Free Rad. Res., Vol. 21, No. 1, pp. 1-8
Reprints available directly from the publisher
Photocopying permitted by license only

INVITED COMMENTARY
IS APOPTOSIS MEDIATED BY REACTIVE OXYGEN SPECIES?

THEODORE A. SARAFIAN* and DALE E. BREDESEN†

*Departments of *Pathology and †Neurology, †Brain Research Institute, and †Molecular Biology Institute, University of California, Los Angeles, California, USA*

(Received February 7th, 1994; in revised form February 18th, 1994)

Apoptosis is a common mode of programmed cell death occurring during development as well as in many pathological conditions, in which the cell plays an active role in its own demise. Although the morphological and biochemical hallmarks of apoptosis are conserved across phyla and cell type, the mechanism(s) of apoptosis is unknown. However, data recently published demonstrate that expression of the anti-apoptotic gene *bcl-2* decreases the net cellular generation of reactive oxygen species, and that reactive oxygen species serve as mediators of apoptosis in at least some cases.

KEY WORDS: Apoptosis, programmed cell death, necrosis, Bcl-2, anti-apoptotic gene, internucleosomal DNA fragmentation.

INTRODUCTION

When biological evolution reached the critical juncture leading to the development of multicellular organisms, the capacity for the controlled termination of individual cells became an important system feature. Morphological development requires the coordinated execution of three fundamental cellular processes: division, differentiation, and death. Controlled cell death is vital not only in morphogenesis but also in the elimination of damaged or neoplastic cells and the optimization of immunological responses.[1]

Developmentally-controlled cell death occurring during insect morphogenesis was termed *programmed cell death* in 1964 by Lockshin.[2] Subsequent studies of cell death following hepatic ischaemia led to the concept of *apoptosis*, from the Greek *apo* = away from, and *ptosis* = falling.[3] Programmed cell death and apoptosis share many morphological features, and the terms are often used interchangeably, but equality has not been demonstrated: specifically, the internucleosomal fragmentation of DNA that characterizes apoptosis has not been demonstrated in programmed cell death, and the requirement for protein synthesis that is characteristic of programmed cell death exists in some, but not all, cells undergoing apoptosis.[2,4] These differences may simply reflect the different organisms in which the two

† To whom reprint requests should be addressed at: University of California, Los Angeles, School of Medicine, Reed Neurological Research Center, 710 Westwood Plaza, Los Angeles, CA 90024-1769, USA. Telephone: (310) 206-7080. Fax: (310) 206-2502.

phenomena were described, or may reflect more fundamental differences. Morphological characteristics of apoptosis include nuclear fragmentation, chromatin condensation, plasma membrane blebbing, relative preservation of organellar structure, and preservation of plasma membrane continuity.[3] Genetic and biochemical characteristics include the internucleosomal fragmentation of DNA (in multiples of approximately 180 base pairs, corresponding to the size of the nucleosome) and enhanced expression of a set of genes, including those encoding sulphated glycoprotein-2, transglutaminase, polyubiquitin.[5] Although this set of expressed genes varies to some extent between cell types, there is a high degree of conservation.

Both programmed cell death and apoptosis have been referred to as cellular suicide, since (1) the morphological, genetic, and biochemical changes that occur are orderly and reproducible,[6] and (2) inhibitors of transcription or translation inhibit the process (except in apoptosis in some cell types), suggesting an active role for the cell in its own demise.[7,8] The mechanism(s) by which the cell effects its own termination is unknown, but appear to be conserved across phyla and cell types.[9,10] Extensive studies of thymocyte apoptosis in response to corticosteroids have led to a model that features calcium entry leading to the activation of a nuclear calcium/magnesium-dependent endonuclease.[11-13] Three observations have suggested the need for a revision of this model: first, in at least some cellular systems, the induction of apoptosis by serum and growth factor withdrawal is not accompanied by a rise in intracellular free calcium concentration[14] (this does not preclude the possibility that subcellular changes in calcium distribution might have been missed by the FURA-2 imaging in that study). Second, in some cells the morphological features of apoptosis are not accompanied by double stranded internucleosomal DNA fragmentation.[15] Third, the expression of the *bcl-2* gene inhibits apoptosis across cell types, phyla, and apoptotic paradigms, yet has no effect on intracellular free calcium.[14,16]

