Syk Tyrosine Kinase Is Required for Immunoreceptor Tyrosine Activation Motif-dependent Actin Assembly*

Dianne Coxt, Peter Chang‡, Tomohiro Kurosaki§, and Steven Greenberg¶

From the Department of Medicine, Pulmonary Division, College of Physicians and Surgeons, Columbia University, New York, New York 10032, the Department of Cardiovascular Molecular Biology, Lederle Laboratories, Pearl River, New York, New York 10965, and Section of Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06510

Clusterings of several multisubunit receptors on hematopoietic cells results in a signaling cascade involving the phosphorylation of immunoreceptor tyrosine activation motifs, or "ITAMs," and actin polymerization. Recent experiments indicate that direct clustering of the ITAM-binding protein, p72\(^{Syk}\), is capable of transmitting a phagocytic signal in COS cells (Greenberg, S., Chang, P., Wang, D., Xavier, R., and Seed, B. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1103-1107). However, the possibility of redundant signaling pathways makes it difficult to test the requirement for Syk in ITAM-dependent actin polymerization in hematopoietic cells. We developed a model system to study ITAM-dependent actin assembly. DT40 lymphocytes were transfected with fusion proteins encoding the transmembrane and cytosolic domains of the ITAM-containing γ subunit of Fc receptors. Clustering of the γ-containing fusion proteins with IgG-coated erythrocytes triggered submembranous actin assembly. This response depended on an intact ITAM, was absent in cell lines that had been engineered to lack Syk, and was augmented in cell lines that stably overexpressed Syk. These experiments demonstrate an absolute requirement for Syk tyrosine kinase in ITAM-dependent actin assembly in transfected lymphocytes.

Actin assembly is a nearly ubiquitous response to the engagement of a diverse array of cell surface receptors that signal chemotaxis, phagocytosis, cell division, or cell adhesion. In hematopoietic cells, ligation of members of the immunoglobulin gene superfamily is accompanied by actin polymerization and several functionally important actin-dependent events. For example, clustering receptors for the Fc portion of IgG (F\(_c\) receptors)\(^1\) by IgG-opsonized targets results in phagocytosis in macrophages (1) and neutrophils (2, 3), while engagement of antigen receptors by target cells results in cytochalasin-sensitive interference secretion by T-lymphocytes (4). Since many biochemical events accompany ligation of these multisubunit receptors, it is important to identify those events among the many that are required for triggering actin assembly. A recent clue was provided by studies demonstrating a requirement for tyrosine kinases in lymphocyte effector functions (5) and phagocytosis (6, 7). However, activation of multiple tyrosine kinases accompanies engagement of F\(_c\) receptors (8-12) and antigen receptors (13), making it difficult to implicate specific tyrosine kinases in triggering actin assembly. Models for signaling by both families of receptors have been proposed which implicate ZAP-70 and Syk tyrosine kinases in several downstream signaling events (13-17), and both kinases are capable of triggering actin assembly when autonomously clustered in COS cell transfectants (18). However, it is not clear whether Syk is required for ITAM-mediated actin assembly in hematopoietic cells. To test a requirement for Syk in cytoskeletal alterations mediated by specific ITAM-containing subunits, such as the γ subunit of Fc receptors, we developed an assay of ITAM-dependent actin assembly using the DT40 lymphocyte cell line. The advantages of this cell line are that it lacks endogenous γ subunits, and it undergoes a high rate of homologous recombination. Thus, by gene targeting and cDNA transfection, it is possible to isolate clones of DT40 cells that lack expression of a given gene product (19), and that express specific ITAM-containing subunits. Plasmid constructs encoding the transmembrane and cytosolic domains of the γ subunit were expressed in wild-type lymphocyte cell lines, or in cell lines that were engineered to either lack, or stably overexpress, Syk. Using these cell lines, we tested whether Syk was required for γ subunit-mediated actin assembly.

