Acta Biochimica Polonica

Vol. 46 No. 1/1999

107-116

QUARTERLY

The role of cholesterol and sphigomyelin in tyrosine phosphorylation of proteins and capping of Fcγ receptor II*°

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Received: 19 November, 1998

Key words: cholesterol, Fc gamma receptor, sphingomyelin, receptor clustering

Cross-linking of cell surface receptors by multivalent ligands, e.g. by antibodies, evokes their clustering – patching. Subsequently, these clusters can be translocated by the acto-myosin machinery toward one pole of the cell and assembly cap. Patching of FcγRII in U937 cells correlates with tyrosine phosphorylation of several proteins while cap assembly correlates with their dephosphorylation. To study the mechanism of activation of tyrosine kinases during FcγRII activation we disturbed the organization of the putative plasma membrane microdomains by depletion of membrane cholesterol and sphingomyelin. Cholesterol was removed with the use of β-cyclodextrin while sphingomyelin was decomposed by exogenous sphingomyelinase. Cyclodextrin at 5–10 mM removed about 70% of cholesterol from the cells and abolished the assembly of FcγRII caps thereby arresting the receptors at the patching stage. Similarly, 70 mU/ml sphingomyelinase inhibited cap formation by 60%. Cholesterol and sphingomyelin depletion also suppressed the tyrosine phosphorylation of proteins which accompanied cross-linking of FcγRII. The observations indicate that cholesterol and sphingomyelin can control the interactions of tyrosine kinases with clustered FcγRII.

Fcγ receptors (FcγRs) are a family of transmembrane proteins (FcγRI–FcγRIII) which recognize and bind the Fc domain of IgG. The interaction between FcγRs and IgG is the first step of phagocytosis of invading microorganisms which are opsonized by host serum antibodies (Silverstein *et al.*, 1989; Kwiatkowska & Sobota, 1999a). It is assumed that the receptors form clusters during ligation since several intracellular responses triggered dur-

*Presented at the 34th Meeting of the Polish Biochemical Society, September, 1998, Białystok, Poland.

°This work was supported by the State Committee for Scientific Research to the Nencki Institute of Experimental Biology.

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Abbreviations: FcγR, Fcγ receptors; FITC, fluorescein isothiocyanate; HBS, Hepes-buffered saline; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate.

ing phagocytosis can be evoked by the cross-linking of FcγRs with specific anti-receptor antibodies (Huang *et al.*, 1992; Agarwal *et al.*, 1993; Kiener *et al.*, 1993). Under the influence of anti-FcγR antibodies the cross-linked receptors form clusters, named patches, which can be accumulated in an acto-myosin dependent way on one pole of the cell and assembly cap (Kwiatkowska *et al.*, 1997; Kwiatkowska & Sobota, 1999a). Clustering of FcγRs is accompanied by a sequential activation of protein kinases of the src- and syk-families (Bewarder *et al.*, 1996; Greenberg *et al.*, 1996; Matsuda *et al.*, 1996). As a result several proteins including FcγRs are phosphorylated (Huang *et al.*, 1992; Grennberg *et al.*, 1994; Strzelecka *et al.*, 1997).

The mechanism by which clustering of FcγRs leads to activation of the src-family tyrosine kinases is not understood. Recent data indicate that these kinases are concentrated on the cytoplasmic surface of the plasma membrane in special Triton X-100-insoluble microdomains (Stefanova *et al.*, 1991; Cinek & Horejsi, 1992; Anderson, 1993; Dráberová & Dráber, 1993; Shenoy-Scaria *et al.*, 1992; Liu *et al.*, 1997). These detergent-insoluble fractions are also enriched in glycosylphosphatidylinositol-anchored proteins, sphingolipids and cholesterol (Brown & Rose, 1992; Dorahy *et al.*, 1996; Fujimoto, 1996; Simons & Ikonen, 1997). It is presumed that such plasma membrane microdomains may serve as centers of recruitment for clustered receptors and enable the association of the receptors with src-family tyrosine kinases (Field *et al.*, 1995).

Participation of cholesterol and sphingolipids is essential for the maintenance of plasma membrane microdomains (Schroeder *et al.*, 1994; Dráberová *et al.*, 1996; Brown, 1998). Therefore, we examined how changes in cholesterol and sphingomyelin content in U937 cells can affect the transduction of signals triggered by cross-linked FcγRII. We demonstrate that depletion of cholesterol and sphingomyelin blocks tyrosine phosphorylation of proteins induced by cross-linking of FcγRII and suppresses capping of the receptor.