Any new model for the biochemical events defining apoptosis must distinguish between an *inducer* of apoptosis – of which there are many – and a *mediator* of apoptosis. Since apoptosis represents the cell's response to virtually any severe injury that does not cause rapid necrosis,[6] the list of apoptosis inducers is large, including hyperthermia, growth factor withdrawal, ischaemia, hypoglycaemia, many toxins, β-amyloid peptide, calcium ionophores, viral infection, and many other factors.[6] Thus, the induction of apoptosis by any given factor offers insight neither into the toxic biochemistry of the given factor nor into the mechanism of apoptosis itself. In contrast, the identification of a mediator of apoptosis would offer some insight into the biochemistry of apoptosis. Such a mediator should satisfy the following criteria:

1. The mediator should demonstrate a change (an increase or decrease in activity) during apoptosis, prior to cell death.
2. Modulation of the putative mediator should modulate apoptosis accordingly.
3. Expression of anti-apoptotic genes should affect the putative mediator, unless the effects of the anti-apoptotic genes are 'downstream' from the putative mediator.
4. The effect of anti-apoptotic genes should be overridden by independent modulation of the putative mediator, unless the effects of the anti-apoptotic genes are 'downstream' from the putative mediator.

REACTIVE OXYGEN SPECIES AS MEDIATORS OF APOPTOSIS

Recent data from two laboratories have implicated reactive oxygen species (ROS) as mediators of apoptosis.[17-19] The proto-oncogene *bcl-2*, which is expressed at high level (due to a translocation) in approximately 60% of B-cell lymphomas,[20] was shown to inhibit apoptosis in hematopoietic cell lines induced by the withdrawal of interleukin-3.[21,22] Subsequently, it was noted that Bcl-2 inhibits cell death from a broad range of insults, across phyla (chordata and arthropoda) and cell origin (e.g., hematopoietic, neural, and prostatic). Furthermore, in some cases necrosis, as well as apoptosis, was inhibited, arguing that Bcl-2 does not inhibit apoptosis per se; rather, Bcl-2 inhibits a cellular process that may result in apoptosis or necrosis.[17,18]

Expression of *bcl-2* was associated with a marked decrease in the net cellular generation of reactive oxygen species, as determined by dichlorofluorescein diacetate,[18] and prevention of lipid peroxidation.[18,19] Bcl-2 also increased the survival of yeast mutants null for superoxide dismutase, especially those deficient in MnSOD. Furthermore, overexpression of *sodl* in a neural cell line (but not *sod2* in a hematopoietic cell line) or glutathione peroxidase in a lymphoid cell line inhibited apoptosis.[18,19]

Although it is possible that Bcl-2 has additional cellular effects, the effect on reactive oxygen species was found to be causally associated with the inhibition of cell death by Bcl-2.[18] First, the rise in ROS in the control cells preceded the cell death. Second, modulation of the rise in ROS modulated the appearance and extent of cell death. Third, in more recent experiments, overcoming the inhibition of ROS generation by Bcl-2 overcame the inhibition of cell death (ref. 24 and Kane *et al.*, in preparation).

These findings argue that ROS serve as mediators of apoptosis, and the findings are compatible with previous studies of apoptosis. First, ionizing radiation induces apoptosis in some cell types, and, based on relative molecular abundance, the principal target of ionizing radiation is water, with the principal cellular damaging species being hydroxyl radical. This free radical species reacts in a diffusion-controlled manner with protein, DNA, and lipids, causing protein oxidation, DNA strand scission, membrane blebbing, and lipid oxidation, the last three of which are characteristic of apoptosis. Although it is unlikely that hydroxyl radical is responsible for the periodically spaced double stranded DNA breaks characteristic of apoptosis, it could be involved in the initial cleavage at 100–300 kilobase intervals which precedes the production of the 180 base pair multiples.