EXPERIMENTAL PROCEDURES

Cells and Materials—DT40 cells, a transformed chicken lymphocyte cell line, were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 1% chicken serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin and maintained at 37 °C in a 5% CO\(_2\) incubator. IgG against sheep erythrocytes was from Dianmedix (Miami, FL). Fluorescein anti-rabbit-IgG was from Jackson Immunoresearch, Inc. (West Grove, PA). Rhodamine-phalloidin was from Molecular Probes (Eugene, OR). Rabbit anti-Syk IgG and protein A-agarose were from Santa Cruz Biotechnology (Santa Cruz, CA). RC20H, a horseradish peroxidase-conjugated recombinant fragment of mAb P1Y20 anti-phosphotyrosine, was from Transduction Laboratories (Lexington, KY). mAb C4 against actin was kindly provided by J. James Lessard (University of Cincinnati, Cincinnati, OH). IgG-RBCs were prepared as described (20). E-3G8, bovine erythrocytes opsonized with F(ab')\(_2\), fragments of mAb 3G8 (Medarex, Annandale, N.J.), was a kind gift of Robert Kimberly (Carnegie University Medical College, New York).

Plasmid Construction and Isolation of Transfected Cell Lines—16, γ, a fusion protein consisting of the extracellular domain of human F\(_c\)R

*This work was supported in part by Grants HL02641 and HL54164 from the National Institutes of Health, a Research Grant from the American Cancer Society, and a grant-in-aid from the American Heart Association, New York City affiliate. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Columbia University, Dept. of Medicine, 630 West 168th St., New York, NY 10032. Tel.: 212-305-1586; Fax: 212-305-1146; E-mail: greenberg@cuccfa.ccc.columbia.edu.

‡ The abbreviations used are: F\(_c\) receptor, receptor for the Fc portion of IgG; ITAM, immunoreceptor tyrosine activation motif; mAb, monoclonal antibody; RBCs, red blood cells; IgG-RBCs, sheep erythrocytes opsonized with anti-sheep erythrocyte IgG; Syk, DT40 cell line that overexpress Syk; Syk- DT40 cell line that lack expression of Syk; 16-γ, fusion proteins containing CD16 ectodomains and γ subunit transmembrane and cytosolic domains; 16-γc, a 16-γ-based construct bearing a Tyr→Phe mutation in tyrosine 76; WT, wild-type DT40 cells.

Received for publication, March 5, 1996, and in revised form, April 22, 1996
Syk Tyrosine Kinase and Actin Assembly

I11A (CD16) and the transmembrane and cytosolic domains of the murine γ subunit of Fc receptors, was constructed by polymerase chain reaction (21) and was inserted into the EcoRI site of pApuro (19). 16:γ−3G8, a construct bearing a Tyr→Phe mutation in tyrosine 76, was created by polymerase chain reaction. Resultant constructs were confirmed by DNA sequencing. DT40 lymphocytes expressing either 16:γ or 16:γ−3G8 were sorted following transfection of F-actin that was 19% apparent and selected in the presence of 0.25 μg/ml puromycin. 16:γ was similarly expressed in Syk− DT40 cells, previously generated by gene targeting (19). To generate 16:γ-expressing cell lines that also overexpressed Syk (Syk+/−/16:γ), one clone of WT/16:γ (clone G1) was cotransfected with pSyk (19) and pRC-CMV, and individual clones were isolated following selection with neomycin G418. Between 5 and 30 individual clones were isolated from each transfection, and individual clones were analyzed for surface expression of the CD16 epitope by flow cytometry and by rosetting with IgG-RBCs. Levels of Syk expression were confirmed by immunoblotting with anti-Syk IgG and were normalized to actin content by immunoblotting with mAb C4 against actin.

