Springer Semin Immunopathol (1998) 19 : 271–278

Springer Seminars
in Immunopathology

Immunopathology of apoptosis – introduction and overview

David L. Vaux

The Walter and Eliza Hall Insistute for Medical Research,
Post Office Royal Melbourne Hospital, Victoria 3050, Australia

Introduction

This edition of Seminars in Immunopathology is devoted to apoptosis, the physiological process by which unwanted cells are removed. Rather than covering the entire field of apoptosis, the chapters focus on the clinical implications of cell death, both in terms of causing disease and possible new treatments. In particular it considers apoptosis in the immune system, in which it has been linked with autoimmune disease, neoplasia, graft rejection and infectious disease.

The current flood of publications on apoptosis has been indicative of the rapid progress in the field, but has also made it difficult for anyone not working directly on cell death to maintain perspective. This problem has been compounded by the significant proportion of claims that have subsequently been shown to be incorrect. Furthermore, although apoptosis can be observed in many circumstances, it does not necessarily have any significance. The chapters in this volume give examples of situations in which apoptosis of cells of the immune system has a relevance beyond mere correlation with physiology or pathology, and is critical to the final outcome.

Historically immunologists have been among the first groups to realise the significance of physiological cell death. One reason may have been that some of the early workers in the field happened to be studying lymphoid cells. Another is that *bcl-2*, which was found because it is commonly rearranged in follicular lymphoma, was the first component of the cell death mechanism to be identified.

The apoptotic effector mechanism

The central mechanisms of apoptosis are highly conserved, as some mammalian cell death genes can function in invertebrates and vice versa. For example, the finding that human *bcl-2* could function in *Caenorhabditis elegans* [59] indicated that apoptosis in mammalian cells was implemented by a process resembling the process of programmed cell death that had been studied by genetic means in the worm [22]. Although the effector mechanisms of cell death are conserved, the effects and impact of dysregulation of cell death pathways vary depending on the cells in which it occurs. Furthermore, the signal transduction pathways that can activate the death effector mechanisms are very diverse, and are only beginning to be elucidated.

Table 1. The apoptotic pathway. The key effector proteins of apoptosis are the caspases which exist in a latent form within the cell. Very many signals can lead to activation of the caspases, culminating in apoptosis, but the pathways are carefully controlled

Death signals	Cytokines (addition or removal), steroids, abnormal nucleic acid, viruses, metabolic changes, free radicals, drugs, anoxia, extracellular matrix, etc.
Receptors	Cytokine receptors, steroid receptors, integrins, p53, etc.
Signal transduction	Jaks, ras, kinases, transcription factors, NFκB, calcium ions, etc.
Regulators	BCL-2 family, FLIP, ?IAPs
Adaptors	CED-4, Apaf-1, FADD, RAIDD
Proteases	Caspases 1–11, granzyme B
Substrates	DFF, PAK2, PARP, huntingtin, PKC theta, DNA dependent protein kinase, lamin A, etc.
Secondary substrates	DNA, proteins

In essence, apoptosis is a carefully controlled program of proteolysis (Table 1). The key effector molecules of apoptosis are a family of caspases: cysteine proteases that cleave their substrates following particular aspartate residues [7, 53]. Precursors of these caspases exist in an inactive state within most of our cells, and can be activated by cleavage, without having to be synthesised [37]. Thus, most of our cells carry the latent seeds for their own destruction. The caspases cleave a large number of substrates within the cell, and this is thought to be the point of no return for the cell. Clearly it is important to make sure that these enzymes are tightly regulated.

Regulation of caspases

The pro-caspases are activated either by an upstream caspase [32, 53], or by a family of adaptor proteins that directly associate with the pro-domains of the caspase precursors. These adaptor proteins include FADD, RAIDD and CED-4/Apaf-1 [4, 10, 14, 21, 29, 67]. Somehow these adaptor proteins link the caspases with apoptosis activation signalling pathways, but the details are only known in a few cases.

