin that peaked after about 30 s (Fig. 2A). Granulocytes that were pre-incubated for 10 min with 1 μM staurosporine prior to the clustering of their β_2 integrins showed no increase in the cellular content of F-actin (Fig. 2A). The chemotactic peptide fMLP also triggered a significant increase in the cellular content of F-actin that peaked after only 15 s (Fig. 2B). In contrast to the F-actin response induced by clustering of β_2 integrins, preincubation with 1 μM staurosporine significantly potentiated the fMLP-induced F-actin response (Fig. 2B). Furthermore, we found that the basal F-actin content in granulocytes was slightly reduced by a 10 min incubation with 1 μM staurosporine alone (Table 1).

A significant amount of CD18 was recovered from a Triton-X100 insoluble cytoskeletal fraction of resting cells (Fig. 3, lane 1). Neither binding of an anti-CD18 antibody nor subsequent cross-linking with a secondary antibody for 15 or 30 s significantly affected the amount of CD18 recovered (Fig. 3, lanes 2–4). However, preincubation with staurosporine did cause an overall loss of CD18 associated with the cytoskeletal fraction (Fig. 3, lanes 4–8).

We have previously shown that phosphorylation of the CD18 chain is correlated with inhibition of the signalling capacity of β_2 integrins (16). Therefore, we

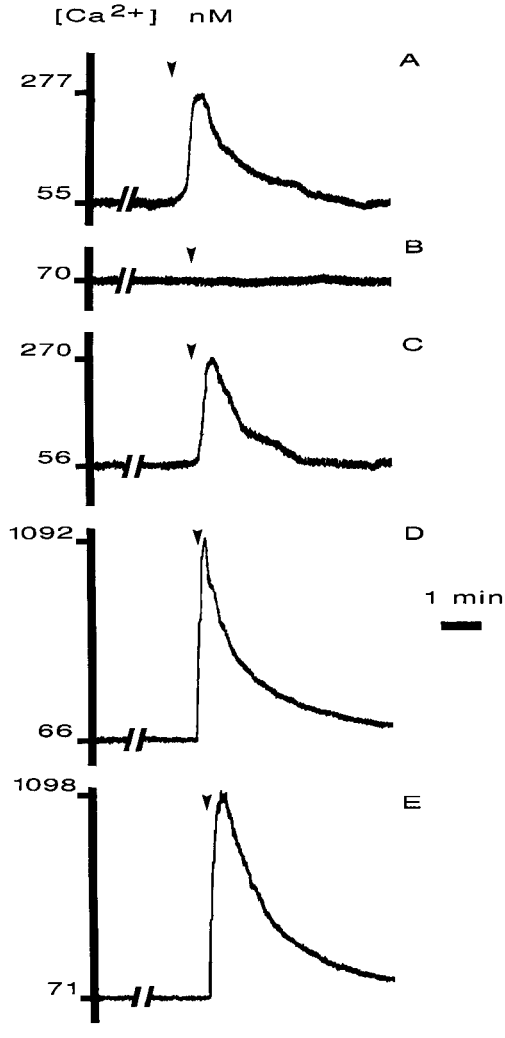

FIG. 1. Effects of staurosporine and H7 on β_2-integrin- and fMLP-induced increases in the cytosolic free Ca^{2+} concentration. Granulocytes were loaded with fura2 and in A–C also incubated with the mAb IB4 (10 μg/ml) in Ca^{2+}-containing medium for 20 min at 37°C, as described under Materials and Methods. The cells were washed and resuspended in 2 ml of Ca^{2+}-containing medium (37°C) and then transferred to cuvettes. Thereafter, the cells were preincubated for 10 min with DMSO alone (A and D), or with1 μM staurosporine (B and E) or H7 (C) dissolved in DMSO, before starting to record fura2 fluorescence. Where indicated, the cells were stimulated by adding RAM antibodies (1:50 dilution; A–C) or 100 nM fMLP (D and E). Representative traces are shown.

