Neutrophils are short-lived leukocytes that die by apoptosis. Whereas stress-induced apoptosis is mediated by the p38 mitogen-activated protein (MAP) kinase pathway (Frasch, S. C., Nick, J. A., Fadok, V. A., Bratton, D. L., Worthen, G. S., and Henson, P. M. (1998) J. Biol. Chem. 273, 8389–8397), signals regulating spontaneous neutrophil apoptosis have not been fully determined. In this study we found increased activation of protein kinase C (PKC)-β and -δ in neutrophils undergoing spontaneous apoptosis, but we show that only activation of PKC-δ was directly involved in the induction of apoptosis. PKC-δ can be proteolytically activated by caspase 3. We detected the 40-kDa caspase-generated fragment of PKC-δ in apoptotic neutrophils and showed that the caspase 3 inhibitor Asp-Glu-Val-Asp-fluoromethylketone prevented generation of the 40-kDa PKC-δ fragment and delayed neutrophil apoptosis. In a cell-free system, removal of PKC-δ by immunoprecipitation reduced DNA fragmentation, whereas loss of PKC-α, -β, or -ζ had no significant effect. Rottlerin and LY379186 inhibit PKC-δ and PKC-β, respectively. Only Rottlerin was able to delay neutrophil apoptosis. Inhibitors of MAP-ERK kinase 1 (PD98059) or p38 MAP kinase (SB202190) had no effect on neutrophil apoptosis, and activation of p42/44 and p38 MAP kinase did not increase in apoptotic neutrophils. We conclude that spontaneous neutrophil apoptosis involves activation of PKC-δ but is MAP kinase-independent.

Neutrophils are short-lived terminally differentiated blood cells that play a vital role in inflammatory responses. Chemo- tactic factors generated at sites of infection induce the movement of neutrophils from the blood into the affected tissues. A variety of molecules, including cytokines and bacterial products, then activate the defense systems of the neutrophil, which include phagocytosis, degranulation, and activation of NADPH oxidase. Although neutrophils are crucial in the defense against infection, they have also been implicated in the pathogenesis of tissue injury seen in inflammatory diseases of the lung, kidney, joints, and other organs (1–3). During the resolution of inflammation, effete neutrophils are removed from inflammatory sites by apoptosis, leading to their recognition and phagocytosis by macrophages (4). Any significant delay to neutrophil apoptosis can lead to excessive neutrophil accumulation and damage to healthy tissue (5). Although apoptosis is an intrinsic cell process, a variety of cytokines, primarily those that prime neutrophils (6), are able to delay apoptosis. How these effects are achieved is not known, although the modulation of signaling pathways regulating apoptosis is an obvious target in such a short-lived cell.

Members of the MAP1 kinase family of signaling enzymes, specifically, p38 MAP kinase, have been shown to be involved in accelerating neutrophil apoptosis in response to stress but do not appear to be involved in spontaneous neutrophil apoptosis (7). An alternative signaling pathway that has been implicated in the regulation of apoptosis in a wide variety of cells (8) is protein kinase C (PKC). PKC is a family of 11 isoenzymes that are well conserved across species, suggesting they have specific functions within cells (9). Neutrophils have been shown to express several PKC isoenzymes including PKC-α, -β, -δ (10, 11), and -ζ (12). Selective involvement of PKC isoenzymes in the regulation of apoptosis has been indicated in recent studies in a variety of cells, including those of myeloid (13–15) and lymphoid (16, 17) origin. In Molt 4 cells, Lee et al. (17) have shown that ceramide-induced apoptosis via the inhibition of PKC-α, and Whelan and Parker (18) have shown that down-regulation of PKC-α induces apoptosis in COS cells, suggesting an anti-apoptotic role for this isoenzyme. In contrast, MacFarlane et al. (13) showed that PKC-β expression was required for promyeloid HL60 cells to undergo differentiation and apoptosis in response to phorbol ester treatment, and the expression of PKC-β was differentially regulated during apoptosis in myelomonocytic U937 cells (14). Also in U937 cells, PKC-δ has been shown to be activated by caspase 3, leading to the generation of a 40-kDa catalytic fragment after the induction of apoptosis by a variety of agents, including Fas ligation (19). Thus, PKC isoenzymes appear to be differentially involved in the regulation of the apoptotic program.

