ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 1, 2002

Forum Review

Redox Regulation of Neutrophil Apoptosis

R.W.G. WATSON

ABSTRACT

The persistence of a neutrophil-mediated inflammatory response is due in part to a delay in their spontaneous rates of apoptosis or cell death. Regulating apoptosis has important implications for the resolution of inflammatory disorders, such as the systemic inflammatory response syndrome or acute respiratory distress syndrome. Neutrophils through their primary function of killing bacteria generate large concentrations of reactive oxygen intermediates and have alterations in the levels of antioxidants. Reactive oxygen intermediates and antioxidants are important regulators of the apoptotic caspases, but the mechanisms involved are still under debate and investigation. This review addresses the role of the cellular redox status of neutrophils on the apoptotic cascade leading to cell death. Antioxid. Redox Signal. 4, 97–104.

INTRODUCTION

POLYMORPHONUCLEAR LEUKOCYTES OR NEUTROPHILS are central in the innate immune response. They represent the first line of defense against bacterial and fungal infection. They kill invading pathogens by the production of reactive oxygen intermediates and proteolytic enzymes. In contrast to this beneficial response, neutrophils have been shown to play an important role in the development of a number of inflammatory diseases ranging from rheumatoid arthritis (49) to acute respiratory distress syndrome (48). This is mediated through the inappropriate persistence of phagocytic cells at the site of inflammation. Ultimately, the removal of these cells from the site is an essential step in the resolution of inflammation.

Mature neutrophils have a very short life span and die rapidly via apoptosis *in vivo* and *in vitro*, resulting in the demise of the entire population within 72 h (18). These cells are replaced by the release of mature neutrophils from the bone marrow. This process is essential for maintaining fully functional neutrophils in the circulation. To mount an inflammatory response and fight a bacterial infection, the apoptotic rates are significantly delayed (59). A number of mediators and intracellular mechanisms that regulate neutrophil apoptosis have been identified, but the complete mechanism still remains unclear. The intracellular redox potential of the cell has been shown to regulate this apoptotic cascade. As the neutrophil has the ability to generate changes in this potential through its primary function of killing bacteria, this may

Department of Surgery, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Mater Misericordiae Hospital, Dublin, Ireland.

have important implications in the regulation of apoptosis and the resolution of inflammatory disorders.

CHARACTERISTICS OF NEUTROPHIL APOPTOSIS

Cells undergoing apoptosis demonstrate similar characteristics; however, individual cell types differ in the extent to which they express these changes. A number of morphologically identifiable stages have been reported. These include exuberant cell-surface protrusion and breaking up of the nucleus to form multiple fragments and compacted chromatin (30, 66). These changes are accompanied by flipping of phosphatidylserine from the inner plasma membrane to the cell surface. Finally, the cell-surface protuberances separate to produce membrane-enclosed apoptotic bodies in which the closely packed cytoplasmic organelles remain well preserved (20, 31). The nuclear collapse that is the hallmark of apoptosis is characterized by fragmentation of DNA induced by endonucleases, producing fragments in the range 300–50 kbp. The DNA cleavage continues with internucleosomal double-stranded cutting to produce the familiar ladder on agarose gel electrophoresis (12).

The end result of apoptosis in the neutrophil is a nonfunctional cell, with a loss in chemotaxis, respiratory burst activity, and degranulation. These apoptotic bodies are then ingested or phagocytosed by the surrounding macrophages or fibroblasts. This prevents the release of their intracellular toxic mediators and results in the resolution of the inflammatory response.

INHIBITORS OF NEUTROPHIL APOPTOSIS

It has been clearly demonstrated that inflammatory mediators, including the cytokines, interleukin-1β (IL-1β), IL-2, IL-6, IL-8, IL-15, interferon-γ, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and the bacterial cell wall product lipopolysaccharide (LPS) can delay spontaneous neutrophil apoptosis (5, 7, 11, 13, 14, 15, 24, 32, 47, 64). How these factors mediate this delay is unknown. It is clear that inhibition of protein synthesis can block the delay, which indicates the production of intracellular survival factors. Activation of the mitogen-activated protin kinase pathway, specifically extracellular signal-regulated kinase 1/2 (ERK1/2), is central to the activation of nuclear factor-κB (NF-κB), which drives the delay in neutrophil apoptosis. Inhibitors of both ERK1/2 and NF-κB have been shown to prevent neutrophil survival (64).

Mcl-1, a member of the Bcl-2 family of antiapoptotic proteins, has been shown to be expressed in the neutrophil (34, 39), and its expression is stabilized in neutrophils with a delay in apoptosis, which may block the apoptotic cascade at the level of the mitochondria.

