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Blockage of the Immune Complex-triggered Transmembrane Proximity Between Complement Receptor Type 3 and Microfilaments by Staurosporine and Methyl-2,5-dihydroxycinnamate

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Recent studies have suggested that integrin (CR3) participates in the signal transduction pathways of certain GPI-anchored phagocytic receptors including FcγRIIIB. One consequence of this functional linkage is an inducible association between CR3 and cortical microfilaments that is triggered by FcγRIIIB binding to immobilized immune complexes (IC). That this signaling event requires the co-expression of FcγRIIIB with CR3 was documented by the use of NIH 3T3 transfectants expressing both CR3 and FcγRIIIB (clone 3-23), CR3 alone (clone 3-19), and FcγRIIIB alone (clone 3-15). Pretreatment of 3-23 cells with protein kinase inhibitors such as staurosporine and methyl 2,5-dihydroxycinnamate (MDHC) blocked IC-stimulated CR3-microfilament proximity without affecting the extent to which FcγRIIIB constrains the lateral membrane mobility of a subset of CR3 on the cell surface (as measured in fluorescence recovery after photobleaching experiments). These data support that CR3 and FcγRIIIB molecules are physically and functionally associated and that ligation of FcgRIIIB triggers CR3-dependent signal transduction.

Keywords: Complement Receptor Type 3 (CR3), FcγRIIIB, Immune complex, Microfilament, Protein kinase inhibitor, Resonance Energy Transfer (r.e.t.), Staurosporine.

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Introduction

Complement receptor type 3 (CR3, $\alpha_m\beta_2$, CD11b/CD18), a member of the integrin supergene family, is a participant in many recognition and adhesive processes of human neutrophils including phagocytosis, cytolysis, chemotaxis, aggregation, and migration across endothelial barriers and through extracellular matrix (Arnaout, 1990; Hynes, 1992). CR3 is a heterodimeric transmembrane glycoprotein composed of an α (CD11b) and a β (CD18) chain that possess apparent M_rs of 155 kDa and 94 kDa, respectively (Springer, 1990; Hynes, 1992).

In addition to binding the complement fragment iC3b and ICAM-1, CR3 recognizes *E. coli* LPS, zymosan, β-glucan, *Leishmania* glycoprotein, and fibrinogen (Petty and Todd, 1993). Recent studies have suggested that CR3 also participates in the signal transduction pathways of other membrane receptors, thus broadening its physiological role in inflammation and host defense (Sehgal *et al.*, 1993; Petty and Todd, 1993; Zhou *et al.*, 1994). An actual physical association between GPI-linked protein Fcγ receptor type IIIB (FcγRIIIB; CD16b) and CR3 on neutrophils has been shown by cocapping and resonance energy transfer (r.e.t.) studies (Zhou *et al.*, 1992). Furthermore, this physical linkage was inhibited by certain soluble saccharides which also partially blocked FcγR-dependent, immune complex stimulated increases in intracellular calcium and release of superoxide radicals (Sehgal *et al.*, 1993). These data support the concept that a lectin–carbohydrate interaction (inhibitable by certain saccharides) is responsible for the signaling partnership between CR3 and FcγRIIIB.

Because neutrophils express FcγRII (CD32) in addition to FcγRIIIB (making it difficult to ascribe FcγR-dependent responses to either FcγR independently), transfectants

expressing either CR3 or FcγRIIIB, or both CR3 and FcγRIIIB were constructed to unambiguously determine the extent to which physical and functional interactions occur between these two receptors (Krauss *et al.*, 1994; Poo *et al.*, 1995). It was shown that the expression level of CR3 and FcγRIIIB on these transfectants was significant by flow cytometry assay even though it was less than that on human neutrophils (Krauss *et al.*, 1994). Fluorescence reconstitution after photobleaching (FRAP) experiments showed that the coexpression of CR3 with FcγRIIIB constrained the lateral mobility of a fraction of FcγRIIIB molecules (Poo *et al.*, 1995). Likewise, as shown in this report, coexpression of FcγRIIIB constrained the lateral mobility of a mobile subset of CR3 molecules, further supporting the existence of a physical association between CR3 and FcγRIIIB. Moreover, the functional dependence of FcγRIIIB on CR3 was demonstrated by the results of experiments in which the only transfectant capable of internalizing IgG-opsonized sheep erythrocytes was the clone coexpressing both CR3 and FcγRIIIB (Krauss *et al.*, 1994).