Although PKC has been implicated in signaling pathways regulating spontaneous apoptosis in promyeloid cells (20), the involvement of PKC isoenzymes in neutrophil apoptosis has not been established. However, the Fas/Fas ligand system has been reported to be involved in mediating spontaneous neutrophil apoptosis (21), although the signaling pathways downstream of Fas ligation in the neutrophil have not been established. Because PKC-δ has been shown to be activated after Fas ligation in promyeloid cells (19), a role for the activation of...
specific PKC isoenzymes in spontaneous neutrophil apoptosis was investigated. In this report, we show that both PKC-β and -δ were activated during apoptosis, although only the inhibition of PKC-δ delayed spontaneous neutrophil apoptosis. We also confirm that p42/44 and p38 MAP kinases are involved in stress-induced apoptosis but are not involved in the regulation of spontaneous neutrophil apoptosis.

EXPERIMENTAL PROCEDURES

Isolation and Culture of Human Peripheral Blood Neutrophils—Venous blood (20–100 ml) was taken from healthy volunteers, and neutrophils were isolated on Percoll gradients as described previously (22). Neutrophil preparations contained >95% neutrophils. Neutrophils were resuspended in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal calf serum (Life Technologies, Inc.) and 100 units/ml penicillin and 100 μg/ml streptomycin (Sigma) and used immediately as healthy control cells, or they were cultured in a humidified 5% CO₂ atmosphere to provide apoptotic cells. Cytospin preparations (3 min, 10 × g; Cytospin 2, Shandon) were made after 6 and 24 h of incubation, differentially stained using a commercial May-Grunwald Giemsa stain (Diff-Quick, Baxter Healthcare Products), and assessed for apoptotic morphology (22).

Treatment of Neutrophils with Inhibitors—PKC inhibitors, G6976 and LY379196, were purchased from Calbiochem and used at the concentrations shown. LY379196 is a novel PKC-β inhibitor (23) and was kindly provided by Eli Lilly and used at 10 μM. The caspase 3 inhibitor DEVD-fmk (Calbiochem) was used at 20 μM. Incubation of caspase 3 was confirmed by assaying caspase 3 activity in 100 μl of neutrophil cell lysates, using a commercial kit and according to manufacturer’s instructions (CaspAce; Promega). The MAP-ERK kinase and p38 MAP kinases were confirmed by assaying caspase 3 activity in 100 μl of neutrophil cell lysates, and the samples were analyzed by Western blotting and was not greater than 5% of any of the isoenzymes tested. Protein concentrations were determined in cytosol and particulate preparations, and the samples were boiled in SDS sample buffer. Equivalent amounts of protein were separated on 12% SDS-polyacrylamide gels and transferred to polyvinylidene fluoride (Immobilon-P, Millipore UK Ltd). PKC isoenzyme immunoreactivity was detected with rabbit polyclonal antibodies to PKC-α, -β, and -δ (Santa Cruz Biotechnology). The anti-PKC-ζ antibody (25) was an anti-peptide antibody raised in rabbits and was a kind gift from Dr. J. Ransom (Syntex Research Ltd., Palo Alto, CA). Horseradish peroxidase-conjugated sheep anti-rabbit IgG antibody (Amersham International) was used as a secondary antibody. Enhanced chemiluminescence (ECL; Amersham International) was used as a secondary antibody. Enhanced chemiluminescence (ECL; Amersham International) was used to reveal immunoreactive bands. 10 ng of human recombinant PKC for each isoenzyme (Calbiochem) was applied to the outer lane in each gel to confirm antibody specificity.