The newly described family of antiapoptotic proteins, the inhibitors of apoptosis, have been identified in the neutrophil at both the mRNA and protein levels. These inhibit caspase activity and block apoptosis. Their expression is not regulated by inflammatory mediators such as LPS or GM-CSF, which delay neutrophil apoptosis (unpublished observations), and their contribution to antiapoptotic effects within the neutrophil remain unclear.

INDUCTION OF NEUTROPHIL APOPTOSIS

The most well known surface receptors that signal for induction of apoptosis are part of the tumour necrosis factor receptor (TNFR) family. TNFR1 and Fas initiate death signals through "death domains" in their receptors on interaction with the corresponding antigens tumour necrosis factor-α (TNF-α) and Fas ligand (42, 43, 52, 56).

The signaling mechanism by which Fas and TNFR1 induce cell death has become clearer over the last few years. Ligation of Fas and TNFR1 results in the binding of MORT1 (or FADD) (10) and TRADD (27) proteins, respectively. These adapter proteins

have been shown to then bind and activate a number of proteolytic enzymes called caspases (1, 6, 25, 41). Over expression of these genes in transformed mammalian cells results in the induction of apoptosis. Likewise inhibition of caspase activity by CrmA viral expression (16) or protease inhibitors blocks the expression of apoptosis (2). To date, 14 members make up the caspase family in human cells (1). All are cysteine proteases with a common pentapeptide sequence -QACRG- that constitutes that active site of the molecule, and all cleave their target proteins at aspartic acid residues. The caspases carry out their destruction of the cell through the cleavage of a number of substrates essential for cellular function. Caspases 1 and 3 have a number of activities, including the cleavage of actin, which gives the cell structure and would result in cell shrinkage and blebbing, and also the cleavage of poly(ADP ribose) polymerase (PARP), which prevents the repair of the fragmented DNA (2).

It has been demonstrated that neutrophils undergoing spontaneous and Fas antibody-induced apoptosis follow a sequence of events that include disruption of the mitochondrial membrane, release of cytochrome *c* (unpublished observations) and activation of caspases 9 and 3 (65). This is supported by a number of studies that have shown activation of caspases 3 and 9 in various stages of the life span of the neutrophil (19, 51). In addition, it is well recognized that caspase inhibition with the zVAD-cmk inhibitor also blocks the induction of both spontaneous and Fas antibody-induced neutrophil apoptosis (19). How the caspases are activated in spontaneous apoptosis remains unclear. One factor believed important in the induction of spontaneous apoptosis was the activation of Fas/Fas ligand on the surface of the neutrophil. But neutrophils from Fas-deficient mice undergo normal rates of spontaneous apoptosis (57).

The role of TNF in the induction of neutrophil apoptosis remains controversial. TNF-α has been shown to induce, delay, and have no effect on neutrophil apoptotic rates (40, 53, 60). Ultimately, it has been suggested that the functional activation of the neutrophil at the time of TNF-α exposure determines its response. NF-κB activation may represent an important determining factor due to its ability to transcribe for a number of survival proteins.

REDOX REGULATION OF THE APOPTOTIC CASCADE

Elevations in reactive oxygen intermediates or depletion of antioxidants can induce alterations in the oxidizing environment of the cell. As the neutrophil has the capacity to produce large amounts of reactive oxygen intermediates through their killing of bacteria (58), it could be hypothesized that this pathway may be important in the regulation of neutrophil function such as apoptosis (see Fig. 1).

Reactive oxygen species have been shown to be an important and possibly a central

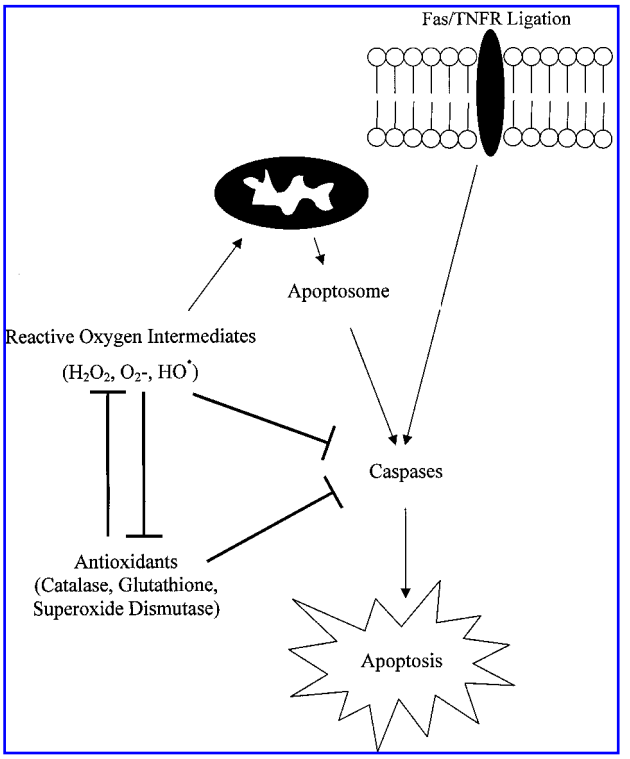

FIG. 1. Possible mechanisms for the role of reactive oxygen intermediates and antioxidants in the regulation of neutrophil apoptosis. See text for details.