Regulation of caspase activation can occur at a number of points. One way the BCL-2 family of proteins appear to inhibit apoptosis is by binding to particular adaptor proteins, preventing them from activating the caspases [43, 44, 62]. Other proteins, such as FLIP, interfere with the function or activation of other adaptors by binding to their protein association domains [30].

There are several viral apoptosis inhibitory proteins (such as CrmA and p35) that function by binding to and thereby inhibiting the active caspases [12, 17, 38, 64]. Some evidence suggests that the IAP family of apoptosis inhibitory proteins protect against cell death in the same way, although it has also been proposed that these proteins act upstream to somehow prevent caspase activation [19, 34].

Apoptosis activation pathways

Cells respond to a wide range of stimuli by activating their suicide mechanism. As apoptosis is used to balance mitosis, and thus maintain a homeostasis of cell number,

cells must be able to respond to signals from other cells [39]. For example, cells may know that they are superfluous when their receptors no longer receive signals from growth factors or the extracellular matrix [11, 13, 58]. Sometimes cytokines or hormones, such as tumour necrosis factor (TNF) or cortisone, are used not to keep cells alive but to tell a cell to kill itself [33, 63].

Some death stimuli may originate within the cell. Cells carefully monitor their own metabolism, and their cell cycle status. If a cell resting in G0 detects activation of S phase genes it may decide to kill itself, assuming that it had been infected by a virus or had suffered a potentially oncogenic mutation to its cell cycle machinery. It is possible that this is one of the roles of the product of the p53 gene [60, 66]. Over 400 drugs and toxins have been shown to induce an apoptotic response in one cell type or another. This probably indicates that apoptosis is used as a "stress response" to remove cells that are metabolically disturbed [57].

Clinical and pathological aspects of apoptosis in the immune system

There are many stages at which lymphocytes may undergo apoptosis (Fig. 1). For example, cells that fail to productively rearrange their antigen receptors are eliminated by apoptosis [48]. Developing T cells that fail to recognise self MHC and cells that recognise self antigens bound to MHC are eliminated in the thymus [51]. Mature lymphocytes have a limited lifespan, and are also removed by apoptosis. Following an immune reaction, lymphocyte numbers return to normal, most likely by apoptosis of the

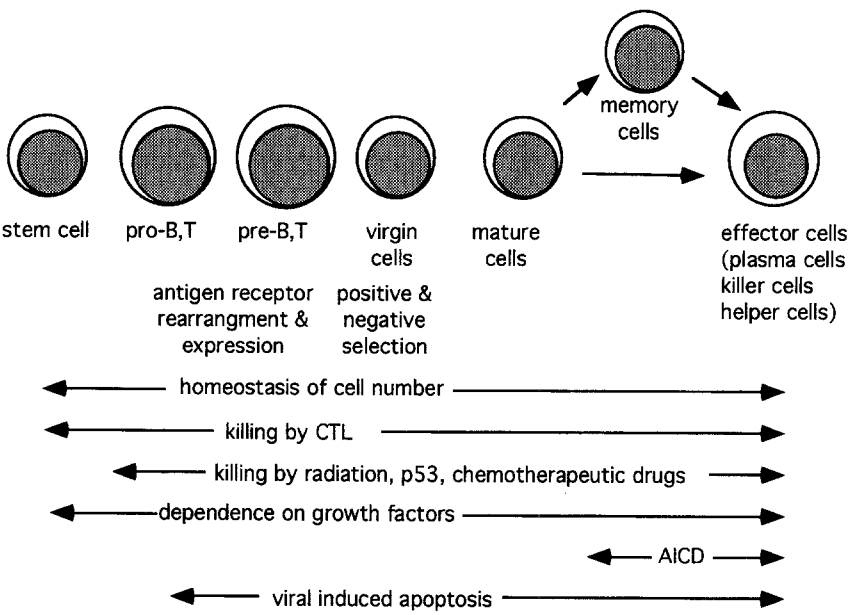

Fig. 1. Occurrence of apoptosis in lymphocytes. Lymphocytes may undergo apoptosis for a variety of reasons at any stage of their differentiation. The number of cells undergoing apoptosis can be altered indirectly, for example when mutations to DNA-dependent protein kinase prevent productive rearrangement of antigen receptors (SCID), or directly, such as when the *bcl-2* gene is rearranged and constitutively expressed