MATERIALS AND METHODS

Antibodies and reagents. The mouse monoclonal antibody to Fcγ receptor II (clone IV.3) was prepared from a culture medium of hybridoma cells obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Monoclonal anti-phosphotyrosine, clone PY20, was from Transduction Laboratories (Lexington, KY, U.S.A.), anti-mouse FITC-conjugated goat IgG was from Jackson ImmunoResearch Laboratory (West Grove, PA, U.S.A.). Fetal calf serum was purchased from Gibco Laboratories (Geithesburg, MD, U.S.A.). Bovine serum albumin was from ICN (Costa Mesa, CA, U.S.A.). Cholesterol, cholesterol oxidase, β-cyclodextrin, neutral sphingomyelinase were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The Enhanced Chemiluminescence Kit was from Pierce (Rockford, IL, U.S.A.).

Cell culture. The U937 human monocytic cells were cultured in RPMI 1640 medium (WSiS, Lublin, Poland) supplemented with 10% heat inactivated fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) in a humidified incubator at 37°C with 5% CO_2. The cells were usually harvested at a density of 10^6 cells/ml, washed twice in Hepes-buffered saline medium (HBS) containing 125 mM NaCl, 5 mM KCl, 10 mM $NaHCO_3$, 1 mM KH_2PO_4, 10 mM glucose, 0.2% bovine serum albumin, 20 mM Hepes, pH 7.4, and used for experiments. Cell viability was tested using 0.5% trypan blue in phosphate-buffered saline (PBS).

Cholesterol and sphingomyelin depletion studies. Measurement of cell unesterified cholesterol. For cholesterol depletion experiments the cells (2×10^6 cells/ml) were pretreated with different concentrations of β-cyclodextrin prepared in HBS medium, for 1 h at 37°C. To destroy cell membrane

sphingomyelin the cells were incubated with neutral sphingomyelinase (70 mU/ml) for 1 h at 37°C. After these treatments the cells were washed twice with HBS and the amount of unesterified cholesterol in cells was analyzed by a fluorimetric method (Gray *et al.*, 1995) with some modifications. In this method cholesterol oxidase oxidizes cholesterol with the formation of 1 mole hydrogen peroxide per mole of cholesterol. The peroxide is decomposed by peroxidase with simultaneous reduction of *p*-hydroxyphenylacetic acid to the fluorescent dimer 6,6′-dihydroxy-3,3′-bisphenyldiacetic acid. For the cholesterol quantification, washed cells were resuspended in 300 μl of isopropyl alcohol, sonicated, and incubated for 15 min at room temperature. The material was centrifuged at 5000 × ***g*** for 5 min. The supernatant (100 μl) was mixed with 1 ml of a fluorescence assay mixture (0.1 U/ml cholesterol oxidase, 2 U/ml horseradish peroxidase, 5 mM sodium cholate, 0.6 mg/ml *p*-hydroxyphenylacetic acid, 50 mM phosphate buffer, pH 7.0) and incubated at 37°C for 30 min. To stop the reaction 175 μl of 0.5 N NaOH was added. The fluorescence intensity was measured with a spectrofluorimeter (Perkin Elmer 5B) with an excitation wavelength of 325 nm, and emission at 415 nm. Cholesterol standards (0.4–1.6 μg/sample), processed as described above for cell cholesterol, were used to prepare a calibration curve. The amounts of cell cholesterol obtained from a calibration curve was expressed in micrograms of unesterified cholesterol per 2×10^6 cells.

Patching and capping of Fcγ receptor II. Cells (1.2×10^7/ml) pretreated with β-cyclodextrin and sphingomyelinase or maintained in HBS medium (control sample) were incubated with mouse IV.3 anti-FcγRII antibody at 0°C for 20 min. After washing with ice-cold HBS the cells were exposed to anti-mouse FITC-conjugated goat IgG at 0°C for 20 min in order to cross-link FcγRII – patching. To induce cap formation of FcγRII the cells were warmed at 20°C for 10 min. At various time points of experiments cell samples were withdrawn and prepared either for immunofluorescence or immunoblotting studies.

Immunofluorescence studies. Cells were fixed in 3% formaldehyde/PBS at 0°C for 10 min, and at room temperature for 20 min. The samples were mounted in Mowiol (Polysciences) containing 2.5% 1,4-diazabicyclo[2.2.2]octane (DABCO) (Sigma) as an antifading agent and examined under a Nikon fluorescence microscope. The percentage of cells having caps, i.e., those in which cross-linked Fcγ receptor II was accumulated on < 25% of the cell surface was determined by counting of 100–150 cells per sample.

Electrophoresis and immunoblotting. Pelleted cells were resuspended in ice-cold Tris-buffered saline containing 0.5 μg/ml leupeptin, 2 μg/ml aprotinin, 10 mM NaF, 1 mM Na_3VO_4, 2 mM EDTA, 0.2 mM PAO, 1 mM phenylmethylsulphonyl fluoride (PMSF), for 2 min at 0°C. After that, samples were mixed with an equal volume of 2 × SDS-sample buffer and boiled for 5 min. Proteins were separated by SDS/polyacrylamide gel electrophoresis and transferred onto nitrocellulose sheets. The blots were developed with anti-phosphotyrosine antibody (Kwiatkowska & Sobota, 1998). Immunoreactive bands were visualized with the Enhanced Chemiluminescence detection system. Molecular masses of separated polypeptides were estimated using prestained molecular mass standards (Bio-Rad).