apoptotic signal (8), as H_2O_2 (33) and radiation (9) both induce apoptosis in different cell systems. Antioxidants have also been shown to inhibit apoptosis induced by these treatments. Further studies have shown that Bcl-2 expression blocks apoptosis by protecting against oxidative stress (26). Kane *et al.* (28) have shown that Bcl-2 blocks GT1–7 neural cell death induced by depletion of glutathione. It is, however, believed that oxygen radicals are not directly responsible for the DNA degradation and membrane damage seen during the final common pathway of apoptosis. Oxygen radicals may deplete thiols that could activate other enzymes responsible for cell death. The endogenous formation of reactive oxygen species and depletion of thiols could be a constitutive factor that tends to drive cells to apoptosis even in the absence of exogenous stimuli. Such a model of apoptosis is consistent with the view of Raff and co-workers (4) that the default state of the cells is to die by programmed cell death unless kept alive by specific signals or anti-apoptotic agents.

Glutathione depletion represents an early event in the induction of apoptosis, occurring before elevations in calcium and increases in reactive oxygen intermediates (51). The loss of glutathione has been shown to be a result of extrusion outside the cell, as GSSG is not increased. In addition, this loss may not trigger the induction of apoptosis, but is essential for the apoptotic cascade to proceed (21). Preventing the loss of glutathione from the cell, by inhibiting the glutathione pumps with methionine or cystathionine, has also been shown to prevent the induction of apoptosis (22). Alternatively, depleting glutathione, with buthionine sulfoximine (GSH synthesis inhibitor), diethyl maleate, or diamide (chemical deactivator of GSH), increases the susceptibility of cells to apoptotic induction (3, 62).

Similar findings are demonstrated in the neutrophil. The ingestion of heat-killed bacteria results in the induction of neutrophil apoptosis and a corresponding increase in cellular reactive oxygen intermediates (61). Addition of H_2O_2, xanthine oxidase (which produces superoxide anion and H_2O_2), glucose oxidase (which produces only H_2O_2), and the nitric oxide donor (*S*-nitrosoglutathione, GSNO) also results in the induction of neutrophil DNA fragmentation and apoptosis (29, 35, 50, 59). Also associated with the increase in reactive oxygen intermediates are decreases in cellular glutathione. This loss of glutathione is seen in neutrophils undergoing spontaneous apoptosis and is more marked in Fas antibody- and TNF-α-treated cells (38). An imbalance in oxidants and antioxidants in neutrophils from elderly people has also been shown to contribute to the increase in spontaneous apoptosis in this population, which have a lower antioxidant shield, leading to an augmented cellular oxidative load (54).

Antioxidants have been shown to have some protective role against the induction of neutrophil apoptosis. Catalase has been shown to prevent both spontaneous neutrophil apoptosis and reactive oxygen-induced apoptosis (29). It has also been shown that Cu,Zn-superoxide dismutase and Mn-superoxide dismutase, important cellular antioxidants, have protective effects (45). Increases in cellular glutathione through incubation with the glutathione precursor *N*-acetylcysteine inhibit Fas antibody-induced apoptosis; however, there was no effect on spontaneous rates (63, 65). Recent studies have demonstrated that for this to occur the glutathione pumps in the neutrophil have to be inhibited, which will then allow the accumulation of glutathione within the cell and the inhibition of spontaneous neutrophil apoptosis (unpublished observations). LPS, which delays neutrophil apoptosis, has also been shown to increase glutathione levels within the cells and may contribute to this delay in the apoptotic cascade (62). Counter to this, there is evidence that exogenous glutathione has been shown to enhance neutrophil apoptosis at an early time point (38).

The direct depletion of glutathione is associated with the induction of neutrophil apoptosis. Diethyl maleate and diamide chemically deplete glutathione and induce neutrophil apoptosis in a dose-dependent manner. This process results in the activation of caspase 3 and is caspase-dependent, as the broad range zVAD caspase inhibitor blocks the apoptotic response (46, 62).