Ionizing radiation has also been demonstrated to activate unspecified tyrosine kinase enzymes which appear to mediate apoptosis in human B-lymphocyte cell lines.[25] Radiation treatment stimulated tyrosine phosphorylation of 6–14 proteins. The tyrosine kinase inhibitor genistein inhibited this phosphorylation and protected cells against DNA degradation and cell death. In contrast, the phosphatase inhibitor, vanadate, resulted in increased sensitivity to ionizing radiation.

Second, the human T-cell line CCRF-CEM undergoes apoptotic cell death when cultured at a density $<10^5$ cells/ml in serum-free medium.[26] However, cell death is prevented by medium conditioned by cells cultured at higher density (3×10^6 cells/ml). Isolation of the active anti-apoptotic factor in the conditioned medium identified a 60 kilodalton protein demonstrating sequence homology to human catalase as well as enzyme activity. Furthermore, catalase from non-human sources could substitute for conditioned medium to block density-dependent apoptosis.[26] These observations suggest that CCRF-CEM cultures contain sufficient hydrogen peroxide to induce apoptosis at low density. Although the possibility of the introduction of

hydrogen peroxide from tissue culture medium was not excluded, the most likely source is the CCRF-CEM cells themselves.

Third, embryonic blastocoele fluid contains a soluble agent capable of killing embryonal carcinoma cells with trophectodermal potential.[27] In contrast, P19 embryonic carcinoma cells are resistant to this soluble agent, due to higher levels of glutathione. Indeed, exposure to buthionine sulphoximine (a specific and essentially irreversible inhibitor of γ-glutamylcystein synthetase[28]) increases the sensitivity of P19 cells to embryonic blastocoele fluid. This cell death is apoptotic, and may be prevented by treating the blastocoele fluid with catalase prior to exposing the cells to the blastocoele fluid. The hydrogen peroxide present in the fluid appears to be generated by a polyamine oxidase.[27]

Fourth, addition of nerve growth factor (NGF) to PC12 pheochromocytoma cells increases the intracellular level of catalase and decreases the sensitivity of PC12 cells to exogenous hydrogen peroxide.[29] Furthermore, addition of the catalase inhibitor 3-aminotriazole counters the protective effect of NGF against hydrogen peroxide. However, NGF inhibits PC12 death due to many agents, including PC12 apoptosis in response to serum withdrawal or NGF withdrawal,[30] and it is unknown whether catalase expression alone is responsible for the inhibition of PC12 cell death following these other insults.

Fifth, tumor necrosis factor (TNF) – which would perhaps more appropriately be designated tumor apoptosis/necrosis factor – induces apoptosis in many neoplastic (and some non-neoplastic) cell types.[31] Intracellular ROS are induced by TNF, and expression of MnSOD inhibits cell death induced by TNF.[32] Ultrastructurally, early degenerative changes of mitochondria occur in L929 cells, and protection against cytotoxicity is afforded by antioxidants such as ascorbate, and by the iron ion chelator desferrioxamine.[33]

SOURCES OF REACTIVE OXYGEN SPECIES IN APOPTOSIS

What is/are the source(s) and chemical structure(s) of the ROS that mediate apoptosis? As noted above, work by Pierce and colleagues,[27] using systems of trophectoderm development and limb bud development, implicates hydrogen peroxide generated by a tissue polyamine oxidase. Studies of tumor necrosis factor cytotoxicity implicate superoxide; furthermore, inhibitors of complex III of the mitochondrial electron transport chain potentiate TNF-mediated cytotoxicity in L929 cells, whereas inhibitors of complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) protect against cytotoxicity, suggesting that superoxide anion produced by electron transfer from ubisemiquinone to dioxygen may be an important mechanism of TNF toxicity.[33] The mechanism by which such electron transfer would be increased following TNF binding is unknown. Studies of the inhibition of TNF-induced apoptosis by the protein products of the adenovirus E1B and E3 genes implicate arachidonic acid metabolism by phospholipase A2.[34] Even from these few examples, it is clear that more than one source of reactive oxygen species generation is likely. In addition, since cells normally generate approximately 10^{11} molecules of superoxide per day,[35] it is of course possible that the net cellular generation of ROS that occurs during apoptosis is the result of a decrease in scavenging and other antioxidant defence systems rather than a true increase in generation.