extra cells. During many stages of their development the survival of lymphoid cells is regulated by cytokines such as interleukin-7, and in the absence of these growth factors the cells die [5, 35]. As these cells can be rescued by BCL-2, once again their death must be due to an apoptotic process that BCL-2 can block. Interestingly, the processes of positive and negative selection, which are also thought to occur by apoptosis, occur normally in *bcl-2* transgenic and *lpr* mice, so it must be signalled by a pathway that does not require CD95 and is not subject to control by BCL-2 [2, 42, 45].

Like other cells, lymphocytes can become infected by viruses. The only way to eliminate such cells is by apoptosis. Some cells may respond to the viral infection by committing suicide, while others are killed by cytotoxic T cells.

It is clear that lymphocytes can undergo apoptosis normally or abnormally at many different stages and under many different circumstances. Pathologies causing abnormal apoptosis and the pathology caused by abnormalities in apoptosis will, therefore, depend on which cells are affected and the molecular nature of the abnormality. Many of the molecules involved at different stages of apoptosis in lymphocytes will be discussed at length in sections of this volume. Some of them are briefly introduced below.

bcl-2

The *bcl-2* gene was first identified as the gene translocated and thereby activated in follicular lymphoma [54, 55]. Genetic studies in *C. elegans* suggest that the BCL-2 homolog CED-9 functions by preventing the adaptor molecule CED-4 from activating the caspase CED-3 [22, 43]. Presumably BCL-2 functions in a similar way.

bcl-2 has been found to be translocated in a number of lymphoid tumors in addition to follicular lymphoma , and is found to be expressed at abnormally high levels in many other types of cancers [50]. Experiments with transgenic mice have shown that *bcl-2* does not directly transform cells, but can cause cells to accumulate, increasing the number of cells susceptible to other oncogenic genetic changes [36, 47]. Cells expressing *bcl-2* are also resistant to induction of death by p53, so if these cells suffer extra genetic mutations they can not be eliminated by p53-dependent apoptosis [15, 49]. Transgenic mice expressing *bcl-2* in their lymphocytes develop cancer and on certain genetic backgrounds develop an autoimmune disease resembling systemic lupus erythematosus [46]. Thus, as well as being oncogenic, loss of the ability to undergo apoptosis can promote autoimmune disease.

While many BCL-2-like proteins (such as BCL-x, BCL-w, etc.) inhibit apoptosis, apoptosis can be promoted by other BCL-2 like proteins (such as BAX, BAD, BIK, etc.) [52, 61, 65]. This has raised the possibility of using these proteins therapeutically as BCL-2 antagonists, hopefully to cause tumor cells overexpressing *bcl-2* to undergo apoptosis [16].

TNF receptor family

TNF was the first cytokine identified that could induce cells to undergo apoptosis [33]. It therefore represents the proximal end of a signal transduction pathway that can culminate in cell suicide. However, receptors for TNF, and other members of this family of receptors including CD95 (Fas/APO-1), are not simply obligate death signallers. In many cases ligation of these receptors does not cause apoptosis, but may cause other

cellular responses including proliferation or cytokine secretion [1, 3, 6, 23]. Mice bearing mutations of CD95 (*lpr*) or its ligand (*gld*) develop lymphadenopathy and autoimmune disease [18]. The same appears to be the case in humans [24, 40]. Although apoptotic signalling pathways originating from CD95 leading to activation of caspase-8 are among the best understood, other signalling pathways activated by ligation of CD95 have been recognised but are only just beginning to be understood. It is not yet fully resolved how much these pathways (rather than the apoptosis pathways) contribute to the phenotype of *lpr* or *gld* mice.