Immunoblotting, Immunoprecipitation, and in Vitro Kinase Assays—

5 × 105 cells were incubated in the presence or absence of 20 μg/ml mAb 3G8 for 30 min at 4°C and further incubated for varying intervals at 37°C with 40 μg/ml rabbit anti-mouse IgG. Following addition of ice-cold lysis buffer (1% Triton X-100, 100 mM NaCl, 2 mM EDTA, 1 mM sodium orthovanadate, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 20 mM Tris-HCl, pH 7.2), samples were processed for either immunoblotting with anti-phosphotyrosine (20) or immunoprecipitation with anti-Syk IgG pre-adsorbed to protein A-agarose. For anti-Syk immunoblotting, 2 × 105 cells were lysed as above and immunoblotted with rabbit anti-Syk IgG. Blots were visualized using enhanced chemiluminescence. Syk protein expression was quantitated by densitometry of anti-Syk immunoblots using NIH-Image. For in vitro kinase assays, anti-Syk immunoprecipitates derived from 107 cells were washed and incubated with a kinase buffer containing 5 μCi of [γ-32P]ATP, 5 mM MnCl2, 5 mM MgCl2, and 25 mM Tris-HCl, pH 7.2. After incubation at 25°C for 5 min, samples were diluted in a buffer containing 10 mM EDTA to inhibit further kinase activity, washed, and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.

F-actin Assays and Fluorescence Microscopy—

Total cellular F-actin was quantitated as described previously (1) with the following modifications: 2 × 105 lymphocytes were allowed to adhere for 30 min at 37°C in 96-well tissue culture plates and were incubated in the presence or absence of 4 × 105 IgG-RBCs for 10 min at 4°C, washed to remove nonadherent RBCs, and incubated for further varying times at 37°C. Cells were fixed in 3.7% formaldehyde and stained with 0.33 μM rhodamine-phalloidin. Rhodamine fluorescence (excitation wavelength: 530 nm, emission wavelength: 590 nm) was measured using a fluorescence plate reader (CytoFluor II; Millipore). To normalize for cell number, nuclei were stained with 5 μM YO-PRO, fluorescence was determined (excitation wavelength: 485 nm, emission wavelength: 530 nm), and F-actin content per unit cell was calculated as the ratio of rhodamine to YO-PRO fluorescence. These results indicate that an intact ITAM is required for the localization of actin and for actin-rich membrane protrusions underlying attached particles. Although surface expression of 16:γ was somewhat higher than that of WT/16:γ−3G8 (Fig. 1), there was considerable overlap in the extent of surface expression of the two constructs, while there were essentially no detectable focal accumulations of F-actin in cells expressing WT/16:γ−3G8. Therefore, differences in surface expression could not account for the lack of focal accumulations of F-actin in cells expressing WT/16:γ−3G8. These results indicate that an intact ITAM is required for the focal accumulation of F-actin mediated by clustered γ subunits.

Syk Is Required for 16:γ-mediated Enhanced Protein Tyro-

**FIG. 1.** Surface expression of 16:γ and 16:γ−3G8, in lymphocyte transfectants. The indicated cell lines were stained for surface expression using mAb 3G8 followed by fluorescein anti-mouse IgG and examined by flow cytometry. Dashed lines represent fluorescence of cells using an isotype-matched control mAb.

Given density multiplied by that pixel intensity. Data represents measurements taken from 40–57 cortical regions from five microscopic fields of the indicated cell lines.

**RESULTS**

An Intact γ Subunit ITAM Is Required for Signaling Enhanced Protein Tyrosine Phosphorylation—

All cell lines transfected with 16:γ or 16:γ−3G8 expressed the CD16 epitope at the cells’ surfaces as determined by flow cytometry. We characterized two individual clones, expressing 16:γ or 16:γ−3G8, in more detail (Fig. 1). WT/16:γ and WT/16:γ−3G8, but not the parental cell line, WT, bound IgG-RBCs (not shown). To determine whether 16:γ was capable of triggering enhanced protein tyrosine phosphorylation, we incubated transfectants or controls with mAb 3G8 followed by anti-mouse IgG. Enhanced tyrosine phosphorylation of multiple proteins was apparent within 1 min of clustering surface-bound mAb 3G8 in WT/16:γ, but not untransfected controls (WT) or WT/16:γ−3G8 cells (Fig. 2). These data indicate a requirement for tyrosine 76 of the γ subunit in transmitting a signal for enhanced protein tyrosine phosphorylation and are consistent with findings of others that do not document a similar requirement for an intact γ subunit ITAM in mediating enhanced protein tyrosine phosphorylation (24, 25).