investigated whether the effect of staurosporine could be due to its reported ability to activate phospholipase D (40), leading to activation of PKC and possibly an altered phosphorylation statues of the β_2 integrin. The CD18 chain of this integrin is not phosphorylated in untreated cells (Refs. 16, 34). With the immunoprecipitation protocol we used, two phosphorylated protein bands with molecular weights of about 165 and 150 kDa, respectively, corresponding to the two α chains CD11b and CD11c, co-precipitated with CD18 (16). Pretreatment of cells with 1 μM staurosporine for 10

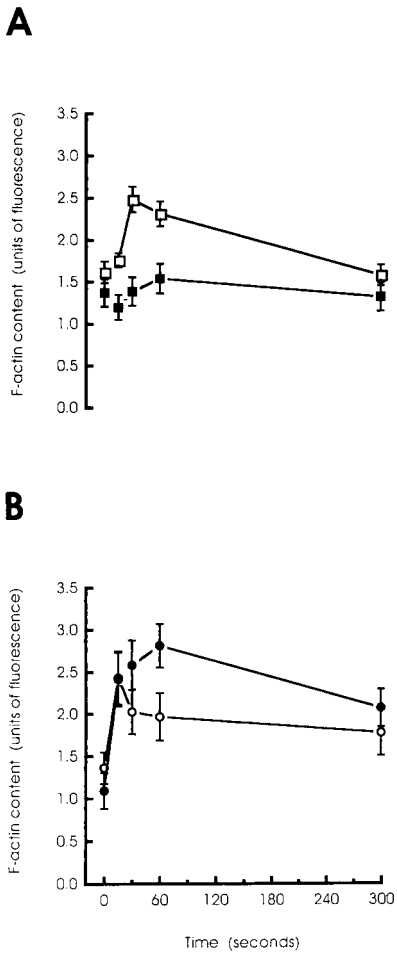

FIG. 2. Effects of staurosporine on actin polymerisation induced by β_2-integrins and fMLP. Granulocytes were suspended in Ca^{2+}-containing medium and in A were also incubated for 20 min with the mAb IB4 (10 μg/ml) as described under Materials and Methods. Thereafter, the cells were preincubated for 10 min with DMSO alone (open symbols) or with 1 μM staurosporine dissolved in DMSO (closed symbols), and subsequently stimulated by adding RAM antibodies (1:50 dilution; A) or 100 nM fMLP (B). The reactions were terminated by adding an equal volume of ice-cold paraformaldehyde (4% w/v), and F-actin was determined as described under Materials and Methods. The values given are means ± SEM of 10 separate experiments.

min, which completely blocks the β_2 integrin-induced Ca^{2+} signal (Fig. 1B) and polymerisation of actin (Fig. 2A), did not affect the phosphorylation status of β_2 integrins in the following: control cells, cells preincubated with an anti-CD18 antibody, or cells with their β_2 integrins engaged by antibody cross-linking (data not shown).

DISCUSSION

The present results show that pre-incubation with staurosporine abolishes both the β_2 integrin-induced Ca^{2+} signal and its downstream effect on the cytoskeleton. An earlier study (16) revealed that PMA-induced activation of PKC results in a phosphorylation of the CD18 chain, a phenomenon coupled to inhibition of β_2 integrin signalling in granulocytes, which is noteworthy considering that staurosporine has been reported to activate phospholipase D (40). Consequently, if a sufficient degree of such activation were to occur, it would result in a downstream accumulation of diacylglycerols and activation of PKC (41) as well as phosphorylation of the CD18 chain, which has a cytoplasmic domain with the characteristic's of a PKC substrate (42). Nonetheless, a mechanism of that type seems unlikely, because staurosporine did not induce detectable phosphorylation of the CD18 chain. Alternatively, it is possible that staurosporine instead inhibits a serine-threonine kinase that is essential for initiation of β_2 integrin signalling. However, in our study clustering of β_2 integrins did not induce a detectable serine-threonine phosphorylation on either the CD11 or the CD18 chain, making such an explanation less likely. More importantly, we noted that preincubation with H7, another broad-spectrum serine-threonine kinase inhibitor, did not affect the integrin-induced Ca^{2+} signal, which refutes the notion that staurosporine mediates its effects via modulation of a serine-threonine protein kinase.