REDOX REGULATION OF CASPASE ACTIVITY

Caspases are central signaling molecules in the induction of neutrophil apoptosis. As these proteases are cysteine-dependent enzymes, they are sensitive to reducing and oxidizing environments. Understanding the effects of oxidants and antioxidants on caspase activity has important implications for the regulation of neutrophil apoptosis.

Glutathione depletion by the thiol oxidant diamide has been shown to induce Jurkat cell apoptosis at low concentrations, but necrosis at high doses (55). This was not associated with an increase in reactive oxygen intermediates, but did result in mitochondrial release of cytochrome *c* and caspase 3 activation. It has also been shown that Fas-mediated apoptosis in T-lymphocytes is inhibited by preculture with *N*-acetylcysteine and enhanced by buthionine sulfoximine (55).

Other studies have demonstrated that the generation of an oxidizing intracellular environment has two distinct effects: it can directly induce apoptosis, which is caspase-dependent due to the cleavage of PARP, but it has also been shown to block apoptosis through the inhibition of the caspases. These effects are supported by two models: firstly, H_2O_2 was shown to block Fas antibody-induced apoptosis and the activation of the caspases, despite the fact that H_2O_2 induces apoptosis. Secondly, dithiocarbamates, which have been shown to inhibit the apoptotic response possibly through their antioxidant effects, have been shown to be potent thiol oxidizing compounds that inhibit procaspase processing as well as mature caspase activity in the cell. However, in both these models the effects are only temporary, and over extended time periods they go on to induce apoptosis (23). Other studies have shown that incubation of cellular extracts, with increased caspase activity, are inhibited to cleave caspase substrates when incubated with increasing concentrations of the reducing agents, 2-mercaptoethanol, glutathione, and thioredoxin (44, 55). These studies point toward the fact that a reduced environment is anticaspase.

Glutathione has been shown to directly inhibit caspase activity, which is reversed by diamide (65) in cellular extracts isolated from neutrophils incubated with Fas antibodies. This is supported by neutrophil glutathione depletion studies, which demonstrate that there is an increase in caspase activity following incubation with diethyl maleate or diamide (46).

In contrast to these studies, neutrophils incubated with diphenyleneiodonium, an inhibitor of the NADPH oxidase system, caused an increase in caspase activity, as determined by DEVD-AMC cleavage, in both spontaneous and Fas antibody-induced apoptosis. It was further demonstrated that reactive oxygen intermediates do not activate caspase activity, but in fact block their activity (19).

Chronic granulomatous disease (CGD) is a rare hereditary disease characterized by severe, protracted, and potentially fatal bacterial and fungal infections due to their inability to produce reactive oxygen intermediates and kill these organisms. It was hoped that neutrophils from these patients would help to unravel the role of reactive oxygen intermediates in neutrophil apoptosis. Studies by Kasahara *et al.* (29) have shown that there is a delay in spontaneous and resistance to Fas antibody-induced neutrophil apoptosis in these patients. However, Fadeel *et al.* demonstrated that although there was slower induction of neutrophil spontaneous and Fas antibody-induced apoptosis in CGD neutrophils, there was no difference overall and caspase 3 activity occurred as normal. Additional work now needs to be carried out with these neutrophils to determine the role of reactive oxygen intermediates on the apoptotic pathway. These studies would, however, demonstrate that reactive oxygen intermediates are not involved in spontaneous apoptosis as the cells do undergo apoptosis, but it does not rule out the possibility that glutathione depletion is centrally involved.

CONCLUSION

From all these studies, it could be possible that reactive oxygen intermediates do inhibit

caspase activity and that their role in inducing apoptosis maybe through the depletion of antioxidants, specifically glutathione. Further research will have to be undertaken to prove this hypothesis conclusively or fuel further debate.

Neutrophils have the ability to produce large concentrations of reactive oxygen intermediates. These species may contribute to either delaying neutrophil apoptosis and thus contributing to the persistence of the inflammatory response or inducing apoptosis and helping in its resolution. Both results have important implications in the treatment of inflammatory diseases, such as bacterial infections and the systemic inflammatory response syndrome.

ABBREVIATIONS

CGD, chronic granulomatous disease; ERK, extracellular signal-regulated kinase; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PARP, poly(ADP ribose) polymerase; TNF-α, tumor necrosis factor-α; TNFR, tumour necrosis factor receptor.