WT/16:γ, but Not WT/16:γ−3G8, Mediates the Submembranous Accumulation of F-actin—

We tested the ability of 16:γ to mediate cytoskeletal alterations when expressed in DT40 cells. Incubation of WT/16:γ cells with IgG-RBCs led to the submembranous accumulation of F-actin (Fig. 3). Most, but not all, cortical regions underlying attached particles demonstrated a broadened-acumulation of F-actin that was first apparent by 1 min (not shown), appeared maximal at 4 min (Fig. 3), and was no longer detectable after 10 min of incubation (not shown). Using phase-contrast optics, rudimentary pseudopods could be seen beneath many bound IgG-RBCs (not shown). The focal accumulation of F-actin in response to the IgG-bearing ligand was blocked by the presence of 1 μM cytochalasin D (not shown), indicating a requirement for barbed-end filament growth. To test the requirement for an intact ITAM in mediating the focal appearance of F-actin, we performed parallel experiments using WT/16:γ−3G8 cells. Despite obvious IgG-RBC binding, there were no discernible focal accumulations of F-actin present beneath the attached particles (Fig. 3). Although surface expression of 16:γ was somewhat higher than that of WT/16:γ−3G8 (Fig. 1), there was considerable overlap in the extent of surface expression of the two constructs, while there were essentially no detectable focal accumulations of F-actin in cells expressing WT/16:γ−3G8. Therefore, differences in surface expression could not account for the lack of focal accumulations of F-actin in cells expressing WT/16:γ−3G8. These results indicate that an intact ITAM is required for the focal accumulation of F-actin mediated by clustered γ subunits.
Syk Tyrosine Kinase and Actin Assembly

Respectively (mean ± S.E., n = 7). These differences were not statistically significant. Quantitation of Syk by immunoblotting confirmed the lack of Syk expression in Syk-/- cells and the overexpression of Syk in Syk+/-/- cells (Fig. 5A). By densitometry, one such clone contained 27 ± 7.9 times more Syk protein than did WT/16; γ cells (mean ± S.E., n = 4). When compared with WT/16; γ cells, Syk+/-/- cells displayed a higher basal level of tyrosine phosphorylation and an accentuated accumulation of phosphorysyl-containing proteins in response to clustering surface-bound 16;γ. In contrast, Syk-/-/16;γ displayed lower basal levels of phosphorysyl-containing proteins, which did not increase further following clustering of 16;γ (Fig. 5B). A similar trend was noted in either the phosphorysyl content of Syk, or Syk tyrosine kinase activity, as assessed by autophosphorylation of Syk derived from resting and mAb 3G8-stimulated cells (Fig. 5, C and D). Thus, the levels of basal and stimulated accumulation of phosphorysyl-containing proteins correlated with the relative expression of Syk.