TABLE 1

Effect of Staurosporine on the Basal F-Actin Content in Granulocytes

Pretreatment	F-actin content (units of fluorescence)
Medium alone (control)	1.53 ± 0.09
Staurosporine (1 μM)	1.28 ± 0.18

Note. Cells were exposed to medium alone or to medium supplemented with 1 μM staurosporine for 10 min at 37°C. The values given are means ± S.E.M. of four separate experiments.

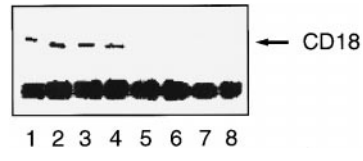

FIG 3. Western blot showing the effect of staurosporine on the amount of β_2 integrins associated with the Triton–X-100-insoluble cytoskeletal fraction. Cells were suspended in Ca^{2+}-containing medium and incubated for 20 min without any additives (lanes 1 and 5), or with the mAb IB4 (10 μg/ml; lanes 2–4, 6–8). After washing, the cells were incubated for 10 min with DMSO alone (lanes 1–4) or 1 μM staurosporine dissolved in DMSO (lanes 5–8), and thereafter incubated with RAM (1:50 dilution) for 15 s (lanes 3 and 7) or 30 s (lanes 4 and 8). The cytoskeletal fractions were prepared as described under Materials and Methods; the proteins in those fractions were separated by electrophoresis and transferred to a nitrocellulose membrane. The presence of β_2 integrins was detected by using a specific anti-CD18 antibody (1:1000 dilution). Representative results are shown.

Roubey *et al.* (43) have reported that staurosporine prevented phagocytosis of iC3b-opsonised particles, which bind to CD11b/CD18 (CR3). Considering the results discussed above, that effect of staurosporine may have involved not only inhibition of PKC, but also initiation of a process upstream of the CR3-induced activation of PKC. The latter represents an additional site of action of staurosporine, which probably exists downstream of ligand-binding or clustering the integrins, since Roubey and co-workers found that binding of iC3b-opsonised particles to CD11b/CD18 on granulocytes was not affected by staurosporine. An alternative explanation for our results, that would agree with the existence of such an additional site of action, is that staurosporine affected β2 integrin signalling by influencing the cytoskeleton in a way that decreased interaction between β2 integrins and cytoskeletal components. That explanation would also be in accordance with previous results showing that staurosporine alone alters the actin-rich cytoskeleton in fibroblasts and endothelial cells (44). It is not yet understood how such alterations occur, but it is known that they are reversible and are not dependent on PKC. In our study, staurosporine abolished the interaction between β2 integrins and the cytoskeleton, whereas the amount of cellular F-actin was only marginally reduced, which suggests that staurosporine primarily affects the cortical cytoskeleton in granulocytes.

It has been proposed that, in granulocytes, interactions between receptors and the actin-rich cytoskeleton regulate the signalling capacity of the chemotactic peptide receptor by mediating its desensitisation (21) The chemotactic peptide receptor can interact with the cytoskeleton in the absence of ligand, if the Gi2-protein, a downstream transducer of the receptor, is activated (22–23). That finding and the present data showing that staurosporine potentiated the fMLP-induced F-actin response further imply that staurosporine affects β_2 integrin signal transduction by disrupting the cortical cytoskeleton and thereby releasing β_2 integrins from the cytoskeleton. The possibility that β_2 integrin signal transduction is modified by the cytoskeleton is also supported by reports stating that an immobile subset of plasma membrane β_2 integrins is crucial for phagocytosis of C3bi-opsonised particles (45) and that granulocytes exposed to TNF exhibit increased association of β_2 integrins with the cytoskeleton and increased β_2 integrin-dependent adhesion (25, 46).

Our results indicate that both chemotactic peptide receptors and β_2 integrins can be simultaneously but differently modulated by their interactions with the cytoskeleton.