Any model of apoptosis that includes ROS as mediators must address the question of how cells derive the remarkable order of the apoptotic program – with the regular

DNA fragmentation, nuclear fragmentation, and appropriate upregulation and downregulation of specific sets of genes (depending on cell type) that characterize apoptosis – from the relative disorder of ROS diffusion and high reactivity (although ROS may be targeted by site-specific metal ion-dependent reactions). One possibility is that coordinated functional changes might be brought about in structural proteins by redox changes, in a situation somewhat analogous to the dissolution of the nuclear envelope that follows the phosphorylation of nuclear lamins.[36] This possibility might explain the plasma membrane blebbing that is prominent early in apoptosis, since oxidation of actin has been associated with plasma membrane blebbing.[37] However, this would leave unexplained the orderly sequence of transcriptional changes that characterizes apoptosis. To account for such changes, and for the finding that the expression of *bcl-2* inhibits both apoptosis and necrosis, we suggest a model in which ROS act as effectors in necrosis but as signaling molecules in apoptosis, leading to the transcriptional changes of apoptosis via redox-sensitive transcription factors. Examples of redox-sensitive transcription factors include AP-1,[38] USF43,[39] and NFκB.[40,41] None of these is known to be specific for the transcriptional changes associated with apoptosis, but it is likely that the existence of other redox-sensitive transcription factors will be recognized.

A link between oxidative activity and activation of the Ca^{2+}/Mg^{2+} dependent endonuclease believed responsible for DNA cleavage during apoptosis has been implicated in human tumour cells which undergo apoptosis when exposed to C-nitroso – compounds.[42] Such compounds oxidize the nuclear enzyme poly (ADP-ribose) polymerase at one zinc finger site, preventing poly-ADP ribosylation of the endonuclease which in turn de-represses the DNA-degrading activity.

This model would explain why similar insults may lead to apoptosis or necrosis, depending on severity and cell type. For example, exposure to low concentrations of hydrogen peroxide may lead to apoptosis, whereas exposure to high concentrations of hydrogen peroxide can lead to necrosis.[43] The model would also explain why Bcl-2 may inhibit both apoptosis and necrosis, yet can be bypassed in some apoptotic paradigms such as that of cytotoxic T-cell killing.[44]

CONCLUSION

If, in fact, ROS are central mediators of apoptosis, this would support recently suggested changes in our perception of the biological roles of these reactive intermediates. For the most part, oxygen free radicals have been considered to be the unavoidable and undesirable consequence of life in the presence of molecular oxygen. Reactive oxygen species have been implicated as the primary destructive intermediates in a wide range of environmental and occupational toxins, as well as an increasing number of human pathological conditions.[45] Their perceived biological utility has until recently been limited to their generation by neutrophils for use in microbial killing. However, a signaling role for nitric oxide has been established in endothelia[46] and in the nervous system.[47,48] Modulation of guanylate cyclase and other signaling systems by reactive oxygen species has also been reported in some other cells.[49–51] These observations have led to the proposal of an 'oxygen radical cycle' whereby superoxide anion and hydrogen peroxide are not completely destroyed, but utilized in messenger functions.[52]

The accumulating evidence for a central role of reactive oxygen species in apoptosis suggests that virtually all eukaryotic cells possess the means for active, regulated

generation of such species and for selective channeling of these intermediates to produce the intricately orchestrated process of apoptosis.