Syk Is Required for 16;γ-mediated Actin Assembly—Based on earlier work (1), we developed an assay for F-actin using rhodamine-phalloidin and YO-PRO, a nuclear stain, and quantitated average F-actin content per cell using a fluorescence plate reader. This assay was linear over a wide range of cell concentrations using the same concentration of rhodamine-phalloidin, indicating that all F-actin binding sites were saturated with phalloidin (Fig. 6A). Incubation of WT/16; γ cells by IgG-RBCs led to a small time-dependent increase in F-actin, which peaked at 4 min (not shown). These changes were more easily observed in Syk+/-/-16;γ cells, which produced a greater magnitude of increase in F-actin upon addition of IgG-RBCs (Fig. 6B). The level of Syk expression influenced the magnitude of accumulation of F-actin in response to the IgG-containing ligand. Absence of Syk did not support significant increases in F-actin, while its overexpression led to an augmented ITAM-induced response (Fig. 6C). Cytochalasin D blocked the ITAM-
mediated increase in total cellular F-actin in WT/16:γ (not shown) and in Syk+/16:γ cells (Fig. 6C), indicating a requirement for barbed end actin filament growth. While the level of Syk expression influenced the basal quantity of phosphotyrosine-containing proteins (Fig. 4), it did not influence the basal F-actin content of the cells (not shown). However, Syk was required for focal F-actin staining beneath the test particles, while overexpression of Syk led to an augmented focal accumulation of F-actin (Fig. 7), which was blocked by cytochalasin D (not shown).

Regional changes in cortical F-actin have been documented in other cells using several stimuli (23, 26, 27). We therefore measured cortical F-actin content in transfectants challenged with IgG-RBCs. We compared the F-actin content in lymphocyte cytoplasm subjacent to attached IgG-RBCs with the F-actin content in other cortical areas of the same cell. Depending on the particular clone examined, cortical F-actin beneath attached IgG-RBCs was enriched 4–8-fold (mean 6.2 ± 0.9) in WT/16:γ cells, 10–14-fold (mean 11.7 ± 1.1) in Syk+/16:γ cells, and 1–1.2-fold (mean 1.1 ± 0.1) in Syk−/16:γ cells (Fig. 8A). Interestingly, the density of F-actin staining per pixel was greatest in Syk+/16:γ cells, followed by WT/16:γ and Syk−/16:γ cells (Fig. 8B), suggesting that maximal Syk expression favors the formation of a dense meshwork of actin filaments upon ITAM clustering.

**DISCUSSION**

Given the multitude of tyrosine kinases present in eukaryotic cells, and the possibility of redundant signaling pathways, it is important to establish functional roles for individual kinases. Previous studies, including those showing that Syk is capable of triggering (18) or enhancing (28) phagocytosis in COS cells, provided evidence that this kinase can trigger phagocytosis in a cell that is not normally phagocytic. This study demonstrates that Syk tyrosine kinase is required for ITAM-mediated actin assembly and Fcγ receptor-mediated phagocytosis and that no redundant pathways exist in these hematopoietic cells to effect these responses.

We did not address the mechanism of Syk-mediated actin assembly in this study, nor did we address the requirement for other kinases in ITAM-mediated signaling pathways. We suspect, however, that one or more members of the Src family is required for these events, since Lyn-negative DT40 cell lines
which were transfected with 16;γ were also incapable of supporting 16;γ-mediated actin assembly (not shown). Furthermore, fusion proteins bearing kinase-deficient Syk constructs do not trigger actin assembly in transfected COS cells (18), indicating that an intact Syk kinase domain is required for mediating actin assembly. It is likely that one or more Syk tyrosine kinase substrates enhance the formation of an as yet unidentified actin-nucleating activity.