RESULTS

Depletion of cell cholesterol by β-cyclodextrin

To remove cholesterol from plasma membrane of U937 cells β-cyclodextrin, a cell-nonpermeable cyclic oligomer of glucose, was applied (Kilsdonk *et al.*, 1995; Atger *et al.*, 1997). The amount of unesterified cholesterol in cells was estimated by a fluorimetric method (Gray *et al.*, 1995) using cholesterol

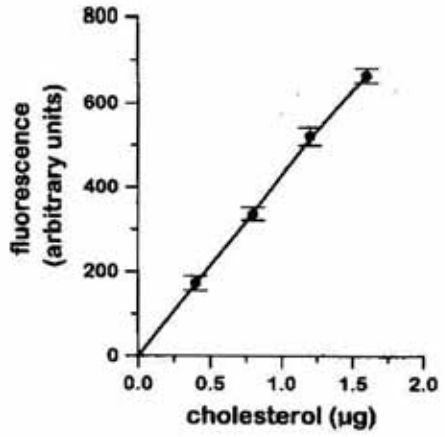

Figure 1. Calibration curve for cell cholesterol estimated by fluorimetric method.

The data shown are means ±S.E.M. of four experiments.

standards in the range 0.4–1.6 µg/sample. Within the concentration range the fluorescence intensity increased in a linear manner (Fig. 1). The level of cellular cholesterol was reduced by 70% during a 1 h incubation of the cells at 37°C with 5 mM β-cyclodextrin. The efficiency of cholesterol removal was only slightly improved when higher concentrations of the drug, up to 10 mM, were examined (Fig. 2). Cyclodextrin was non-toxic to the cells, as established by the trypan blue viability test (not shown).

An influence of depletion of sphingomyelin on cell cholesterol content was next tested since cholesterol is likely to associate with sphingolipids in the plasma membrane. However, the enzymatic digestion of sphingomyelin by exogenously added neutral sphingomyelinase (70 mU/ml, 1 h at 37°C) did not change the level of cellular cholesterol (Fig. 2).

Cholesterol and sphingomyelin depletion suppress capping of FcγRII

Cross-linking of FcγRII with the specific mouse IV.3 anti-FcγRII and anti-mouse FITC-conjugated IgG at 0°C induced clustering of the receptors – patching (Fig. 3A). The patches uniformly distributed over the cell surface were translocated toward one pole of the cell upon cell warming to 20°C – cap formation (Fig. 3B).

Depletion of cell cholesterol by 5–10 mM β-cyclodextrin had a prominent effect on the receptor redistribution. The drug almost completely abolished, by over 96%, the assembly of FcγRII caps (Fig. 4). The cross-linked receptors were arrested at the patching stage (Fig. 3C). Formation of FcγRII caps was also inhibited under treatment of cells with exogenous sphingomyelinase. Cap assembly was reduced by 60% (Fig. 4). In the cells preserving residual capping ability the caps were not fully developed and part of the receptor patches remained scattered over the cell surface (Fig. 3D).

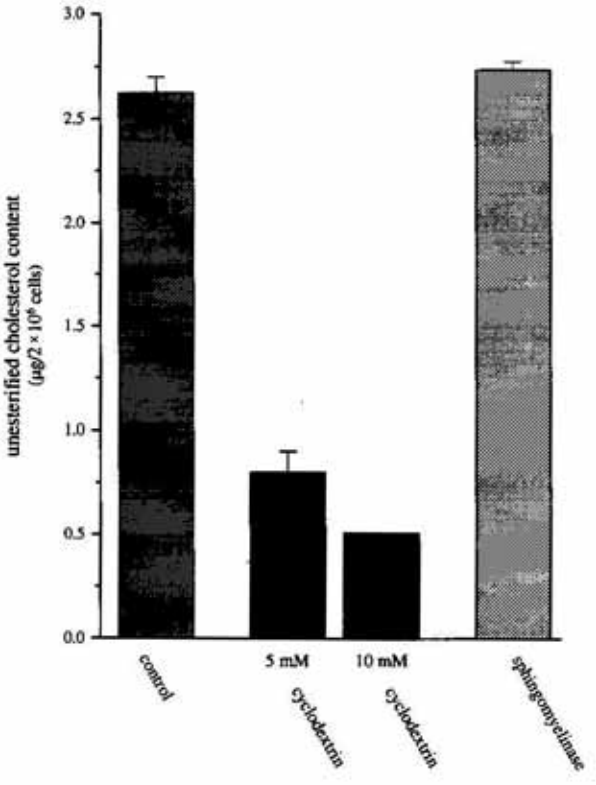

Figure 2. Effect of incubation of U937 cells with β-cyclodextrin and sphingomyelinase on cholesterol depletion.