The CR3-dependent signaling events that are triggered by FcγRIIIB ligation and which led to cellular effector responses such as phagocytosis remain ill-defined. One intermediate signaling step that occurs as a consequence of immune complex binding to FcγR is an inducible association between CR3 and cytoskeletal actin. This was shown in r.e.t. experiments performed by this laboratory in which the physical proximity between CR3 and actin microfilaments rose as a result of neutrophil adherence to immobilized immune complexes (Zhou *et al.*, 1992). The involvement of cytoskeletal elements in integrin-dependent signaling is also suggested by the tyrosine phosphorylation of cytoskeletal proteins that occurs after integrin activation (Fuortes *et al.*, 1994; Graham *et al.*, 1994) Tyrosine phosphorylation is one well-known consequence of receptor-mediated transmembrane signaling (Petty, 1993) and is also known to influence neutrophil function (Kusunoki *et al.*, 1992; Fuortes *et al.*, 1993). Tyrosine phosphorylation of certain neutrophil lysate proteins increases from exposure to certain CR3-activating factors including N-formyl-methionyl-leucyl-phenylanaline, β-glucan, tumor necrosis factor (TNF), and phorbol esters (Kusunoki *et al.*, 1992; Gresham *et al.*, 1994; Fuortes *et al.*, 1994).

The experiments described in this report were designed to further examine the role of protein kinases in the CR3-dependent signaling process triggered by immune complex stimulation of FcγRIIIB. In 3T3 transfectants expressing both CR3 and FcγRIIIB, inhibitors of protein kinase C and tyrosyl kinase activity (staurosporine, MDHC, and genestein) blocked the inducible increase in r.e.t. between CR3 and cytoskeletal actin stimulated by immobilized immune complexes (but had no significant effect on lateral membrane mobility of CR3 molecules constrained by FcγRIIIB). These data support the concept that the activity of protein kinases leading to tyrosine phosphorylation plays a critical role in CR3-dependent-FcγRIIIB-mediated transmembrane signaling.

Materials and Methods

Monoclonal antibodies and materials $F(ab')_2$ fragments of mouse anti-CD11b antibody (Mol, clone 44) were prepared and labeled with fluorescein isothiocyanate (FITC) (Molecular Probes, Eugen, OR) as previously described (Zhou *et al.*, 1993). Tetramethylrhodamine (TRITC) conjugated phalloidin was purchased from Molecular Probes (Eugene USA). Anti-phosphotyrosine mAb (4G10) was purchased from Upstate Biotechnology (Lake Placid, USA).

Staurosporine, aprotinin, phenylmethylsulfonyl fluoride (PMSF), hydrogen peroxide (30%), N-acetyl-D-glucosamine (NADG), and sodium orthovanadate were purchased from Sigma Chemical Co. (St. Louis, MO). Methyl 2,5-dihydroxycinnamate (MDHC) and genestein were purchased from Gibco (Grand Island, USA). A stock solution of 5 mM vanadyl hydroperoxide (pervanadate) was prepared from sodium orthovanadate and hydrogen peroxide as described by Bennett *et al.* (1993).