Immunostaining and Confocal Microscopy—The subcellular distribution of PKC isoenzymes was assessed using indirect immunofluorescence staining and confocal microscopy. Cytosin preparations were made of apoptotic and non-apoptotic neutrophils and air dried for at least 1 h before fixing in 4% paraformaldehyde for 10 min. Cells were incubated with antibody to PKC-δ (Santa Cruz Biotechnology) for 30 min at room temperature in a humidified atmosphere and incubated with fluorescein isothiocyanate-conjugated secondary antibody for an additional 30 min. Nuclei were counterstained with propidium isodide. Fading of fluorescence was retarded by treatment of stained cells with 2.5% (w/v) 1,4-diazobicyclo(2,2,2)-octane (DABCO; BDH Ltd.) in 80% glycerol. Immunofluorescence was analyzed by laser scanning confocal microscopy using a MRC 500 confocal microscope (Bio-Rad).

Assay for Active p42/44 and p38 MAP Kinase—Protein extracts from freshly isolated neutrophils and neutrophils cultured overnight were separated on 10% SDS-polyacrylamide gel electrophoresis gels and analyzed by Western blotting using antibodies specific for the phosphorylated, active forms of p42/44 and p38 MAP kinase (Promega). Immunoreactive bands were revealed by enhanced chemiluminescence.

Cell-free Apoptosis System—Nuclei were isolated from neutrophils using a rapid procedure developed for hemopoietic cells (26). Cytoxic was prepared from neutrophils incubated for 24 h to give >75% apoptotic cells. Neutrophils were lysed in Buffer A and spun at 100,000 × g to recover the cytosol (supernatant) fraction. Healthy nuclei were combined with apoptotic cytosol, and DNA fragmentation was measured after 30 min using an enzyme-linked immunosorbent assay-based commercial kit (Roche Molecular Biochemicals). To deplete PKC isoenzymes, the apoptotic cytosol was incubated with PKC isoenzyme antibodies (Santa Cruz Biotechnology) at 10 μg/ml extract for 30 min at 4 °C, followed by a mouse anti-rabbit IgG antibody and protein A coupled to agarose beads (Upstate Biotechnology). Immunocomplexes were removed by centrifugation, and the remaining cytosol was combined with the healthy nuclei. Depletion of PKC was confirmed by Western blotting. Mouse immunoglobulin G was used as control.

Statistics—Data presented here represent a minimum of three experiments, and, where appropriate, data are expressed as mean ± S.D. Statistical significance was assessed by Student’s t test, and p < 0.05 was taken as a significantly different value.

RESULTS

PKC Isoenzymes and Neutrophil Apoptosis—The level of apoptosis in freshly isolated neutrophils (Fig. 1) was low (2.2 ± 0.7%; n = 5) and increased significantly as cells were aged in culture to 11.6 ± 5.1% after 6 h (p < 0.05; n = 5) and 68.0 ± 15% after 24 h (p < 0.001; n = 5).

PKC resides in the cytosol in the inactive state and is translocated to the membrane fraction upon activation. Assessment of particulate (membrane) and cytosolic PKC can thus give an indication of enzyme activation. Immunoblotting of neutrophil particulate and cytosolic protein extracts revealed an increase in PKC-δ and -ζ isoenzymes associated with the particulate fraction during apoptosis (Fig. 2A). Translocation of PKC-α was not detected, and the majority of PKC-α was located in the cytosol. PKC-ζ was also detected only in the cytosol fraction of neutrophils, but its subcellular localization did not alter as neutrophils were aged in culture (Fig. 2A).

PKC-δ can also be activated by caspase 3. A 40-kDa fragment of PKC-δ was not detected in freshly isolated neutrophils, and only the full-length 78-kDa form was seen. However, as cells were aged overnight in culture and entered apoptosis, a 40-kDa fragment was detected, and the level of the 78-kDa PKC-δ was reduced concomitantly (Fig. 2B). The appearance of the 40-kDa PKC-δ fragment was reduced when DEVD-fmk was included in the medium (Fig. 2B), confirming that generation of the active

![Fig. 1. Neutrophils die by apoptosis when cultured in vitro.](image-url)
PKC Isoenzymes and Neutrophil Apoptosis