The cells were incubated with the tested compounds for 1 h at 37°C after which the lipid fraction was extracted and the content of unesterified cholesterol was determined by fluorimetric method. Values are mean ±S.E.M. of two experiments.

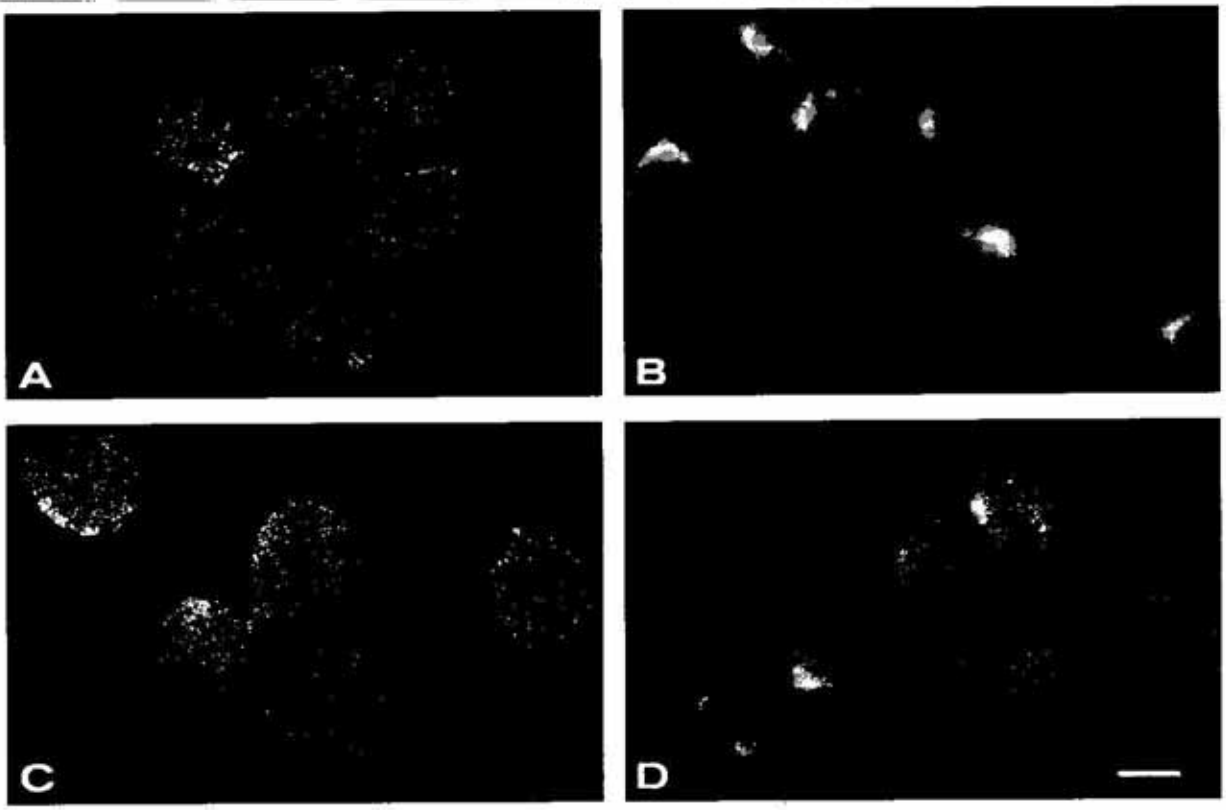

Figure 3. β-Cyclodextrin and shingomyelinase inhibit the assembly of FcγRII caps — immunofluorescence studies.

(A, B) Untreated, control cells were sequentially exposed to mouse anti-FcγRII and anti-mouse goat IgG-FITC at 0°C to cross-link the receptor — patching (A) and next were shifted to 20°C for10 min to induce formation of FcγRII caps (B). **(C)** Cells treated with 5 mM β-cyclodextrin for 1 h at 37°C before FcγRII cross-linking failed to form the receptor caps on 10 min warming at 20°C. **(D)** Cells preincubated with 70 mU/ml neutral sphingomyelinase (1 h, 37°C) followed by anti-FcγRII antibodies preserved residual capping ability. Scale bar = 10 μm.

Protein tyrosine phosphorylation is blocked upon cholesterol and sphingomyelin depletion

Cross-linking of FcγRs and related immunoreceptors with specific antibodies induces the phosphorylation of tyrosine residues of several proteins (Huang *et al.*, 1992; Kiener *et al.*, 1993; Zenner *et al.*, 1995). Accordingly, patching of FcγRII in U937 cells at 0°C evoked an increase of tyrosine phosphorylation of 150, 130, 110, 95, 28 kDa polypeptides (Fig. 5, compare lane 2 and 1). Assembly of the receptor caps at 20°C correlated with the dephosphorylation of these proteins (Fig. 5, lane 6).