Transfected cells An NIH 3T3 transfectant cell line which expresses both CR3 (CD11b/CD18) and FcγRIIIB (CD16b) (line 3-23), and a transfectant expressing CR3 alone (line 3-19) were prepared as previously described (Krauss *et al.*, 1994). NA2B (3T3 transfectant expressing CD16b alone) was constructed as described (Chen and Okayama, 1987). Briefly, 3T3 cells were transfected with calcium precipitated DNA mixture of 25 μg of CD16 cDNA and 5 μg of pSV_2neo and incubated overnight under 3% CO_2 at 37°C. These tranfectants were maintained in D-MEM supplemented with 10% fetal calf serum (Hyclone Corp., Logan, USA) and 1% gentamicin (Gibco Laboratories, Grand Island, USA). Prior to assay, transfectant cells were seeded on 25 mm^2 tissue culture plates (Costar Co., Cambridge, USA), allowed to grow near confluence ($4–6 \times 10^5$), washed with sterile PBS, and harvested by gentle scraping after culture in 3 ml of Puck's EDTA at 37°C for 3 min. For certain experiments, transfectants were grown in glass coverslips as described previously (Krauss *et al.*, 1994).

FRAP analysis of CR3 FRAP (fluorescence recovery or redistribution after photobleaching) was used to analyze the lateral diffusion of CR3 in transfectants expressing CR3 alone or co-expressed with FcγRIIIB. Transfectant cells adherent to coverslips were labeled with saturating amount of FITC-$F(ab')_2$ fragments of CD11b monoclonal antibody in PBS buffer alone or in PBS containing NADG (0.15 M), or staurosporine (0.5 μM) and incubated for 15 min at 37°C followed by washing with PBS. FRAP measurements were made as previously described (Poo *et al.*, 1995) with an ACAS 470 interactive laser cytometer (Meridian Instruments, Okemos, USA). Parameters for FRAP experiments were set as follows: laser wavelength, 488 nm; laser power, 200 mW; blast structure, 50%; and scan structure, 10%. Recovery of FITC fluorescence within bleached area due to translational diffusion of neighboring intact fluorescent molecules was measured by repetitive scanning of the cell surface with an

attenuated laser beam. All experiments were repeated more than three times.

Resonance energy transfer Transfectants were incubated in the presence or absence of staurosporine (0.5 μM) or MDHC (50 μg/ml) for 15 min at 37°C and then labeled with non-saturating amounts of FITC-conjugated $F(ab')_2$ fragments of anti-CD11b on ice for 30 min. These labeled cells were incubated on untreated or immune complex (BSA + anti-BSA antibody)-coated surfaces on glass coverslips prepared as previously described (Zhou *et al.*, 1993) at 37°C for 20 min. Cells were fixed with 3.7% paraformaldehyde in PBS for 10 min and then extracted with acetone at −20°C for 15 min. Microfilaments were labeled with 2 units of TRITC conjugated phalloidin (Molecular Probes, Eugene, OR) at 37°C for 20 min. Image collection and quantitation of r.e.t. between CR3 and cortical microfilaments were performed with an automated Zeiss axiovert inverted microscope and photomultiplier tube as previously described (Poo *et al.*, 1994). R.e.t. photon count rate derived from single cells was obtained by calculating the difference between photon count rate of a cell and the background photon count rate of the microscopic field adjacent to the cell. Thirty to fifty randomly selected cells were observed for photon counting in each experiment.