While γ subunit-expressing macrophages are capable of Fcγ receptor-mediated phagocytosis, 16;γ-expressing DT40 cells did not support phagocytosis, as assessed by resistance to hypotonic lysis of cell-associated IgG-RBCs. DT40 cells transfected with human FcγR IIA, another ITAM-containing transmembrane protein, were equally potent in inducing actin assembly, but incapable of mediating phagocytosis (not shown). Why are DT40 lymphocyte transfecants capable of ITAM-directed actin assembly, yet incapable of ITAM-mediated phagocytosis? It could be argued that the levels of surface expression of 16;γ fusion proteins were insufficient to support phagocytosis. We were unable to isolate clones of DT40 cells that expressed higher levels of 16;γ to test this hypothesis directly. We think this explanation is unlikely, however, since macrophages are capable of some degree of particle ingestion, even when surface Fcγ receptor expression is decreased by nearly 90% when the cells are plated on adherent immune complexes (29). Since mammalian B-lymphocytes express FcγRIIB (30), a receptor that transmits an inhibitory signal to antigen receptors (31–33), we also considered the possibility that DT40 cells express the avian homolog of FcγRIIB. This receptor might be expected to dampen the phagocytic response by its co-ligation of IgG-RBCs. However, this explanation is unlikely, since untransfected DT40 cells were incapable of binding IgG-RBCs. Furthermore, addition to WT/16;γ cells of E-3G8, erythrocytes that bear F(ab)γ2 fragments of mAb 3G8 rather than intact IgG (34) and cannot ligate endogenous Fcγ receptors, also resulted in binding, but not ingestion. Finally, overexpression of Syk in 16;γ-expressing cells led to an augmented local accumulation of F-actin in response to IgG-RBCs, but did not confer to the transfected lymphocytes the ability to mediate particle ingestion (not shown), suggesting that the level of Syk tyrosine expression was not the limiting factor in promoting phagocytosis. Although we did not perform a direct comparison between the quantitative increases in F-actin due to the interaction of IgG-containing ligands with DT40 cells and macrophages, it is interesting to note that regional accumulations in F-actin beneath 16;γ-expressing lymphocytes appeared even more prominent than similar regions in FcγR-expressing macrophages (cf. Figs. 3 and 7 of the current study with Fig. 2 in Ref. 1).

While actin assembly is clearly required for pseudopod extension, these data suggest that actin assembly alone is insufficient for mediating phagocytosis. Other events, such as recruitment of new membrane components to the growing pseudopod, may be required for productive pseudopod extension. Alternatively, the newly formed submembranous actin network may need to be coupled to mechanotransducing proteins in order to achieve a significant degree of pseudopod extension, as suggested in a review of neuronal growth cone motility (35). Candidates for this activity include the MARCKS family of proteins (36) and members of the myosin I superfamily (37). Recent experiments have implicated one or both families of proteins in pseudopod extension (38) and phagocytosis (38, 39). We are currently testing these possibilities.

Acknowledgment—We thank Robert Kimberly (Cornell University Medical College) for E-3G8, Michael Cammer of the Analytic Imaging Facility of the Albert Einstein College of Medicine for help in image analysis, and Samuel C. Silverstein for a careful reading of the manuscript.