Pretreatment of the cells with 5 mM β-cyclodextrin precluded protein tyrosine phosphorylation which occurred during the cross-linking of FcγRII in untreated cells (Fig. 5, lanes 3, 4). Similarly, depletion of plasma membrane sphingomyelin by exogenous sphingomyelinase reduced the level of protein phosphorylation (Fig. 5, lane 5). When β-cyclodextrin and sphingomyelinase pretreated cells were shifted to 20°C in order to induce cap formation, no significant changes in the pattern of protein tyrosine phosphorylation were observed (not shown).

Inhibition of protein tyrosine phosphorylation by cholesterol and sphingomyelin depletion correlated with the inability of the cells to assembly FcγRII caps (compare Fig. 5 with Figs. 3 and 4).

DISCUSSION

We have recently demonstrated that patching (cross-linking) of FcγRII by specific anti-

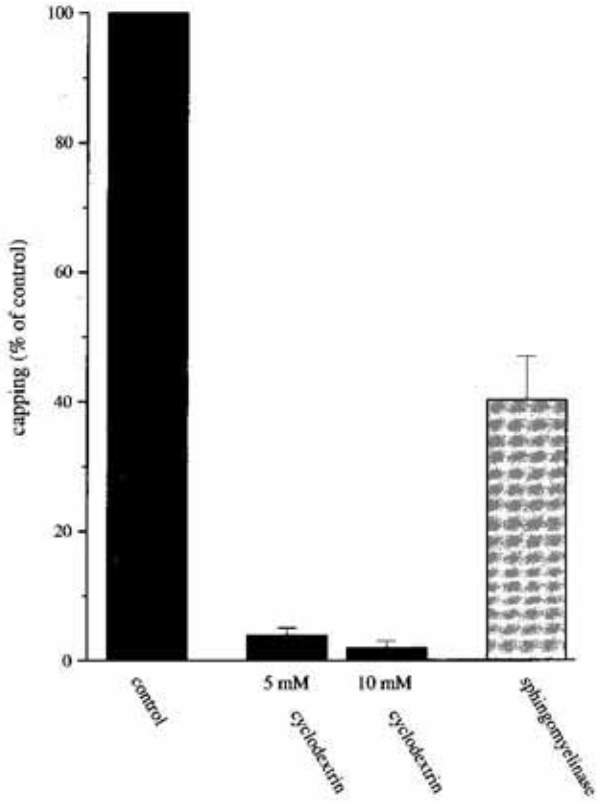

Figure 4. Estimation of inhibitory influence of β-cyclodextrin and sphingomyelinase on assembly of FcγRII caps.

The cells with caps were scored under a fluorescence microscope assuming that cap was formed when the cross-linked receptors occupied less than 25% of the cell surface. Data show the mean ±S.E.M. from six experiments.

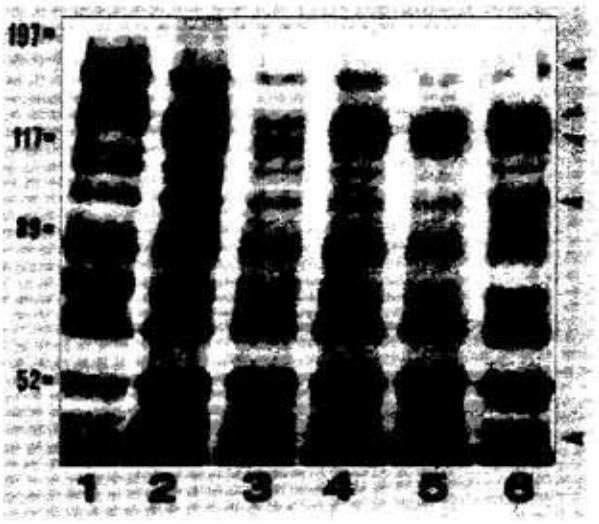

Figure 5. Cholesterol and sphingomyelin depletion disturbs protein tyrosine phosphorylation during cross-linking of FcγRII.

Lane 1: Unstimulated, resting cells displayed low level of protein tyrosine phosphorylation. **Lane 2:** Cross-linking of FcγRII with mouse anti-FcγRII and anti-mouse goat IgG-FITC at 0°C triggered tyrosine phosphorylation of several proteins (arrowheads). **Lanes 3, 4, 5:** Before cross-linking of FcγRII cells were exposed to 5 mM cyclodextrin (lane 3), 2 mM cyclodextrin (lane 4) or 70 mU/ml sphingomyelinase (lane 5). Note lack of the rise in protein tyrosine phosphorylation which accompanied receptor cross-linking in untreated cells (compare with lane 2). **Lane 6:** Assembly of FcγRII caps at 20°C in cells unexposed to the compounds was correlated with dephosphorylation of protein tyrosine residues. On the left, molecular masses of prestained standards (myosin, 197 kDa; β-galactosidase, 117 kDa; bovine serum albumin, 89 kDa; ovalbumin, 52 kDa) are shown.

bodies in U937 cells is accompanied by intensive phosphorylation of tyrosine residues of several proteins (Kwiatkowska *et al.*, 1997; Kwiatkowska & Sobota, 1999b). Inhibitors of protein tyrosine kinases abolished this phosphorylation and simultaneously blocked translocation of cross-linked FcγRII, arresting the receptors at the patching stage. These data indicated that the activity of tyrosine kinases is a prerequisite for capping of FcγRII to occur.