Results and Discussion

Proximity of CR3 with cortical microfilaments A functional association between CR3 and Fcγ receptors expressed by human neutrophils was suggested by the results of experiments in which FcγR-dependent adherence to immune complex-coated surfaces triggered an inducible linkage between CR3 and cytoskeletal actin (Zhou *et al.*, 1992). These data were consistent with our hypothesis that CR3 serves as a mechanotransducer of signals for GPI-anchored FcγRIIIB in neutrophils (Petty and Todd, 1993). However, since neutrophils express FcγRII in addition to FcγRIIIB, the contribution of the former to CR3-actin linkage was not excluded. In order to directly determine if FcγRIIIB-dependent immune complex stimulation induces a physical association between CR3 and cytoskeletal actin (independent of FcγRII), transfectant cell lines expressing CR3 alone, or CR3 plus FcγRIIIB were generated and subjected to analysis. For these experiments, transfectant cells were labeled with FITC-conjugated F(ab')2 anti-CD11b, allowed to adhere to immobilized immune complexes composed of BSA plus anti-BSA antibody (or control glass substrate), permeablized by fixation, and labeled with rhodamine-conjugated phalloidin as a marker for actin. Resonance energy transfer (r.e.t., reflecting close physical proximity) between donor (FITC) and acceptor (rhodamine) fluorochromes would indicate an inducible association between CR3 and cytoskeletal microfilaments. As shown by fluorescence microscopy (Fig. 1) and quantitative photon counting (Fig. 2), immune complex stimulation did indeed result in r.e.t. between FITC-labeled CR3 and rhodamine-labeled actin in transfectant line 3-23 co-expressing both receptors (Fig. 1, Panel h; Fig. 2, IC [3-23]) as compared to 3-23 cells exposed to controlled substrates (Fig. 1, Panel d; Fig. 2, Control [3-23]). Similar results were observed in neutrophils assayed in parallel (data not shown). Conversely, there was no significant increase in CR3-actin r.e.t. seen when transfectant line 3-19, expressing CR3 alone, was allowed to adhere to the immune complex-coated substrate (Fig. 2, IC [3-19] vs. control [3-19]). These data indicate that the close proximity of CR3 with cytoskeletal microfilaments induced by immune complexes requires the co-expression of FcγRIIIB, and that FcγRIIIB plus CR3 expression is sufficient for the signaling event to occur.

Effect of protein kinase inhibitors on the induced proximity between CR3 and cytoskeleton We then tested the hypothesis that protein kinase inhibitors such as staurosporine (0.5 μM), methyl 2,5-dihydroxycinnamate (MDHC 0.26 mM), and genestein (0.19 mM) block the FcγRIIIB-dependent association of CR3 with microfilaments triggered by immune complexes. After pre-treatment of the 3-23 transfectant with 0.5 μM staurosporine, an inhibitor of protein kinase C and tyrosyl kinase activity, the CR3-actin r.e.t. level in response to contact with immune complex-coated surfaces was reduced ($p < 0.001$) to basal levels, indistinguishable from that exhibited by the cells exposed to untreated surfaces (Figs. 1 and 2). Pre-incubation of 3-23 cells with tyrosyl kinase inhibitors, MDHC or genestein, also prevented the immune complex-triggered increase in CR3 to microfilament r.e.t. in similar rate with staurosporine (Fig. 2, IC + MDHC [3-23] and data not shown).

Since staurosporine has been reported to cause an increase in the expression of CR3 on neutrophils, it was important to exclude the possibility that changes in the number of CR3 sites on the plasma membrane of the 3-23 transfectant might be responsible for the effect of this agent in inhibiting CR3-to-actin association. However, by quantitative flow cytometric analysis comparing CD11b expression on 3-23 cells incubated in the presence and absence of staurosporine, there was no significant difference in surface CR3 expression (data not shown) as is also demonstrated qualitatively in Fig. 1 comparing panels f and n.

Lateral diffusion of CR3 on transfectants; effect of staurosporine Fluorescence redistribution after photobleaching (FRAP) experiments were performed to assess the extent to which the co-expression of FcγRIIIB constrains the lateral mobility of CR3 in transfectants and the influence that staurosporine has on the physical interaction between these two receptors. As shown in Table 1, the percent recovery of CR3 fluorescence after photobleaching was significantly less (36%) in 3-23 transfectants coexpressing FcγRIIIB than in 3-19