REFERENCES

1. Greenberg, S., El Khoury, J., Di Virgilio, F., Kaplan, E. M., and Silverstein, S. C. (1991) J. Cell Biol. 113, 757–767
2. Sheterline, P., Rickard, J. E., and Richards, R. C. (1984) Eur. J. Cell Biol. 34, 80–87
3. Salmon, J. E., Brogé, N. L., Edberg, J. C., and Kimberly, R. P. (1991) J. Immunol. 146, 997–1004
4. Vidalutti, S., Dessing, M., Aktories, K., Galliati, H., and Lanzavecchia, A. (1995) J. Exp. Med. 181, 577–584
5. Howe, L. R., and Weiss, A. (1995) Trends Biochem. Sci. 20, 59–64
6. Greenberg, S., Chang, P., and Silverstein, S. C. (1993) J. Exp. Med. 177, 529–534
7. Hutchinson, M. J., Harrison, P. T., Floto, R. A., and Allen, J. M. (1995) Eur. J. Immunol. 25, 481–487
8. Agarwal, A., Salen, P., and Robbins, K. C. (1993) J. Biol. Chem. 268, 15900–15905
9. Darby, C., Gaehlen, R. L., and Schreiber, A. D. (1994) J. Immunol. 152, 5429–5437
10. Hamada, F., Aoki, M., Akiyama, T., and Toyoshima, K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6305–6309
11. Marchall, A., Rivero-Lefranco, O. M., Agarwal, A., and Robbins, K. C. (1995) J. Biol. Chem. 270, 9115–9120
12. Wang, A. V. T., Schol, P. R., and Geha, R. S. (1994) J. Exp. Med. 180, 1165–1170
13. Weiss, A. (1993) Cell 73, 209–212
14. Cambier, J. C. (1995) J. Immunol. 155, 3281–3285
15. Greenberg, S. (1995) Trends Cell Biol. 5, 93–99
16. Chan, A. C., Iwashima, M., Turck, C. W., and Weiss, A. (1992) Cell 71, 649–652
17. Taniguchi, T., Kobayashi, T., Kondo, J., Takahashi, K., Nakamura, H., Suzuki, J., Nagai, K., Yamada, T., Nakamura, S., and Yamamura, H. (1993) J. Biol. Chem. 268, 15790–15796
18. Greenberg, S., Chang, P., Wang, D., Xavier, R., and Seed, B. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1103–1107
19. Takata, M., Sabe, H., Hata, A., Inazu, T., Homma, Y., Nukada, T., Yamamura, H., and Kurosaki, T. (1994) EMBO J. 13, 1341–1349
20. Greenberg, S., Chang, P., and Silverstein, S. C. (1994) J. Biol. Chem. 269, 3897–3902
21. Kurosaki, T., Gander, I., and Ravetch, J. V. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3837–3841
22. Theriot, J. A., and Mitchison, T. (1991) Nature 352, 126–131
23. Segall, J. E., Tyerech, S., Boselli, L., Masselling, S., Hefti, J., Chan, A., Jones, J., and Condeelis, J. (1996) Clin. Exp. Metastasis 14, 61–72
24. Bonnerot, C., Amigorena, S., Choquet, D., Pavlovich, R., Choukrourn, V., and Fridman, W. H. (1992) EMBO J. 11, 2747–2757
25. Park, J. G., Murray, R. K., Chien, P., Darby, C., and Schreiber, A. D. (1993) J. Clin. Invest. 92, 2073–2079
26. Redmond, T., and Zimgorn, S. H. (1993) Cell Motil. Cytoskeleton 26, 7–18
27. Norman, J. C., Price, L. S., Ridley, A. J., Hall, A., and Koffler, A. (1994) J. Cell Biol. 126, 1005–1015
28. Indik, Z. K., Park, J. G., Pan, X. Q., and Schreiber, A. D. (1995) Blood 85, 1175–1180
29. Michi, J., Unkeless, J. C., Pieczonka, M. M., and Silverstein, S. C. (1983) J. Exp. Med. 157, 1746–1757
30. Ravetch, J. V., and Kinet, J. (1991) Annu. Rev. Immunol. 9, 457–492
31. Amigorena, S., Bonnerot, C., Drake, J. R., Choquet, D., Hunziker, W., Guiliet, J. G., Webster, P., Sautes, C., Mellman, I., and Fridman, W. H. (1992) Science 256, 1808–1812
32. Muta, T., Kurosaki, T., Misulovin, Z., Sanchez, M., Nussenzweig, M. C., and Ravetch, J. V. (1994) Nature 368, 70–73
33. Dambrosio, D., Hipp, K. L., Minkoff, S. A., Mellman, I., Pani, G., Siminovich, K. A., and Cambier, J. C. (1995) Science 268, 293–297
34. Edberg, J. C., and Kimberly, R. P. (1992) J. Immunol. Methods 148, 179–184
35. Sheetz, M. P., Wayne, D. B., and Pearlman, A. L. (1992) Cell Motil. Cytoskeleton 22, 160–169
36. Aderem, A. (1992) Cell 71, 713–716
37. Wolenski, J. S. (1995) Trends Cell Biol. 5, 310–316
38. Allen, L. A. H., and Aderem, A. (1995) J. Exp. Med. 182, 829–840
39. Zhu, Z., Bao, Z., and Li, J. (1995) J. Biol. Chem. 270, 17652–17655