Here, we report that protein tyrosine phosphorylation, as well as capping of FcγRII, is inhibited upon depletion of cellular cholesterol and sphingomyelin. The lack of tyrosine phosphorylation of proteins during patching of FcγRII could result either from inactivation of tyrosine kinases or from the inaccessibility of their target proteins. Since, in our hands, depletion of cholesterol and sphingomyelin was non-toxic to the cells (confirmed by trypan blue exclusion test) we assumed that disturbances in kinase-substrate interactions are likely to take place. Tyrosine kinases of the src-family initiate a signaling cascade triggered by cross-linked FcγRs (Bewarder *et al.*, 1996; Matsuda *et al.*, 1996). The palmitoylated and myristoylated N-terminus of these kinases provides an anchor by which

the enzymes are docked into detergent-insoluble microregions of the plasma membrane (Shenoy-Scaria *et al.*, 1994; Resh, 1994). The integrity of these membrane microdomains is maintained by cholesterol and sphingolipids which laterally separate within the glycerophospholipid environment (Schroeder *et al.*, 1994; Edidin, 1997; Simons & Ikonen, 1997; Brown, 1998). We assume that the removal of cholesterol by cyclodextrin, or the destruction of sphingomyelin by sphingomyelinase, leads to disorganization of the plasma membrane microdomains and to the dispersion of membrane anchored src-family kinases. Under these conditions, FcγRs cross-linked by externally added antibodies, fail to interact with src-family kinases; this blocks signal transduction and as a result no capping of the receptor occurs. Our current knowledge about the role of cholesterol-sphingolipids microdomains in tyrosine kinase function sheds new light on the results reported almost 20 years ago by Karnovsky and colleagues (Klausner *et al.*, 1980; Hoover *et al.*, 1983). In their studies the removal of cholesterol from lymphocytes by incubation with lipid vesicles inhibited the capping of cell surface immunoglobulin. Although, Karnovsky's group proposed that lipid domains of the plasma membrane are essential for capping, these structures were considered as calcium enriched compartments. Release of ions during patching was proposed to control the interaction between plasma membrane proteins and the actin-based cytoskeleton during cap formation.

In our studies we depleted cellular cholesterol with the use of β-cyclodextrin. This compound associates with, but does not penetrate through, the plasma membrane and specifically sequesters cholesterol (Kilsdonk *et al.*, 1995; Atger *et al.*, 1997; Christian *et al.*, 1997). Due to a high affinity of β-cyclodextrin for sterols it does not remove membrane phospholipids (Yancey *et al.*, 1996). Since the plasma membrane contains 90% of total cellular cholesterol (Lange *et al.*, 1989) cells can be efficiently deprived of cholesterol with the use of β-cyclodextrin without disturbing cell integrity. Despite the well documented use of β-cyclodextrin for the manipulation of the plasma membrane cholesterol content, there are few reports examining the influence of this process on plasma membrane function (Neufel *et al.*, 1996; Harder *et al.*, 1998; Keller & Simon, 1998). On the other hand, lowering cellular cholesterol by growing the cells under cholesterol depletion conditions as well as oxidation of membrane cholesterol affect the clustering of some plasma membrane receptors, organization of caveolae and caveolae-dependent internalization of these receptors (Chang *et al.*, 1992; Smart *et al.*, 1994). Cyclodextrins, in view of their specificity and efficiency in cholesterol removal from the plasma membrane, provide a convenient tool for the studies of the role of cholesterol in cell surface receptor behavior.

We thank Dr. Edward Czuryło for valuable advice concerning the use of the spectrofluorimeter, and Kazimiera Mrozińska for an excellent technical assistance.

REFERENCES

Agarwal, A., Salem, P. & Robbins, K.C. (1993) Involvement of $p72^{syk}$, a protein-tyrosine kinase, in Fcγ receptor signaling. *J. Biol. Chem.* **268**, 15900–15905.

Anderson, R.G.W. (1993) Caveolae: where incoming and outgoing messengers meet. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10909–10913.

Atger, V.M., de la Llera Moya, M., Stoudt, G.W., Rodrigueza, W.V., Phillips, M.C. & Rothblat, G.H. (1997) Cyclodextrins as catalysts for removal of cholesterol from macrophage foam cells. *J. Clin. Invest.* **99**, 773–780.