Although much attention has been focused on CD95 and the TNF receptors, other members of this rapidly growing family are also certain to be involved in human disease.

Cytotoxic T cells

The role of cytotoxic T lymphocytes (CTL) is to protect the body from infected and mutated cells by causing them to undergo apoptosis. If they fail in this task infections can spread, and there may be a higher incidence of cancer [56]. CTL can also be harmful, for example by causing graft rejection.

Because apoptosis is a process of cell suicide, it was initially puzzling why the target cells killed by CTL displayed classical apoptotic morphology [41]. Observations that granzyme B, a serine protease in the granules of CTL, resembles the caspases in substrate specificity, suggested that one of the ways CTL kill is by using an enzyme that replaces or activates the caspases when introduced into the target cell [59]. Precisely how cytotoxic granules are released from CTL, and how their components function within the target cell is an area of intense investigation. It has become apparent that CTL may also kill in some circumstances through membrane-bound CD95L or by secretion of cytokines [9, 31].

Apoptosis in HIV infection

Observations that many viruses carry genes encoding anti-apoptotic proteins support the notion that apoptosis is used as a cellular defense against viral infection, with the infected cell altruistically killing itself to protect other cells in the organism [26, 57]. Nevertheless, apoptosis seen in the context of a viral infection does not necessarily indicate that it is occurring as an attempt at self defence. Apoptosis of CD4 T cells is certainly seen in people with AIDS, but the death of these cells may be caused in a number of ways, and not all the cells that die are necessarily infected [8, 25]. Several proposals have been made to attempt to save these cells using either pharmacological or genetic inhibitors of cell death. Until this is done, either in animal models or in humans, it will not be possible to know whether the "saved" cells retain the ability to function normally. Of course, more effective therapies will require determining the pathways by which HIV infection leads to apoptosis.

p53

The p53 tumor gene is the most frequently mutated gene in human cancers [60]. Mice lacking p53 commonly develop thymic lymphomas [20]. p53 is able to cause cell cy-

cle arrest allowing DNA repair, but may also induce apoptosis, so it is thought that loss of these activities allows the growth of mutated cells and their development into cancers. Apoptosis induced by p53 can almost invariably be inhibited by expression of *bcl-2*, so it must occur by the caspase-mediated mechanism that *bcl-2* can control [15]. It is not yet known, however, what connects p53 to the apoptotic pathway. More progress has been made in understanding how p53 regulates the cell cycle [27, 28].

The significance of apoptosis seen in diseases affecting the immune system

Ultimately, every disease process involves either an increase or decrease in cell number, and as apoptosis is one side of the equation determining cell number, every disease process will involve alterations in the number of cells undergoing apoptosis. In most cases abnormal survival or death of cells is a late consequence of the disease process, so therapeutic approaches that aim at the apoptotic mechanisms will not alter the number of cells that die, or will not have a beneficial effect on the disease process. However, a growing number of diseases are being recognised in which apoptotic processes play a direct role in either causing or exacerbating disease.

The first step is to discover the nature of the mechanisms of apoptosis, and how they are regulated. Progress in this area has been extremely rapid. The next step is to determine which pathways operate in different disease states. The sections of this volume describe how this is being done for cells of the immune system, and at times they also describe how this knowledge is being applied in attempts to find novel therapies.