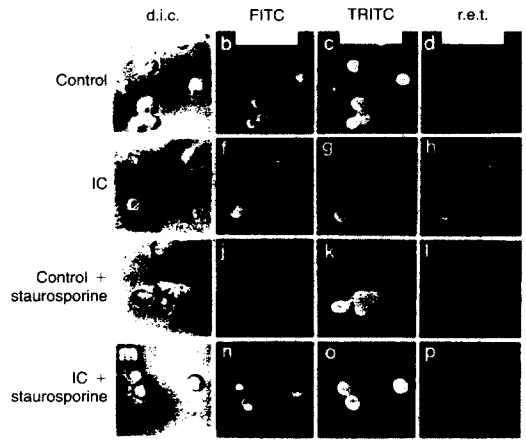

Fig. 1. Representative micrographs of resonance energy transfer between CR3 and cortical microfilaments on transfectants (3-23) expressing CR3 and FcγRIIIB. Cells were labeled with FITC-conjugated anti-CR3 antibody (clone 44) and TRITC-conjugated phalloidin on various surfaces. a–d and i–l are cells on control surface (PBS) and e–h and m–p are cells incubated on immune complex coated surfaces. a–h are cells incubated in the absence of staurosporine and i–p are cells incubated in the presence of staurosporine (0.5 μM). a, e, i, and m show differential interference contrast (d.i.c.), b, f, j, and n show flourescein fluorescence of anti-CR3 (Fab')$_2$ fragments. c, g, k, and o show rhodamine fluorescence of phalloidin bound to f-actin, and d, h, i, and p show r.e.t.

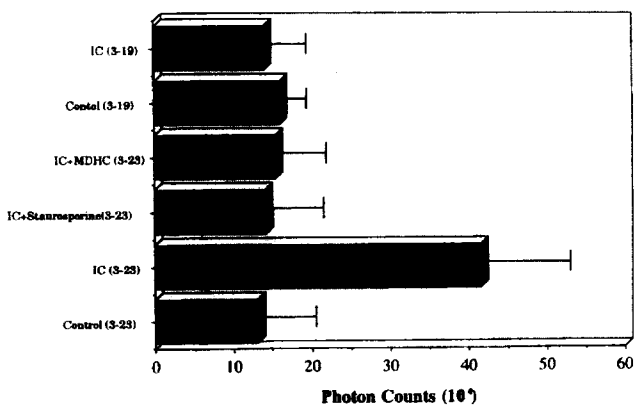

Fig. 2. Quantitation of resonance energy transfer between CR3 and F-actin by photon counts. IC refers to cells incubated on immune complex-coated surface (BSA + anti-BSA). Control surface was prepared with PBS. 3-23 cells express CR3 and FcγRIIIB and 3-19 cells express only CR3. 3-23 cells incubated on IC coated surface shows significant increase in r.e.t. ($p < 0.01$) between CR3 and cortical F-actin, as compared to cells incubated on control surface.

transfectants expressing CR3 alone (67%). This suggests that the co-expression of FcγRIIIB constrains the lateral mobility of a proportion of CR3 molecules and is consistent with other evidence supporting a physical linkage between a sub-population of CR3 and FcγRIIIB receptors (Poo *et al.*, 1995). Exposure of 3-23 cells to 0.15 mM NADG, which we have shown to inhibit the CR3-FcγRIIIB association in neutrophils, produced a rise in CR3 recovery to the level seen in 3-19 cells. However, exposure of 3-23 cells to staurosporine had no significant effect on CR3 mobility. The diffusion coefficient of CR3 did not differ significantly between 3-23 and 3-19 transfectants or as a result of either NADG or staurosporine exposure, suggesting no difference in the intrinsic mobility of CR3 in these cells. In aggregate, these data support the concept that a subset of CR3 is physically linked with FcγRIIIB and that staurosporine's inhibitory effect on the FcγRIIIB-dependent association between CR3 and microfilaments is not due to a direct inhibition of the physical association between CR3 and FcγRIIIB.

Table 1. FRAP analysis of CR3 on transfectants.