References

1. Y.J. Liu, D.E. Joshua, G.T. Williams, C.A. Smith, J. Gondon and I.C. MacLennan (1989) Mechanism of antigen-driven selection in germinal centres. *Nature*, **342**, 21–28.
2. R.A. Lockshin and C.M. Williams (1964) Programmed cell death II. Endocrine potentiation of the breakdown of the intersegmented muscles of silkmoths. *The Journal of Insect Physiology*, **10**, 643.
3. J.F.R. Kerr, A.H. Wyllie and A.R. Currie (1972) Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Brain Journal of Cancer*, **26**, 239.
4. L.M. Schwartz, S.W. Smith, M.E. Jones, B.A. Osborne (1993) Do all programmed cell deaths occur via apoptosis? *Proceedings of the National Academy of Sciences of the United States of America*, **90**(3), 380–4.
5. R. Buttyan (1991) Genetic response of prostate cells to androgen deprivation: insights into the cellular mechanism of apoptosis. *Apoptosis: The Molecular Basis of Cell Death*, 157–173.
6. J.F.R. Kerr and B.V. Harmon (1991) Definition and incidence of apoptosis: An historical perspective. *Apoptosis: The Molecular Basis of Cell Death*, 5–29.
7. J.R. Tata (1966) Requirement for RNA and protein synthesis for induced regression of tadpole tail in organ culture. *Developmental Biology*, **13**, 77.
8. D.P. Martin, R.E. Schmidt, P.S. DeStefano, O.H. Lowry, J.G. Carter and E.M. Johnson (1988) Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. *Journal of Cell Biology*, **106**, 829–844.
9. D.L. Vaux, I.L. Weissman and S.K. Kim (1992a) Prevention of programmed cell death in caenorhabditis elegans by human bcl-2. *Science*, **258**, 1955–1957.
10. S. Rabizadeh, D.J. La Count, P.D. Friesen and D.E. Bredesen (1993) Expression of the baculovirus *p35* gene inhibits mammalian neural cell death. *Journal of Neurochemistry*, **61**, 2318–2321.
11. D.J. McConkey, P. Hartzell, S.K. Duddy, H. Hakansson and S. Orrenius (1988) 2,3,7,8-Tetrachlorodibenzo-p-dioxin Kills Immature Thymocytes by Ca^{2+}-Mediated Endonuclease Activation. *Science*, **2**, 256–259.
12. D.J. McConkey, P. Hartzell, P. Nicotera and S. Orrenius (1989) Calcium-activated DNA fragmentation kills immature thymocytes. *Federation of American Societies for Experimental Biology Journal*, **3**, 1843–1849.
13. D.J. McConkey, P. Hartzell, J.F. Amador-Perez, S. Orrenius and J. Mondal (1989) Calcium-dependent killing of immature thymocytes by stimulation via the CD3/T cell receptor complex. *The Journal of Immunology*, **143**, 1801–1806.
14. L.T. Zhong, T. Sarafian, D.J. Kane, A.C. Charles, S.P. Mah, R.H. Edwards and D.E. Bredesen (1993a) Expression of *bcl-2* inhibits neural death induced by multiple agents. *Proceedings of the National Academy of Science, USA*, **90**, 4533–4537.
15. L.D. Tomei, J.P. Shapiro and F.O. Cope (1992) Apoptosis in C3H/10T 1/2 mouse embryonic cells: Evidence for internucleosomal DNA modification in the absence of double-strand cleavage. *Proceedings of the National Academy of Science, USA*, **90**, 853–857.
16. L. Zhong, D. Kane and D.E. Bredesen (1993) BCL-2 glutamate toxicity in neural cell lines. *Molecular Brain Research*, **19**, 353–355.
17. D.J. Kane, T. Ord, R. Anton, T. Sarafian and D.E. Bredesen (1993) BCL2 inhibits necrotic cell death. *Submitted to Journal of Neuroscience Research.*
18. D.J. Kane, T.A. Sarafian, R. Anton, H. Hahn, E.B. Gralla, J.S. Valentine, T. Ord and D.E. Bredesen (1993) Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science*, **262**, 1274–1277.
19. D. Hockenbery, Z.N. Oltvai, X. Yin, C.L. Milliman and S. J. Korsmeyer (1993) Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell*, **75**, 241–251.
20. Y. Tsujimoto, L. Finger, J. Yunis, P. Nowell and C. Croce (1984) Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science*, **226**, 1097–1099.
21. D. Hockenbery, G. Nuñez, C. Milliman, R. Schreiber and S. Korsmeyer (1990) Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature*, **348**, 334–336.
22. G. Nuñez, L. Loncon, D. Hockenbery, M. Alexander, J.P. McKearn and S.J. Korsmeyer (1990) Deregulated bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. *The Journal of Immunology*, **144**, 3602–3610.