REFERENCES

1. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, and Yuan J. Human ICE/CED-3 protease nomenclature. *Cell* 87: 171, 1996.
2. Armstrong RC, Aja T, Xiang J, Gaur S, Krebs JF, Hoang K, Bai X, Korsmeyer SJ, Karanewsky DS, Fritz LC, and Tomaselli KJ. Fas-induced activation of the cell death-related protease CPP32 is inhibited by Bcl-2 and by ICE family protease inhibitors. *J Biol Chem* 271: 16850–16855, 1996.
3. Banki K, Hutter E, Colombo E, Gonchoroff NJ, and Perl A. Glutathione levels and sensitivity to apoptosis are regulated by changes in transaldolase expression. *J Biol Chem* 271: 32994–33001, 1996.
4. Barres BA, Hart IK, Coles HS, Burne JF, Voyvedic JT, Richartson WD, and Raff MC. Cell death and control of cell survival in the oligodendrocyte lineage. *Cell* 70: 31–46, 1992.
5. Biffl WL, Moore EE, Moore FA, and Barnett CC. Interleukin-6 suppression of neutrophil apoptosis is neutrophil concentration dependent. *J Leukoc Biol* 58: 582–584, 1995.
6. Boldin MP, Goncharov TM, Goltsev YV, and Wallach D. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. *Cell* 85: 803–815, 1996.
7. Brannigan A, O'Connell R, Hurley H, O'Neill A, Fitzpatrick JM, Brady HR, and Watson RWG. Neutrophil apoptosis is delayed in patients with inflammatory bowel disease. *Shock* 5: 361–366, 2000.
8. Buttke TM and Sandstrom PA. Oxidative stress as a mediator of apoptosis. *Immunol Today* 15: 7–10, 1994.
9. Chang DJ, Ringold GM, and Heller RA. Cell killing and induction of manganous superoxide dismutase by tumor necrosis factor-alpha is mediated by lipoxygenase metabolites of arachidonic acid. *Biochem Biophys Res Commun* 188: 538–546, 1992.
10. Chinnaiyan AM, O'Rourke K, Tewari M, and Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 81: 505–512, 1995.
11. Chitnis DC, Dickerson C, Munster AM, and Winchurch RA. Inhibition of apoptosis in polymorphonuclear neutrophils from burn patients. *J Leukoc Biol* 59: 835–839, 1996.
12. Cohen JJ. Apoptosis. *Immunol Today* 14: 126–130, 1993.
13. Colotta F, Re F, Polentarutti N, Sozzani S, and Mantovani A. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood* 80: 2012–2020, 1992.
14. Cox G. Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. *J Immunol* 154: 4719–4725, 1995.
15. Cox G, Gauldie J, and Jordana M. Bronchial epithelial cell-derived cytokines (G-CSF and GM-CSF) promote the survival of peripheral blood neutrophils in vitro. *Am J Respir Cell Mol Biol* 7: 507–513, 1992.
16. Dbaibo GS, Perry DK, Gamard CJ, Platt R, Poirier GG, Obeid LM, and Hannun YA. Cytokine response modifier A (CrmA) inhibits ceramide formation in response to tumor necrosis factor (TNF)-α: CrmA and Bcl-2 target distinct components in the apoptotic pathway. *J Exp Med* 185: 481–490, 1997.
17. DiPietrantonio AM, Hsieh T, and Wu JM. Activation of caspase 3 in HL-60 cells exposed to hydrogen peroxide. *Biochem Biophys Res Commun* 255: 477–482, 1999.
18. Edwards SW. *Biochemistry and Physiology of the Neutrophil.* New York: Cambridge University Press, 1994.
19. Fadeel B, Ahlin A, Henter J-I, Orrenius S, and Hampton MB. Involvement of caspases in neutrophil apoptosis: regulation by reactive oxygen species. *Blood* 92: 4808–4818, 1998.
20. Gerschenson LE and Rotello RJ. Apoptosis: a different type of cell death. *FASEB J* 6: 2450–2455, 1992.
21. Ghibelli L, Coppola S, Rotilio G, Lafavia E, Maresca V, and Ciriolo MR. Non-oxidative loss of glutathione in apoptosis via GSH extrusion. *Biochem Biophys Res Commun* 216: 313–320, 1995.
22. Ghibelli L, Fanelli C, Rotilio G, Lafavia E, Coppola S, Colussi C, Civitareale P, and Ciriolo MR. Rescue of cells from apoptosis by inhibition of active GSH extrusion. *FASEB J* 12: 479–486, 1998.