Moreover, our data suggest that the cross-talk Eierman *et al.* (15) noted between chemotactic peptide receptors and β_2 integrins, which entailed transient inhibition of the signalling capacity of β_2 integrins by fMLP, was due to the well-known properties of Ca^{2+} and/or cAMP, in this case generated by the fMLP receptor, to depolymerise and reduce the F-actin network in granulocytes (14, 47) and thereby release β_2 integrins from the cytoskeleton and block their signalling capacity.

The directed locomotion of leukocytes in a chemotactic gradient requires efficient desensitisation of chemotactic factor receptors, mediated via interaction of these receptors with the cytoskeleton, and release from integrin attachments in the tail of the moving cell (48). The present results add to this concept by suggesting that, as part of the detachment process, β_2 integrins are also rendered incapable of generating intracellular signals. Accordingly, it is reasonable to assume that, in addition to providing the driving force for cell locomotion, the actin framework also constitutes a matrix for the integration of signals generated by integrins and chemotactic peptide receptors.

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REFERENCES

1. Schwartz, M. A. (1992) *Trends Cell Biol.* **2,** 304–307.
2. Juliano, R. L., and Haskill, S. (1993) *J. Cell Biol.* **120,** 577–585.
3. Ng-Sikorski, J., Lindén, L., Eierman, D., Franzén, L., Molony, L., and Andersson, T. (1996) *J. Cell Sci.* **109,** 2361–2369.
4. Sanchez-Madrid, F., Nagy, J. A., Robbins, E., Simon, P., and Springer, T. A. (1983) *J. Exp. Med.* **158,** 1785–1803.
5. Gahmberg, C. G., Tolvanen, M., and Kotovuori, P. (1997) *Eur. J. Biochem.* **245,** 215–232.
6. Berton, G., Fumagalli, L., Laudanna, C., and Sorio, C. (1994) *J. Cell Biol.* **126,** 1111–1121.
7. Lowell, C. A., Fumagalli, L., and Berton, G. (1996) *J. Cell Biol.* **133,** 895–910.
8. Hellberg, C., Molony, L., Zheng, L., and Andersson, T. (1996) *Biochem. J.* **317,** 403–409.
9. Petersen, M., Williams, J. D.. and Hallet, M. B. (1993) *Immunology* **80,** 157–159.
10. Fällman, M., Andersson, R., and Andersson, T. (1993) *J. Immunol.* **151,** 330–338.
11. Löfgren, R., Ng-Sikorski, J., Sjölander, A., and Andersson, T. (1993) *J. Cell Biol.* **123,** 1597–1605.
12. Zheng, L., Sjölander, A., Eckerdal, J., and Andersson, T. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 8431–8436.
13. Laudanna, C., Campbell, J. J., and Butcher, E. C. (1996) *Science* **271,** 981–983.
14. Nathan, C., and E. Sanchez. (1990) *J. Cell Biol.* **111,** 2171–2181.
15. Eierman, D., Hellberg, C., Sjölander, A., and Andersson, T. (1994) *Exp. Cell Res.* **215,** 90–96.
16. Hellberg, C., Eierman, D., Sjölander, A., and Andersson, T. (1995) *Exp. Cell Res.* **217,** 140–148.