23. H. Hahn, S. Rabizadeh, E.B. Gralla, J.S. Valentine, T. Huang, C.J. Epstein and D.E. Bredesen (1993) Prevention of apoptosis by superoxide dismutase; rescue of yeast *sod* mutants by the anti-apoptotic gene p35. *Submitted for publication to Science.*
24. T.A. Sarafian, L. Vartavarian, D.J. Kane, D.E. Bredesen and M.A. Verity (1993) Bcl-2 expression decreases methyl mercury-induced free-radical generation and cell killing in a neural cell line. *Submitted for publication to Toxicology Letters.*
25. F.M. Uckun, L. Tuel-Ahlgren, C.W. Song, K. Waddick, D.E. Myers, J. Kirihara, J.A. Ledbetter and G.L. Schieven (1992) Ionizing radiation stimulates unidentified tyrosine-specific protein kinases in human B-lymphocyte precursors, triggering apoptosis and clonogenic cell death. *Proceedings of the National Academy of Sciences, USA*, **89**, 9005–9009.
26. P.A. Sandstrom and T.M. Butke (1993) Autocrine production of extracellular catalase prevents apoptosis of human CEM T-cell line in serum-free medium. *Proceedings of the National Academy of Sciences*, **90**, 4708–4712.
27. G.B. Pierce, R.E. Parchment and A.L. Lewellyn (1991) Hydrogen peroxide as a mediator of programmed cell death in the blastocyst. *Differentiation*, **46**, 181–186.
28. O.W. Griffith (1982) Mechanism of action, metabolism, and toxicity of buthionine sulfoximine and its higher homologs potent inhibitors of glutathione synthesis. *Journal of Biological Chemistry*, **257**, 13704–13712.
29. G.R. Jackson, L. Apffel, K. Werrbach-Perez and J.R. Perez-Polo (1990) Role of nerve growth factor in oxidant-antioxidant balance and neuronal injury. Stimulation of hydrogen peroxide resistance. *Journal of Neuroscience Research*, **25**, 360–368.
30. A. Batistatou, C. Volonte, L.A. Greene (1992) Nerve growth factor employs multiple pathways response genes in PC12 cells. *Molecular Biology of the Cell*, **3**, 363–71.
31. E.A. Carswell, L.J. Old, R.L. Kassel, S. Green, N. Fiore and B. Williamson (1975) An endotoxin-induced serum factor that causes necrosis of tumors. *Proceedings of the National Academy of Sciences, USA*, **72**, 3666–3670.
32. G.H. Wong, T. McHugh, R. Weber and D.V. Goeddel (1991) Tumor necrosis factor α selectively sensitizes human immunodeficiency virus-infected cells to heat and radiation. *Proceedings of the National Academy of Sciences, USA*, **88**, 4372–6.
33. K. Schulze-Osthoff, A.C. Bakker, B. Vanhaesebroeck, R. Beyaert, W.A. Jacob, W. Fiers (1991) Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. *The Journal of Biological Chemistry*, **267**, 5317–5323.
34. L. Gooding, S. Laster and W. Wold (1993) Control of immunologically mediated apoptosis by human adenoviruses. Paris Conference on Apoptosis in Aids and Cancer, December 2, 3, 4, 1993.
35. B.N. Ames (1989) Endogenous oxidative DNA damage, aging and cancer. *Free Radical Research Communications*, **7**, 121.
36. M. Peter, J.S. Sanghera, S.L. Pelech, E.A. Nigg (1992) Mitogen-activated protein kinases phosphorylate nuclear lamins and display sequence specificity overlapping that of mitotic protein kinase p34cdc2. *European Journal of Biochemistry*, **205**(1), 287–94.
37. F. Mirabelli, A. Salis, M. Perotti, F. Taddei, G. Bellomo and S. Orrenius (1988) Alterations of surface morphology caused by the metabolism of menadione in mammalian cells are associated with the oxidation of critical sulfhydryl groups in cytoskeletal proteins. *Biochemical Pharmacology*, **37**(18), 3423–3427.
38. C. Abate, L. Patel, F.J. Rauscher III, and T. Curran (1990) Redox regulation of fos and jun DAN-binding activity *in vitro*. *Science*, **249**, 1157–1161.
39. P. Pognonec, H. Kato and R.G. Roeder (1992) The helix-loop-helix/leucine repeat transcription factor USF can be functionally regulated in a redox-dependent manner. *Journal of Biological Chemistry*, **267**, 24563–24567.
40. F.J. Staal, M. Roederer, L.A. Herzenberg (1990) Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immonodeficiency virus. *Proceedings of the National Academy of Sciences of the United States of America*, **87**(24), 9943–7.
41. R. Schreck, K. Albermann and P.A. Baeuerle (1991) Nuclear Factor kB: An Oxidative Stress-Responsive Transcription Factor of Eukaryotic Cells (A Review) *Free Radical Research Communications*, **17**, 221–237.
42. W.G. Rice, C.D. Hillyer, B. Harten, C.A. Schaeffer, M. Dorminy, D.A. Lackey III, E. Kirsten, J. Mendeleyev, K.G. Buki, A. Hakam, and E. Kun (1992) Induction of endonuclease-mediated apoptosis in tumor cells by C-nitroso-substituted ligands of poly (ADP-ribose) polymerase. *Proceedings of the National Academy of Sciences, USA*, **89**, 7703–7707.
43. S.V. Lennon, S.J. Martin, T.G. Cotter (1991) Dose-dependent induction of apoptosis in human tumor cell lines by widely diverging stimuli. *Cell Proliferation*, **24**, 203–214.