**Effect of PKC-specific Inhibitors on Neutrophil Apoptosis**—To determine whether the activation of PKC-β and -δ seen in apoptotic cells was involved in the apoptotic process, three PKC isoenzyme-selective inhibitors were used. Go6976 inhibits the classical PKC isoenzymes (PKC-α, -β, and -γ), LY379196 is a PKC-δ-specific inhibitor (23), and Rottlerin inhibits PKC-δ (27). Because PKC-δ can be activated by caspase 3, the effect of the caspase 3 inhibitor (DEVD-fmk) on neutrophil apoptosis was also determined. Go6976 and LY379196 did not decrease apoptosis in neutrophil cultures after an overnight incubation (Fig. 3A). However, Rottlerin and DEVD-fmk inhibited neutrophil apoptosis significantly (Fig. 3A). The inhibition of PKC isoenzymes by the inhibitors used was confirmed by Western blotting of neutrophil cytosol and particulate extracts (Fig. 3B).

**Effect of Removal of PKC Isoenzymes on DNA Fragmentation in a Cell-free System**—To attempt to confirm the data gained with pharmacological inhibitors of PKC isoenzymes, a cell-free system was used. Nuclei were isolated from non-apoptotic neutrophils and combined with cytosol from apoptotic neutrophils after the removal of individual PKC isoenzymes by immunoprecipitation (Fig. 4A). Apoptosis was then assessed using an enzyme-linked immunosorbent assay method to detect DNA fragmentation. Cytosol from apoptotic neutrophils (Apo) induced a significant increase in DNA fragmentation in non-apoptotic nuclei, compared with the addition of cytosol from healthy neutrophils (Fig. 4B). The removal of PKC-δ by immunoprecipitation significantly decreased DNA fragmentation induced by the apoptotic cytosol. Loss of PKC-β gave a slight but not significant reduction in DNA fragmentation, and removal of PKC-α or -γ or the addition of a mouse IgG had no effect (Fig. 4B).

**Involvement of p42/44 MAP Kinase and p38 MAP Kinase in Spontaneous Neutrophil Apoptosis**—Inhibitors of MAP-ERK kinase 1 (PD98059), which lies upstream of p42/44 MAP kinase, and p38 MAP kinase (SB202190) were used to determine whether members of the MAP kinase family were involved in the regulation of spontaneous neutrophil apoptosis. Neither of these agents affected the level of neutrophil apoptosis when they were included in the culture medium for 6 h (data not shown) or overnight (Fig. 5A). In addition, activated p42/44 MAP kinase and p38 MAP kinase detected by antibodies specific for the active forms of these kinases were barely detectable in freshly isolated or spontaneously apoptotic neutrophils (Fig. 5B). In contrast, activated p38 MAP kinase was present at a high level in neutrophils induced to die by UV irradiation (Fig. 5B), and p42/44 MAP kinase was activated by treatment of neutrophils with phorbol myristic acid (Fig. 5B).
PKC Isoenzymes and Neutrophil Apoptosis

Fig. 4. Effect of PKC isoenzyme depletion on nuclear DNA fragmentation in a cell-free system. Cytosol and nuclei were isolated from apoptotic and non-apoptotic neutrophils, respectively, and combined (A,+) or they were isolated after the depletion of PKC iso-enzymes by immunoprecipitation. DNA fragmentation was then measured using a commercial enzyme-linked immunosorbent assay-based kit (A). Healthy nuclei were combined with non-apoptotic cytosol (A,−) as a control for the fractionation procedure, and a mouse anti-rabbit IgG antibody (Ms IgG) was a control for the depletion procedure. The removal of PKC iso-enzymes was confirmed by immunoblotting of the depleted cytosol and analysis of band intensity determined by scanning densitometry and expressed as absorbance units per mm² (B). Results are the mean ± S.D. of three separate experiments, and * denotes a value of p < 0.05.