17. Ydrenius, L., Molony, L., Ng-Sikorski, J., and Andersson, T. (1997) *Biochem. Biophys. Res. Commun.* **235,** 445–450.
18. Jesaitis, A. J., Nakamura, J. R., Sklar, L. A., Cochrane, C. G., and Painter, R. G. (1984) *J. Cell Biol.* **98,** 1378–1387.
19. Rijken, P. J., Hage, W. J., van Bergen en Henegouwen, P. M. P., Verkleij, A. J., and Boonstra, J. (1991) *J. Cell Sci.* **100,** 491–499.
20. Sampath, R., Gallagher, P. J., and Pavalko, F. M. (1998) *J. Biol. Chem.* **273,** 33588–33594.
21. Jesaitis, A. J., and Klotz, K. N. (1993) *Eur. J. Haematol.* **51,** 288–293.
22. Särndahl, E., Bokoch, G. M., Stendahl, O., and Andersson, T. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6552–6556.
23. Särndahl,E., Bokoch, G. M., Boulay, F., Stendahl, O., and Andersson T. (1996) *J. Biol. Chem.* **271,** 15267–15271.
24. Goldsmith-Clermont, P. J., Kim. J. W., Machesky, L. M., Rhee, S. G., and Pollard, T. D. (1991) *Science* **251,** 1231–1233.
25. Yan, S. R., Huang, M., and Berton G. (1997) *J. Immunol.* **158,** 1902–1910.
26. Wright, S. D., Rao, P-E.,van Vorris, W. C., Craigmyle, L. S., Iida, K., Talle, M. A., Westberg, E. F., Goldstein, G., and Silverstein, S. C. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5699–5703.
27. Collins, S. J. (1987) *Blood* **70,** 1233–1244.
28. Andersson, T., Dahlgren, C., Pozzan, T. Stendahl, O., and Lew, D. P. (1986) *Mol. Pharmacol.* **30,** 437–443.
29. Grynkiewicz, G. Poenie, M., and Tsien, R. Y. (1985) *J. Biol. Chem.* **260,** 3440–3450.
30. Wallace, P. J., Wersto, R. P., Packman, C. H., and Lichtman, M. A. (1984) *J. Cell Biol.* **99,** 1060–1065.
31. Bengtsson, T., Stendahl, O., and Andersson, T. (1986) *Eur. J. Cell Biol.* **42,** 338–343.
32. Bengtsson, T., Särndahl, E., Stendahl, O., and Andersson, T. (1990) *Proc. Natl. Acad. Sci.USA* **87,** 2921–2925.
33. Changelian, P. S., and Fearon, D. T. (1986) *J. Exp. Med.* **163,** 101–115.
34. Chatila, T. A., Geha, R. S., and Arnaout, M. A. (1989) *J. Cell Biol.* **109,** 3435–3444.
35. Buyon, J. P., Slade, S. G., Reibman, J., Abramson, S. B., Philips, M. R., Weissman, G., and Winchester, R. (1990) *J. Immunol.* **144,** 191–197.
36. White, J. R., Naccache, P. H., and Sha'afi, R. I. (1983) *J. Biol. Chem.* **258,** 14041–14047.
37. Särndahl, E., Lindroth, M., Bengtsson, T., Fällman, M., Gustavsson, J., Stendahl, O., and Andersson, T. (1989) *J. Cell Biol.* **109,** 2791–2799.
38. Laemmli, U. K. (1970) *Nature* **227,** 680–685.
39. Kawamoto, S., and Hidaka, H. (1984) *Biochem. Biophys. Res. Commun.* **125,** 258–264.
40. Kanaho, Y., Takahashi, K. Tomita, U., Iiri, T., Katada, T., Ui, M., and Nozawa, Y. (1992) *J. Biol. Chem.* **267,** 23554–23559.
41. Billah, M. M., Pai, J.-K., Mullman, T. J., Egan, R. W., and Siegal, M. I. (1989) *J. Biol. Chem.* **264,** 9069–9076.
42. House, C., Wettenhall, R. E. H., and Kemp, B. E. (1987) *J. Biol. Chem.* **262,** 772–777.
43. Roubey, R. A. S., Ross, G. D., Merrill, J. T., Walton, F., Reed, W., Winchester, R. J., and Buyon J. B. (1991) *J. Immunol.* **146,** 3557–3562.
44. Hedberg, K. K., Birrell, G. B., Habliston, D. L., and Griffith, O. H. (1990) *Exp. Cell Res.* **188,** 199–208.
45. Graham, I. L., Gresham, H. D., and Brown, E. J. (1989) *J. Immunol.* **142,** 2352–2358.
46. Yan, S. R., Fumagalli, L., Dusi, S., and Berton, G. (1995) *J. Leukocyte Biol.* **58,** 595–606.
47. Stossel, T. P. (1992) *in* Inflammation: Basic Principles and Clinical Correlates (Gallin, J. I., Goldstein, I. M., and Snyderman, R., Eds.), 2nd ed., pp. 459–475 Raven Press Ltd., New York, NY.
48. Cassimeris, L., and Zigmond, S. H. (1990) *Semin. Cell Biol.* **1,** 125–134.