44. D.L. Vaux, H.L. Aguila and I.L. Weissman (1992b) Bcl-2 prevents death of factor-deprived cells but fails to prevent apoptosis in targets of cell mediated killing. *International Immunology*, **4**, 821–824.
45. B. Halliwell (1992) Reactive oxygen species and the central nervous system. *Journal of Neurochemistry*, **59**, 1609–1623.
46. S. Moncada, R.M.J. Palmer and E.A. Higgs (1989) Biosynthesis of nitric oxide from L-Arginine. A pathway for the regulation of cell function and communication. *Biochemical Pharmacology*, **38**, 1709–1715.
47. S.H. Synder and D.B. Bredt (1991) Nitric oxide as a neuronal messenger. *Trends in Pharmacological Science*, **14**, 60–68.
48. J. Garthwaite (1991) Glutamate, nitric oxide and cell-cell signaling in the nervous system. *Trends in Neuroscience*, **14**, 60–68.
49. M.K. Haddox, J.H. Stephensen, M.E. Moser and N.E. Goldberg (1978) Oxidative-reductive modulation of guinea pig splenic cell guanylate cyclase. *Journal of Biological Chemistry*, **253**, 3143–3152.
50. D.L. Vesely, B. Watson and G.S. Levey (1979) Activation of liver guanylate cyclase by paraquat. Possible role of superoxide ion. *Journal of Pharmacological Experimental Therapy*, **209**, 162–164.
51. T.M. Burke and M.S. Wolin (1989) H_2O_2 and cGMP may function as an O_2 sensor in the pulmonary artery. *Journal of Applied Physiology*, **66**, 167–170.
52. G. Barja (1993) Oxygen radicals, a failure or a success of evolution? *Free Radical Research Communications*, **18**, 63–70.

Accepted by Professor B. Halliwell