Nuclear Localization of PKC-δ during Neutrophil Apoptosis—The Western blotting data indicating PKC-δ translocation to the particulate fraction were investigated further by immunohistochemistry. Comparison of PKC-δ immunostaining in freshly isolated neutrophils (Fig. 6A) and neutrophils cultured for 8 h (Fig. 6B) revealed an increase in PKC-δ associated with the nucleus in many cells. The increase in nuclear PKC-δ was quantitated by determining the percentage of total PKC-δ fluorescence that was coincident with the red fluorescence of the propidium iodide nuclear counterstain. In each study, 100 cells were examined, and the mean value for nuclear PKC-δ increased from 7.7 ± 0.4% in healthy neutrophils to 21.4 ± 1.5% in neutrophils cultured for 8 h. Staining of neutrophils aged for a longer time period in culture was not possible because these cells were more fragile and were not suitable for immunostaining.

DISCUSSION

Much research has focused on the apoptotic program in proliferating cells, but little is known about the regulation of apoptosis in fully differentiated cells, such as neutrophils. However, the pathological consequences of an altered neutrophil life span are considerable (1–3). In this study, the involvement of PKC iso-enzymes and MAP kinases in spontaneous neutrophil apoptosis has been investigated. We confirmed previous reports that p38 MAP kinase is involved in inducing signals for neutrophil death in response to stress (7). However, the p38 MAP kinase pathway is not involved in spontaneous apoptosis, suggesting that this pathway in the neutrophil may be activated solely in response to stress-induced apoptosis.

PKC is known to be involved in neutrophil activation and in the regulation of apoptosis in a variety of cells. The 11 PKC iso-enzymes appear to play various roles in the regulation of apoptosis (8), but PKC-δ is consistently a pro-apoptotic PKC iso-enzyme. PKC-δ can be cleaved by caspase 3, and transfection of cells with the caspase-generated catalytic fragment of PKC-δ is sufficient to induce apoptosis (28). The data reported here show that PKC-δ is activated during spontaneous neutrophil apoptosis. Furthermore, inhibition of PKC-δ activation by either use of a selective inhibitor or inhibition of caspase 3 delayed neutrophil apoptosis significantly. The caspase 3 inhibitor produced a greater delay in apoptosis than the PKC-δ inhibitor. This is not surprising, because PKC-δ is one of the many targets of this protease. Other substrates of caspase 3 include ICAD, the inhibitor of the apoptosis-specific endonuclease caspase-activated DNase (29), and cytoskeletal proteins such as actin (30) and fodrin (31). Thus, inhibition of PKC-δ may remove one of the elements of the apoptotic program but would not be able to prevent it altogether.

Until recently, PKC-δ and the very closely related isoform PKC-ζ were the only PKC iso-enzymes reported to undergo proteolytic cleavage and activation by caspase 3. It has now been shown that PKC-ζ can also be cleaved by caspase 3, but in this case, apoptosis was associated with inhibition of PKC-ζ enzyme activity rather than activation (32). Thus, activation of caspase 3 during apoptosis can result in the cleavage of both PKC-δ and -ζ, simultaneously effecting the activation of a pro-apoptotic PKC and the inhibition of an anti-apoptotic PKC iso-enzyme. Although PKC-ζ was expressed in neutrophils, we did not detect proteolytic cleavage in spontaneously apoptotic neutrophils. Therefore, this pathway may either operate only
in certain cells or be activated only during stress-induced apoptosis.

We detected translocation of PKC-β in apoptotic neutrophils but did not detect proteolytic fragmentation of this isoenzyme. In a cell-free system, depletion of PKC-β from apoptotic cytosol did not significantly reduce DNA fragmentation in healthy nuclei, and the PKC-β inhibitor LY379196 did not delay neutrophil apoptosis. These data do not support a primary role for PKC-β in spontaneous apoptosis. Moreover, PKC-β has been proposed to play a role in neutrophil activation rather than apoptosis and may be involved in NADPH complex assembly and superoxide generation (33, 34). We excluded the possibility that PKC-β translocation was associated with activation of neutrophils in these studies by measuring superoxide generation. None was detected, suggesting that the translocation of PKC-β is an apoptosis-associated event.