23. Hampton MB and Orrenius S. Dual regulation of caspase activity by hydrogen peroxide: implication for apoptosis. *FEBS Lett* 414: 552–556, 1997.
24. Hart SP, Ross JA, Haslett C, and Dransfield I. Molecular characterization of the surface of apoptotic neutrophils: implication for functional down regulation by phagocytes. *Cell Differ* 5: 493–503, 2000.
25. Hengartner MO. The biochemistry of apoptosis. *Nature* 407: 770–776, 2000.
26. Hockenbery DM, Oltvai ZN, Yin XM, Milliman CL, and Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75: 241–251, 1993.
27. Hsu H, Xiong J, and Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-κB activation. *Cell* 81: 495–504, 1995.
28. Kane DJ, Sarafian TA, Anton R, Hahn H, Graalla EB, Valentine JS, Ord T, and Bredesen DE. Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* 262: 1274–1277, 1993.
29. Kasahara Y, Iwai K, Yachie A, Ohta K, Konno A, Seki H, Miyawaki T, and Taniguchi N. Involvement of reactive oxygen intermediates in spontaneous and CD95(Fas/APO-1)-mediated apoptosis of neutrophils. *Blood* 89: 1748–1753, 1997.
30. Kerr JF, Wyllie AH, and Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26: 239–257, 1972.
31. Kerr JFR, Winterford CM, and Harmon BV. Apoptosis: its significance in cancer and cancer therapy. *Cancer* 73: 2013–2026, 1994.
32. Lee A, Whyte MKB, and Haslett C. Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. *J Leukoc Biol* 54: 283–288, 1993.
33. Lennon SV, Martin SJ, and Cotter TG. Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. *Cell Prolif* 24: 203–214, 1991.
34. Leuenroth SJ, Grutkoski PS, Ayala A, and Simms HH. The loss of Mcl-1 expression in human polymorphonuclear leukocytes promotes apoptosis. *J Leukoc Biol* 68: 158–166, 2000.
35. Lundqvist-Gustafsson H and Bengtsson T. Activation of the granule pool of the NADPH oxidase accelerated apoptosis in human neutrophils. *J Leukoc Biol* 65: 196–204, 1999.
36. Macho A, Hirsch T, Marzo I, Marchetti P, Dallaporta B, Susin SA, Zamzami N, and Kroemer G. Glutathione depletion is an early and calcium elevation is a late event of thymocyte apoptosis. *J Immunol* 158: 4612–4619, 1997.
37. McGowan AJ, Ruiz-Ruiz MC, Gorman AM, Lopez-Rivas A, and Cotter TG. Reactive oxygen intermediate(s) (ROI): common mediator(s) or poly(ADP-ribose)polymerase (PARP) cleavage and apoptosis. *FEBS Lett* 392: 299–303, 1996.
38. Misso NL, Peacock CD, Watkins DN, and Thompson PJ. Nitrite generation and antioxidant effects during neutrophil apoptosis. *Free Radic Biol Med* 28: 934–943, 2000.
39. Moulding DA, Quayle JA, Hart CA, and Edwards SW. Mcl-1 expression in human neutrophils: regulation by cytokines and correlation with cell survival. *Blood* 92: 2495–2502, 1998.
40. Murray J, Barbara JAJ, Dunkley SA, Lopez AF, Ostade XV, Condliffe AM, Dransfield I, Haslett C, and Chilvers ER. Regulation of neutrophil apoptosis by tumour necrosis factor-α: requirement for TNFR55 and TNFR75 for induction of apoptosis in vitro. *Blood* 90: 2772–2783, 1997.
41. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME, and Dixit VM. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell* 85: 817–828, 1996.
42. Nagata S. Fas and Fas ligand: a death factor and its receptor. *Adv Immunol* 57: 129–144, 1994.
43. Nagata S and Golstein P. The Fas death factor. *Science* 267: 1449–1456, 1995.
44. Nobel CS, Burgess DH, Zhivotovsky B, Burkitt MJ, Orrenius S, and Slater AFG. Mechanism of dithiocarbamate inhibition of apoptosis: thiol oxidation by dithiocarbamate disulfides directly inhibits processing of the caspase-3 proenzyme. *Chem Res Toxicol* 10: 636–643, 1997.
45. Oishi K and Machida K. Inhibition of neutrophil apoptosis by anti-oxidants in culture medium. *Scand J Immunol* 45: 21–27, 1997.
46. O'Neill AJ, O'Neill S, Hegarty NJ, Coffey RNT, Gibbons N, Brady HR, Fitzpatrick JM, and Watson RWG. Glutathione depletion induced neutrophil apoptosis is caspase 3 dependent. *Shock* 14: 605–609, 2000.
47. Pericle F, Liu JH, Diaz JI, Blanchard DK, Wei S, Forni G, and Djeu JY. Interleukin-2 prevention of apoptosis in human neutrophils. *Eur J Immunol* 24: 440–444, 1994.
48. Repine JE. Scientific perspectives on adult respiratory distress syndrome. *Lancet* 339: 466–469, 1992.
49. Robinson J, Watson F, Bucknall RC, and Edwards SW. Activation of neutrophils reactive oxidants production by synovial fluid from patients with inflammatory joint disease: soluble and insoluble immunoglobulin aggregates activate different pathways in primed and unprimed cells. *Biochem J* 46: 2401–2406, 1992.
50. Rollet-Labelle E, Grange MJ, Elbim C, Marquetty C, Gougerot-Pocidalo MA, and Pasquier C. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. *Free Radic Biol Med* 24: 563–572, 1998.
51. Sanghavi DM, Thelen M, Thornberry NA, Casciola-Rosen L, and Rosen A. Caspase-mediated proteolysis during apoptosis: insights from apoptotic neutrophils. *FEBS Lett* 422: 179–184, 1998.
52. Suda T and Nagata S. Purification and characterization of the Fas-ligand that induces apoptosis. *J Exp Med* 179: 873–879, 1994.
53. Takeda Y, Watanabe H, Yonehara S, Yamashita T, Saito S, and Sendo F. Rapid acceleration of neutrophil