Bewarder, N., Weinrich, V., Budde, P., Hartmann, D., Flaswinkel, H., Reth, M. & Frey, J. (1996) *In vitro* and *in vivo* specificity of pro-

tein tyrosine kinases for immunoglobulin G receptor (FcγRII) phosphorylation. *Mol. Cell. Biol.* **16**, 4735–4743.

Brown, D.A. & Rose, J.K. (1992) Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* **68** 533–544.

Brown, R.E. (1998) Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. *J. Cell Sci.* **111**, 1–9.

Chang, W.-J., Rothberg, K.G., Kamen, B.A. & Anderson, R.G.W. (1992) Lowering the cholesterol content of MA104 cells inhibits receptor-mediated transport of folate. *J. Cell Biol.* **118**, 63–69.

Christian, A.E., Haynes, M.P., Phillips, M.C. & Rothblat, G.H. (1997) Use of cyclodextrins for manipulating cellular cholesterol content. *J. Lipid Res.* **38**, 2264–2272.

Cinek, T. & Horejsi, V. (1992) The nature of large noncovalent complexes containing glycosyl-phosphatidylinositol-anchored membrane glycoproteins and protein tyrosine kinases. *J. Immunol.* **149**, 2262–2270.

Dorahy, D.J., Lincz, L.F., Meldrum, C.J. & Burns, G.F. (1996) Biochemical isolation of a membrane microdomain from resting platelets highly enriched in the plasma membrane glycoprotein CD36. *Biochem. J.* **319**, 67–72.

Dráberová, L. & Dráber, P. (1993) Thy-1 glycoprotein and src-like protein-tyrosine kinase $p53/p56^{lyn}$ are associated in large detergent-resistant complexes in rat basophilic leukemia cells. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3611–3615.

Dráberová, L., Amoui, M. & Dráber, P. (1996) Thy-1-mediated activation of rat mast cells: The role of Thy-1 membrane microdomains. *Immunology* **87**, 141–148.

Edidin, M. (1997) Lipid microdomains in cell surface membranes. *Curr. Opin. Struct. Biol.* **7**, 528–532.

Field, K.A., Holowka, D. & Baird, B. (1995) FcγRI-mediated recruitment of $p53/56^{lyn}$ to detergent-resistant membrane domains accompanies cellular signaling. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9201–9205.

Fujimoto, T. (1996) GPI-anchored proteins, glycosphingolipids, and sphingomyelin are sequestered to caveolae only after crosslinking. *J. Histochem. Cytochem.* **44**, 929–941.

Gray, M.C., Plant, A.L., Nicholson, J.M. & May, W.E. (1995) Microenzymatic fluorescence assay for serum cholesterol. *Anal. Biochem.* **224**, 286–292.

Greenberg, S., Chang, P. & Silverstein, S.C. (1994) Tyrosine phosphorylation of the γ subunit of Fcγ receptors, $p72^{syk}$, and paxilin during Fc receptor-mediated phagocytosis in macrophages. *J. Biol. Chem.* **269**, 3897–3902.

Greenberg, S., Chang, P., Wang, D.C., Xavier, R. & Silverstein, S.C. (1996) Clustered Syk kinase domains trigger phagocytosis. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1103–1107.

Harder, T., Scheiffele, P., Verkade, P. & Simons, K. (1998) Lipid domain structure of the plasma membrane revealed by patching of membrane components. *J. Cell Biol.* **141**, 929–942.

Hoover, R.L., Dawidowicz, E.A., Robinson, J.M. & Karnovsky, M.J. (1983) Role of cholesterol in the capping of surface immunoglobulin receptors on murine lymphocytes. *J. Biol.* **97**, 73–80.

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

Keller, P. & Simons, K. (1998) Cholesterol is required for surface transport of influenza virus hemagglutinin. *J. Cell Biol.* **140**, 1357–1367.

Kiener, P.A., Rankin, B.M., Burkhardt, A.L., Schieven, G.L., Gilliland, L.K., Rowley, R.B., Bolen, J.B. & Ledbetter, J.A. (1993) Cross-linking of Fcγ receptor I (FcγRI) and receptor II (FcγRII) on monocytic cells activates a signal transduction pathway common to both Fc receptors that involves the stimulation of p72 syk protein tyrosine kinase. *J. Biol. Chem.* **268**, 24442–24448.

Kilsdonk, E.P.C., Yancey, P.G., Stoudt, G.W., Wan Bangerter, F., Johnson, W.J., Phillips, M.C. & Rothblat, G.H. (1995) Cellular cholesterol efflux mediated by cyclodextrins. *J. Biol. Chem.* **270**, 17250–17256.