Cell	Treatment	Diffusion Coefficient (cm^2/sec)	Recovery (%)
3-19[a]	none	$3.3 \pm 2.9 \times 10^{-9}$	67 ± 19
3-23[b]	none	$2.7 \pm 2.5 \times 10^{-9}$	36 ± 16
3-23	NADG	$6.3 \pm 4.9 \times 10^{-9}$	71 ± 15[c]
3-23	staurosporine	$4.9 \pm 3.8 \times 10^{-9}$	42 ± 11[d]

[a] NIH 3T3 transfected cell line expressing CR3 only.
[b] NIH 3T3 transfected cell line expressing CR3 and FcRIIIB.
[c] $p + 0.01$ in comparison of the % recovery of NADG (0.15 M) treated 3-23 cells with untreated 3-23 cells.
[d] $p = 0.216$ in comparison of the % recovery of staurosporine (0.5 μM) treated 3-23 cells with untreated 3-23 cell.

The presence of an immobile subpopulation of CR3 which participates in the phagocytosis of CR3 targets was previously shown by Graham *et al.* (1989). Our FRAP studies support the concept that there is an immobile subset of CR3 expressed on transfectants. The immobile population of CR3 may be physically associated with other membrane proteins (Petty and Todd, 1993; Poo *et al.*, 1995). Here we show that the immobilized fraction of CR3 molecules is associated with FcγRIIIB and can be regulated by NADG which, by competitively inhibiting a lectin–carbohydrate-mediated linkage, may block the association between CR3 and FcγRIIIB. In epithelial cells, GPI-linked proteins are clustered in caveolae which are small membranous pore-like structures that may represent centers for signal transduction (Travis, 1993). Even though the presence of caveolae has not been demonstrated on neutrophils, it is possible that the immobile fraction of CR3 which has a physical association with FcγRIIIB is located in the caveolae-like structures where it is able to transduce intracellular signals mediated by GPI-linked proteins.

A physical and functional association between FcγRIIIB and CR3 has been suggested in many studies using human neutrophils (Zhou *et al.*, 1992; Petty and Todd, 1993; Sehgal *et al.*, 1993; Stöckl *et al.*, 1995). Since human neutrophils express not only FcγRIIIB but also FcγRII, transfectants expressing CR3 and FcγRIIIB were constructed to document an interaction between CR3 and FcγRIIIB. The studies using these transfectants confirmed a physical and functional linkage between CR3 and FcγRIIIB (Krauss *et al.*, 1994; Poo *et al.*, 1995), and support the concept that FcγRIIIB induces intracellular signaling via CR3.

What constitutes the signaling events resulting from this inducible inter-receptor interaction is unclear. The role of cytoskeletal elements in CR3-dependent FcγRIIIB-mediated signaling was suggested by the results of previous studies using neutrophils in which ligation of FcγRIIIB with immune complexes induced the proximity between CR3 and cytoskeletal actin (Zhou *et al.*, 1992). In the present study, we show that immune complex stimulation also induces the proximity between CR3 and cortical microfilaments of transfectant cells expressing both CR3 and FcγRIIIB. The activity of protein kinases resulting in tyrosine phosphorylation was implicated in the signaling phenomenon since staurosporine and other kinase inhibitors blocked the immune complex-triggered association between CR3 and microfilaments as detected by a reduction in inter-receptor r.e.t. As shown in parallel FRAP experiments, kinase inhibition had no significant effect on the association between CR3 and FcγRIIIB. Thus, we suggest that protein kinase activity is involved in the inducible linkage between CR3 and the cytoskeleton but is not required to preserve the physical linkage between CR3 and FcγRIIIB.

References

Arnaout, M. A. (1990) Structure and function of the leukocyte adhesion molecules CD11/CD18. *Blood* **75**, 1037–1050.