The data reported here suggest that PKC isoenzymes play several roles in the regulation of spontaneous neutrophil apoptosis, with PKC-β playing a key role. Because p38 MAP kinase was not activated during spontaneous neutrophil apoptosis, we propose that PKC-β represents a primary signaling pathway, maintaining a basal rate of spontaneous apoptosis. Additional pathways can be recruited to increase the rate of neutrophil death, including the MAP kinase pathway, in situations requiring accelerated apoptosis. The upstream mechanisms leading to activation of caspase 3 and PKC-β in spontaneous neutrophil apoptosis are still unclear. Ligation of Fas has been suggested (21) because neutrophils express both Fas and its ligand. However, this is unlikely because Fas-blocking antibodies are ineffective (35). An alternative explanation is that spontaneous apoptosis is a result of cytokine deprivation; several pro-inflammatory cytokines are able to prevent neutrophil apoptosis (6, 36). Furthermore, we have shown recently that cytokine deprivation-induced apoptosis of T cells also involves activation of PKC-β (37).

The association of PKC-δ with the nucleus during apoptosis and its subsequent activation by caspase 3 have recently been reported in U937 cells after ionizing irradiation (38) and during cytokine deprivation and Fas-induced apoptosis in T cells (37). Nuclear translocation of PKC-δ may therefore be an early event in the apoptotic program, preceding the common pathway that follows caspase 3 activation. The tyrosine kinase c-abl can be activated by ionizing irradiation, resulting in phosphorylation of PKC-δ and translocation to the nucleus. Tyrosine phosphorylation may be a general mechanism for nuclear translocation of PKC-δ.

In summary, PKC isoenzymes play several distinct roles in the regulation of spontaneous neutrophil apoptosis. The translocation of PKC-δ to the nucleus and its activation by caspase 3 appear to be crucial events. PKC-δ may therefore represent a useful therapeutic target in conditions of disregulated neutrophil apoptosis.

Acknowledgment—We are grateful to Mike Salmon for critical discussion of the manuscript.

REFERENCES

1. Edwards, S. E. (1994) Biochemistry and Physiology of the Neutrophil 1st Ed., Cambridge University Press, Cambridge and New York.
2. Hogg, J. C. (1987) Physiol. Rev. 67, 1249–1295.
3. Weiss, S. J. (1989) N. Engl. J. Med. 320, 365–376.
4. Savill, J. S., Wyllie, A. H., Henson, J. E., Walport, M. J., Henson, P. M., and Haslett, C. (1989) J. Clin. Invest. 83, 865–875.
5. Hallett, M. B., and Lloyd, D. (1995) Immunol. Today 16, 264–268.
6. Stringer, R. E., Hart, C. A., and Edwards, S. W. (1996) Br. J. Hematol. 92, 159–175.
7. Frasch, S. C., Nick, J. A., Fadok, V. A., Bratton, D. L., Worthen, G. S., and Henson, P. M. (1996) J. Biol. Chem. 271, 8369–8377.
8. Deacon, E. M., Pongracz, J., Griffiths, G., and Lord, J. M. (1997) J. Clin. Pathol. Mol. Pathol. 50, 124–131.
9. Nishizuka, Y. (1992) Science 258, 607–614.
10. Majumdar, S., Rossi, M. W., Fujiki, T., Phillips, W. A., Disa, S., Queen, C. F., Johnston, R. B., Jr., Rosen, O. M., Corkey, B. E., and Korchak, H. M. (1991) J. Biol. Chem. 266, 9285–9293.
11. Smallwood, J. I., and Malawista, S. E. (1992) J. Leukocyte Biol. 51, 84–92.
12. Dang, P. M. C., Hakim, J., and Perianin, A. (1995) FEBS Lett. 349, 338–342.
13. MacFarlane, D. E., and Manzel, L. (1994) J. Biol. Chem. 269, 4327–4331.
14. Pongracz, J., Deacon, E. M., Johnson, G. D., Burnett, D., and Lord, J. M. (1996) Leuk. Res. 20, 319–326.
15. Evans, C. A., Lord, J. M., Owen-Lynch, P. J., Johnson, G., Dive, C. and Whetton, A. D. (1995) J. Cell Sci. 108, 2591–2598.
16. Knox, K. A., Johnson, G. D., and Gordon, J. (1998) Exp. Cell Res. 297, 68–73.
17. Lee, J. Y., Hannon, Y. A., and Obeid, L. M. (1996) J. Biol. Chem. 271, 13169–13174.
18. Whelan, R. D. H., and Parker, P. J. (1998) Oncogene 16, 1939–1944.
19. Ghayur, T., Hugunin, M., Talanian, R. V., Batinsky, S., Quinlan, C., Emoto, Y., Pandey, P., Datta, R., Huang, Y., Kharbanda, S., Allen, H., Kamen, R., Wong, W., and Kufe, D. (1996) J. Exp. Med. 184, 2399–2404.
20. Pongracz, J., Tuffley, W., Johnson, G. D., Deacon, E. M., Burnett, D., Stockley, R. A., and Lord, J. M. (1995) Exp. Cell Res. 218, 430–438.
21. Iwai, K., Miyawaki, T., Takizawa, T., Kanno, A., Okta, K., Yachie, A., Seki, H., and Taniguchi, N. (1994) Blood 84, 1201–1208.
22. Afford, S. C., Pongracz, J., Stockley, R. A., Crocker, J., and Burnett, D. (1992) J. Biol. Chem. 267, 21612–21616.
23. Jirousek, M. R., Gillig, J. R., Heath, W. F., Gonzalez, C. M., McDonald, J. H., III, Neel, D. A., Rito, C. J., Stramm, L. E., Singh, U., Melikian-Badalozian, A.
PKC Isoenzymes and Neutrophil Apoptosis