apoptosis by tumor necrosis factor. *Int Immunol* 5: 691–694, 1993.
54. Tortorella C, Piazzolla G, Spaccavento F, Jirillo E, and Antonaci S. Age-related effects of oxidative metabolism and cyclic AMP signaling on neutrophil apoptosis. *Mech Ageing Dev* 110: 195–205, 1999.
55. Ueda S, Nakamura H, Masutani H, Sasada T, Yonehara S, Takabayashi A, Yamaoka Y, and Yodoi J. Redox regulation of caspase-3(-like) protease activity: regulatory roles of thioredoxin and cytochrome *c*. *J Immunol* 161: 6689–6695, 1998.
56. Vignaux F, Vivier E, Malissen B, Depraetere V, Nagata S, and Golstein P. TCR/CD3 coupling to Fas-based cytotoxicity. *J Exp Med* 181: 781–786, 1995.
57. Villunger A, O'Reilly LA, Holler N, Adams J, and Strasser A. Fas ligand, Bcl2, granulocyte colony-stimulating factor, and p38 mitogen-activated protein kinase: regulators of distinct cell death and survival pathways in granulocytes. *J Exp Med* 192: 647–658, 2000.
58. Ward C, Dransfield I, Chilvers ER, Haslett C, and Rossi AG. Pharmacological manipulation of granulocyte apoptosis: potential therapeutic target. *Trends Pharmacol Sci* 20: 503–509, 1999.
59. Ward C, Wong TH, Murry J, Rahman J, Haslett C, Chilvers IR, and Rossi AG. Induction of human neutrophil apoptosis by nitric oxide donors: evidence for a caspase-dependent, cycle-GMP-independent mechanism. *Biochem Pharmacol* 59: 305–314, 2000.
60. Watson RWG, Redmond HP, Wang JH, and Bouchier-Hayes D. Bacterial ingestion, tumor necrosis factor-alpha, and heat induced programmed cell death in activated neutrophils. *Shock* 5: 47–51, 1996.
61. Watson RWG, Redmond HP, Wang JH, and Bouchier-Hayes DJ. Neutrophils undergo apoptosis following ingestion of *Escherichia coli*. *J Immunol* 156: 3986–3992, 1996.
62. Watson RWG, Rotstein OD, Nathens AB, Dackiw APB, and Marshall JC. Thiol-mediated redox regulation of neutrophil apoptosis. *Surgery* 120: 150–158, 1996.
63. Watson RWG, Rotstein OD, Jimenez M, Parodo J, and Marshall JC. Augmented intracellular glutathione inhibits Fas-triggered apoptosis of activated human neutrophils. *Blood* 89: 1175–1181, 1997.
64. Watson RWG, Rotstein OD, Parodo J, Bitar R, and Marshall JC. The interleukin-1β-converting enzyme (Caspase-1) inhibits apoptosis of inflammatory neutrophils through activation of interleukin-1β. *J Immunol* 161: 957–962, 1998.
65. Watson RWG, O'Neill A, Brannigan A, Coffey R, Marshall JC, Brady HR, and Fitzpatrick JM. Regulation of Fas antibody induced neutrophil apoptosis is both caspase and mitochondrial dependent. *FEBS Lett* 453: 67–71, 1999.
66. Wyllie AH, Kerr JF, and Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 68: 251–306, 1980.

Address reprint requests to:
Dr. R.W.G. Watson
Department of Surgery
University College Dublin
Mater Misericordiae Hospital
47 Eccles Street
Dublin 7
Ireland.

E-mail: research@profsurg.iol.ie

Received for publication July 30, 2001; accepted September 4, 2001.