Amingorena, S., Salamero, J., Davoust J., Friedman, W. H., and Bonnerot, C. (1992) Tyrosine-containing motif that transduce cell activation signals also determines internalization and antigen presentation via type III receptors for IgG. *Nature* **358**, 337–341.

Bennett, P. A., Dixon, R. J., and Kellie, S. (1993) The phosphotyrosine phosphotase inhibitor vanadyl hydroperoxide induces morphological alterations, cytoskeletal rearrangements and increased adhesiveness in rat neutrophil leukocytes. *J. Cell Sci.* **106**, 891–901.

Berkow, R. L. and Dodson, R. W. (1990) Tyrosine-specific protein phosphorylation during activation of human neutrophils. *Blood* **75**, 2445–2452.

Buyon, J. P., Slade, S. G., Reibman, J., Abramson, S. B., Philips, M. R., Weissmann, G., and Winchester, R. (1990) Constitutive and induced phosphorylation of the ... and ... chains of the CD11/CD18 leukocyte integrin family: relationship to adhesion-dependent functions. *J. Immunol.* **144**, 191–197.

Chatila, T. A., Geha, R. S., and Arnout, M. A. (1989) Constitutive and stimulus-induced phosphorylation of CD 11/CD18 leukocyte adhesion molecules. *J. Cell Biol.* **109**, 3435–3444.

Chen, C. and Okayama, H. (1987) High-efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell Biol.* **7**, 2745–2752.

Clark, E. A. and Brugge, J. S. (1995) Integrins and signal transduction pathways: the road taken. *Science* **268**, 233–239.

Ferrell, J. E. and Martin, G. S. (1989) Tyrosine-specific protein phosphorylation is regulated by glycoprotein IIb-IIIa in platelets. *Cell* **86**, 2234–2238.

Fuortes, M., Jin, W. W., and Nathan, C. (1993) Adhesion-dependent protein tyrosine phosphorylation in neutrophils treated with tumor necrosis factor. *J. Cell Biol.* **120**, 777–784.

Fuortes, M., Jin, W. W., and Nathan, C. (1994) ... integrin-dependent tyrosine phosphorylation of paxillin in human neutrophils treated with tumor necrosis factor. *J. Cell Biol.* **127**, 1477–1483.

Graham, I. L., Anderson, D. C., Holers, V. M., and Brown, E. J. (1994) Complement receptor 3 is required for tyrosine phophorylation of paxillin adherent and nonadherent neutrophils. *J. Cell Biol.* **127**, 113–114.

Graham, I. L., Gresham, H. D., and Brown, E. J. (1989) An immobile subset of plasma membrane CD11b/ CD18 (Mac-1) is involved in phagocytosis of targets recognized by multiple receptors. *J. Immunol.* **142**, 2352–2358.

Huang, M. M., Indik, Z., Brass L. F., Hoxie, J. A., Shreiber, A. D., and Brugge, J. S. (1992) Activation of FcγRII induces tyrosine phosphorylation of multiple proteins including FcγRII. *J. Biol. Chem.* **267**, 5467–5473.

Hynes, R. O. (1992) Integrins: versatility, modulation, and signaling — in cell adhesion. *Cell* **69**, 11–25.

Kimberly, R. P., Ahlstrom, J. W., Click, M. E., and Edberg, J. C. (1990) The glycosyl phosphatidylinositol linked FcγIII mediates transmembrane signaling events distinct from FcγI. *J. Exp. Med.* **171**, 1239–1255.

Krauss, J. C., Poo, H., Xue, W., Mayo-Bond, L., Todd, R. F., and Petty, H. R. (1994) Reconstitution of antibody-dependent phagocytosis in fibroblast expression Fcγ receptor IIIB and the complement receptor type 3. *J. Immunol.* **153**, 1769–1777.