24. Griffiths, G., Garrone, B., Deacon, E., Owen, P., Pongracz, J., Mead, G., Bradwell, A. R., Watters, D., and Lord, J. (1996) Biochem. Biophys. Res. Commun. 222, 892–898
25. Tsutsumi, A., Kubo, M., Fujii, H., Freire-Maor, J., Turck, C. W., and Ransom, J. T. (1993) J. Immunol. 150, 1746–1754
26. Bunce, C. M., Thick, J. A., Lord, J. M., Mills, D., and Brown, G. (1988) Anal. Biochem. 175, 67–73
27. Gscwendt, M., Muller, H. J., Kielbassa, K., Zang, R., Kittstein, W., Rincke, G., and Marks, F. (1994) Biochem. Biophys. Res. Commun. 199, 93–97
28. Emoto, Y., Manome, Y., Meinhardt, G., Kisaki, H., Kharbanda, S., Robertson, M., Ghayur, T., Wong, W. W., Kamen, R., Weichselbaum, R., and Kufe, D. (1995) EMBO J. 14, 6148–6156
29. Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., and Nagata, S. (1998) Nature 393, 396–400
30. Mashima, T., Naito, M., and Tsuruo, T. (1999) Oncogene 18, 2423–2430
31. Janicke, R. U., Ng, P., Sprengart, M. L., and Porter, A. G. (1998) J. Biol. Chem. 273, 15540–15545
32. Frutos, S., Moscat, J., and Diaz-Meco, M. T. (1999) J. Biol. Chem. 274, 10765–10770
33. Parmer, T. G., Ward, M. D., and Hait, W. N. (1997) Cell Growth Differ. 8, 327–334
34. Nairn, A. C., and Picciolto, M. R. (1994) Semin. Cancer Biol. 5, 295–303
35. Brown, S. B., and Savill, J. (1999) J. Immunol. 162, 480–485
36. Lee, A., Whyte, M. K. B., and Haslett, C. (1993) J. Leukocyte Biol. 54, 283–288
37. Scheel-Toellner, D., Filling, D., Akbar, A. N., Hardie, D., Lombardi, D., Salmon, M., and Lord, J. M. (1999) Eur. J. Immunol. 29, 2603–2612
38. Bharti, A., Kraeft, S-K., Gounder, M., Pandey, P., Jin, S., Yuan, Z.-M., Lees-Miller, S. P., Weichselbaum, R., Weaver, D., Chen, L. B., Kufe, D., and Kharbanda, S. (1998) Mol. Cell. Biol. 18, 6719–6728