This article has been cited by:

1. Arup Sarkar, Eresso Aga, Uta Bussmeyer, Asima Bhattacharyya, Sonja Möller, Lars Hellberg, Martina Behnen, Werner Solbach, Tamás Laskay. 2012. Infection of neutrophil granulocytes with Leishmania major activates ERK 1/2 and modulates multiple apoptotic pathways to inhibit apoptosis. *Medical Microbiology and Immunology* . [CrossRef]
2. Marcelo Paes Barros, Douglas Popp Marin, Anaysa Paola Bolin, Rita de Cássia Santos Macedo, Thais Regina Campoio, Claudio Fineto, Beatriz Alves Guerra, Tatiana Geraldo Polotow, Cristina Vardaris, Rita Mattei, Rosemari Otton. 2012. Combined astaxanthin and fish oil supplementation improves glutathione-based redox balance in rat plasma and neutrophils. *Chemico-Biological Interactions* **197**:1, 58-67. [CrossRef]
3. El#bieta Jab#onowska, Kamila Wójcik, Barbara Kur, Przemys#aw Lewkowicz, Marek Nocu#. 2011. Neutrophil Function and Apoptosis in Patients with Chronic Hepatitis C Treated with Pegylated Interferon # and Ribavirin. *Archivum Immunologiae et Therapiae Experimentalis* . [CrossRef]
4. A. Paige Davis Volk, Brieanna M. Barber, Kelli L. Goss, Jake G. Ruff, Christine K. Heise, Jessica S. Hook, Jessica G. Moreland. 2011. Priming of Neutrophils and Differentiated PLB-985 Cells by Pathophysiological Concentrations of TNF-# Is Partially Oxygen Dependent. *Journal of Innate Immunity* **3**:3, 298-314. [CrossRef]
5. Nada Oršoli#, Vesna Benkovi#, Duje Lisi#i#, Domagoj #iki#, Julija Erhardt, Anica Horvat Kneževi#. 2010. Protective effects of propolis and related polyphenolic/flavonoid compounds against toxicity induced by irinotecan. *Medical Oncology* **27**:4, 1346-1358. [CrossRef]
6. François D. Graham, Karl-Rudolf Erlemann, Sylvie Gravel, Joshua Rokach, William S. Powell. 2009. Oxidative stress-induced changes in pyridine nucleotides and chemoattractant 5-lipoxygenase products in aging neutrophils. *Free Radical Biology and Medicine* **47**:1, 62-71. [CrossRef]
7. Craig B. Webb, Kelly W. McCord, David C. Twedt. 2009. Assessment of oxidative stress in leukocytes and granulocyte function following oral administration of a silibinin-phosphatidylcholine complex in cats. *American Journal of Veterinary Research* **70**:1, 57-62. [CrossRef]
8. O KHYMENETS, M COVAS, M FARRE, K LANGOHR, M FITO, R DELATORRE. 2008. Role of sex and time of blood sampling in SOD1 and SOD2 expression variability. *Clinical Biochemistry* **41**:16-17, 1348-1354. [CrossRef]
9. Miriam S. Giambelluca, Oscar A. Gende. 2008. Hydrogen peroxide activates calcium influx in human neutrophils. *Molecular and Cellular Biochemistry* **309**:1-2, 151-156. [CrossRef]
10. Lizzia Raffaghello, Giordano Bianchi, Maria Bertolotto, Fabrizio Montecucco, Alessandro Busca, Franco Dallegri, Luciano Ottonello, Vito Pistoia. 2008. Human Mesenchymal Stem Cells Inhibit Neutrophil Apoptosis: A Model for Neutrophil Preservation in the Bone Marrow Niche. *Stem Cells* **26**:1, 151-162. [CrossRef]
11. M KAPISZEWSKA, A CIERNIAK, M ELAS, A LANKOFF. 2007. Lifespan of etoposide-treated human neutrophils is affected by antioxidant ability of quercetin. *Toxicology in Vitro* **21**:6, 1020-1030. [CrossRef]
12. Maria Augusta Arruda, Pedro Barcellos-de-Souza, André Luiz Franco Sampaio, Adriano G. Rossi, Aurélio V. Graça-Souza, Christina Barja-Fidalgo. 2006. NADPH oxidase-derived ROS: Key modulators of heme-induced mitochondrial stability in human neutrophils. *Experimental Cell Research* **312**:19, 3939-3948. [CrossRef]
13. E Arenas-Ríos, M A León-Galván, P E Mercado, A Rosado. 2005. Superoxide dismutase, catalase, and glutathione peroxidase during epididymal maturation and prolonged storage of spermatozoa in the Mexican big-eared bat (Corynorhinus mexicanus). *Canadian Journal of Zoology* **83**:12, 1556-1565. [CrossRef]
14. M Baker. 2004. The importance of redox regulated pathways in sperm cell biology. *Molecular and Cellular Endocrinology* **216**:1-2, 47-54. [CrossRef]