Kusunoki, T., Higashi, H., Hosoi, S., Hata, D., Sugie, K., Mayumi, M., and Mikawa, H. (1992) Tyrosine phosphorylation and its role in superoxide production by human neutrophils stimulated with FMLP and IgG. *Biochem. Biophys. Res. Commm.* **183**, 789–796.

Merrill, J. T., Slade, S. G., Weissman, G., Winchester, R., and Buyon, J. P. (1990) Two pathways of CD11b/CD18-mediated neutrophil aggregation with different involvement of protein kinase C-dependent phophorylation. *J. Immunol.* **145**, 2608–2615.

Petty, H. R. (1993) *Molecular Biology of Membranes: Structure and Function.* Plenum Press, New York.

Petty, H. R. and Todd, R. F. (1993) Receptor–receptor interaction of complement receptor type 3 in neutrophil membranes. *J. Leukoc. Biol.* **54**, 492–494.

Poo, H., Fox, B. A., and Petty, H. R. (1994) Ligation of CD3 triggers transmembrane proximity between LFA-1 and cortical microfilaments in cytotoxic tumor infiltrating T cells: a quantative resonance energy transfer microscopy study. *J. Cell. Physiol.* **159**, 176–180.

Poo, H., Krauss, J. C., Mayo-Bond, L., Todd, R. F., and Petty, H. R. (1995) Interaction of Fcγ receptor type IIIB with complement receptor type 3 in fibroblast transfectants: evidence from lateral diffusion and resonance energy transfer studies. *Mol. Biol.* **247**, 597–603.

Rouby, R. A. S., Ross, G. D., Merrill, J. T., Walton, F., Reed, W., Winchester, R. J., and Buyon, J. P. (1991) Staurosporine inhibits neutrophil phagocytosis but not iC3b binding mediated by CR3 (Cd11b/CD18). *J. Immunol.* **146**, 3557–3562.

Sehgal, G., Zhang, K., Todd, R. F., Boxer, L. A., and Petty, H. R. (1993) Lectin-like inhibition of immune complex receptor-mediated stimulus of neutrophils: effect on cytosolic calcium release and superoxide production. *J. Immunol.* **150**, 4571–4580.

Springer, T. A. (1990) Adhesion receptors of immune system. *Nature* **346**, 425–433.

Stöckl, J., Majdic, O., Pickl, W. F., Rosenkranz, A., Prager, E., Gschwantler, E., and Knapp W. (1995) Granulocyte activation via a binding site near the c-terminal region of complement receptor type 3 ... chain (CD11b) potentially involved in intramembrane complex formation with glycosylphosphatidylinositol-anchored Fcγ IIIB (CD16) molecules. *J. Immunol.* **154**, 5452–5465.

Travis, J. (1993) Cell biologist explore "tiny caves." *Science* **262**, 1208–1209.

Wilson, J. M., Ping, A. J., Krauss, J. C., Mayo-Bond, L., Rogers, C. E., Anderson, D. C., and Todd, R. F. (1990) Correction of CD 18-deficient lymphocytes by retrovirus-mediated gene transfer. *Science* **248**, 1413–1416.

Yamada, K., Jesesema L. C., and Beaven, M. A., (1992) Certain inhibitors of protein serine/threonine kinases also inhibits tyrosine phosphorylation of phospholipase C and other proteins and reveal distinct roles for kinase(s) and protein kinase C in stimulated, rat basophilic RBL-2H3 cells. *J. Immunol.* **149**, 1031–1037.

Zhou, M. J., Todd, R. F., and Petty, H. R. (1991) Detection of transmembrane linkage between immunoglobulin or complement receptors and the neutrophil's cortical microfilaments by resonance energy transfer microscopy. *J. Mol. Biol.* **218**, 263–268.

Zhou, M. J., Poo, H., Todd, R. F., and Petty, H. R. (1992) Surface-bound immune complexes trigger transmembrane proximity between complement receptor type 3 and the neutrophil's cortical microfilaments. *J. Immunol.* **148**, 3550–3553.