Klausner, R.D., Bhalla, D.K., Dragsten, P., Hoover, R.L. & Karnovsky, M.J. (1980) Model for capping derived from inhibition of surface receptor capping by free fatty acids. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 437–441.

Kwiatkowska, K., Pyrzyńska, B. & Sobota, A. (1997) Capping of Fcγ receptor IIA is controlled by tyrosine phosphorylation/dephosphorylation of proteins in U937 cells. *Biophys. J.* **72**, A153.

Kwiatkowska, K. & Sobota, A. (1998) Participation of myosin I, spectrin analogue and tyrosine-phosphorylated proteins at early stages of phagocytosis in *Acanthamoeba castellanii*. *Acta Protozool.* **37**, 191–199.

Kwiatkowska, K. & Sobota, A. (1999a) Signaling pathways in phagocytosis. *BioEssays* **21**, (in press).

Kwiatkowska, K. & Sobota, A. (1999b) Tyrosine phosphorylation/dephosphorylation controls capping of Fcγ receptor II in U937 cells. *Cell Motil. Cytoskeleton* **42**, 298–314.

Lange, Y., Swaisgood, M.H., Ramos, B.V. & Steck, T.L. (1989) Plasma membranes contain half the phospholipid and 90% of the cholesterol and sphingomyelin in cultured human fibroblasts. *J. Biol. Chem.* **264**, 3786–3793.

Liu, J., Oh, P., Horner, T., Rogers, R.A. & Schnitzer, J.E. (1997) Organized endothelial cell surface signal transduction in caveolae distinct from glycosylphosphatidylinositol-anchored protein microdomains. *J. Biol. Chem.* **272**, 7211–7222.

Matsuda, M., Park, J.G., Wang, D.C., Hunter, S., Chien, P. & Schreiber, A.D. (1996) Abrogation of the Fcγ receptor IIA-mediated phagocytic signal by stem-loop Syk antisense oligonucleotides. *Mol. Biol. Cell* **7**, 1095–1106.

Neufeld, E.B., Cooney, A.M., Pithal, J., Dawidowicz, E.A., Dwyer, N.K., Pentchev, P.G. & Blanchette-Mackie, J. (1996) Intracellular trafficking of cholesterol monitored with a cyclodextrin. *J. Biol. Chem.* **271**, 21604–21613.

Resh, M.D. (1994) Myristylation and palmitylation of Src family members: The fats of the matter. *Cell* **76**, 411–413.

Shenoy-Scaria, A.M., Kwong, J., Fujita, T., Olszowy, M.W., Shaw, A.S. & Lublin, D.M. (1992) Signal transduction through decay-accelerating factor. Interaction of glycosylphosphatidylinositol anchor and protein tyrosine kinases $p56^{lck}$ and $p59^{fyn}$. *J. Immunol.* **149**, 3535–3541.

Shenoy-Scaria, A.M., Dietzen, D.J., Kwong, J., Link, D.C. & Lublin, D.M. (1994) $Cysteine^3$ of src family protein tyrosine kinases determines palmitoylation and localization in caveolae. *J. Cell Biol.* **126**, 353–363.

Schroeder, R., London, E. & Brown, D. (1994) Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior. *Proc. Natl. Acad. Sci. U.S.A* **91**, 12130–12134.

Silverstein, S.C., Greenberg, S., Di Virgilio, F. & Steinberg, T.H. (1989) Phagocytosis; in *Fundamental Immunology* (Paul, W.E., ed.) 2nd edn., pp. 703–720, Raven Press Ltd., New York.

Simons, K. & Ikonen, E. (1997) Functional rafts in cell membranes. *Nature* **387**, 569–872.

Smart, E.J., Ying, Y.-S., Conrad, P.A. & Anderson, R.G.W. (1994) Caveolin moves from caveolae to the golgi apparatus in response to cholesterol oxidation. *J. Cell Biol.* **127**, 1185–1197.

Stefanova, I., Horejsi,V., Ansotegui, I.J., Knapp, W. & Stockinger, H. (1991) GPI-anchored cell-surface molecules complexed to protein tyrosine kinases. *Science* **254**, 1016–1019.

Strzelecka, A., Pyrzyńska, B., Kwiatkowska, K. & Sobota, A. (1997) Syk kinase, tyrosine-phosphorylated proteins and actin filaments accumulate at forming phagosomes during Fcγ receptor-mediated phagocytosis. *Cell Motil. Cytoskeleton* **38**, 287–296.

Yancey, P.G., Rodriqueza, W.V., Kilsdonk, E.P.C., Stoudt, G.W., Johnson, W.J., Phillips, M.C. & Rothblat, G.H. (1996) Cellular cholesterol efflux mediated by cyclodextrins. *J. Biol. Chem.* **271**, 16026-16034.

Zenner, G., zur Hausen, J.D., Burn, P. & Mustelin, T. (1995) Toward unraveling the complexity of T cell signal tranduction. *BioEssays* **17**, 967-975.