References

1. Abreu MM, Vidrich A, Lynch DH, Targan SR (1995) Divergent induction of apoptosis and IL-8 secretion in HT-29 cells in response to TNF-alpha and ligation of Fas antigen. J Immunol 155: 4147
2. Adachi M, Suematsu S, Suda T, Watanabe D, Fukuyama H, Ogasawara J, Tanaka T, Yoshida N, Nagata S (1996) Enhanced and accelerated lymphoproliferation in fas-null mice. Proc Natl Acad Sci USA 93: 2131
3. Aggarwal BB, Singh S, Lapushin R, Totpal K (1995) Fas antigen signals proliferation of normal human diploid fibroblasts and its mechanism is different from tumor necrosis factor receptor. FEBS Lett 364: 5
4. Ahmad M, Srinivasula SM, Wang LJ, Talanian RV, Litwack G, Fernandes-Alnemri T, Alnemri ES (1997) CRADD, a novel human apoptotic adaptor molecule for caspase-2 and FasL tumor necrosis factor receptor-interacting protein RIP. Cancer Res 57: 615
5. Akashi K, Kondo M, Vonfreeden JU, Murray R, Weissman IL (1997) Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. Cell 89: 1033
6. Alderson MR, Armitage RJ, Maraskovsky E, Tough TW, Roux E, Schooley K, Ramsdell F, Lynch DH (1993) Fas transduces activation signals in normal human T lymphocytes. J Exp Med 178: 2231
7. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan, J (1996) Human ICE/CED-3 protease nomenclature. Cell 87: 171
8. Ameisen JC (1992) Programmed cell death, AIDS: from hypothesis to experiment. Immunol Today 13: 388
9. Apasov S, Redegeld F, Sitkovsky M (1993) Cell-mediated cytotoxicity – contact and secreted factors. Curr Opin Immunol 5: 404
10. Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH, Wallach D (1995) A novel protein that interacts with the death domain of Fas/APO-1 contains a sequence motif related to the death domain. J Biol Chem 270: 7795
11. Boudreau N, Sympson CJ, Werb Z, Bissell MJ (1995) Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. Science 267: 891
12. Bump NJ, Hackett M, Hugunin M, Seshagiri S, Brady K, Chen P, Ferenz C, Franklin S, Ghayur T, Li P, Licari P, Mankovich J, Shi LF, Greenberg AH, Miller LK, Wong WW (1995) Inhibition of ICE family proteases by baculovirus antiapoptotic protein p35. Science 269: 1885

13. Cardone MH, Salvesen GS, Widmann C, Johnson G, Frisch SM (1997) The regulation of anoikis – MEKK-1 activation requires cleavage by caspases. Cell 90: 315
14. Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM (1995) FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 81: 505
15. Chiou SK, Rao L, White E (1994) Bcl-2 blocks p53-dependent apoptosis. Mol Cell Biol 14: 2556
16. Clarke MF, Apel IJ, Benedict MA, Eipers PG, Sumantran V, Gonzalezgarcia M, Doedens M, Fukunaga N, Davidson B, Dick JE, Minn AJ, Boise LH, Thompson CB, Wicha M, Nunez G (1995) A recombinant bcl-x(s) adenovirus selectively induces apoptosis in cancer cells but not in normal bone marrow cells. Proc Natl Acad Sci USA 92: 11024
17. Clem RJ, Fechheimer M, Miller LK (1991) Prevention of apoptosis by a baculovirus gene during infection of insect cells. Science 254: 1388
18. Cohen PL, Eisenberg RA (1992) The *lpr* and *gld* genes in systemic autoimmunity: life and death in the Fas lane. Immunol Today 13: 427
19. Deveraux QL, Takahashi R, Salvesen GS, Reed JC (1997) X-linked IAP is a direct inhibitor of cell-death proteases. Nature 388: 300
20. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CJ, Butel JS, Bradley A (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356: 215
21. Duan H, Dixit VM (1997) RAIDD is a new death adaptor molecule. Nature 385: 86
22. Ellis RE, Yuan JY, Horvitz HR (1991) Mechanisms and functions of cell death. Annu Rev Cell Biol 7: 663
23. Fiers W (1991) Tumor necrosis factor. Characterization at the molecular, cellular and in vivo level. FEBS Lett 285: 199
24. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, Strober W, Lenardo MJ, Puck JM (1995) Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 81: 935
25. Groux H, Torpier G, Monte D, Mouton Y, Capron A, Ameisen JC (1992) Activation-induced death by apoptosis in CD4+ T cells from human immunodeficiency virus-infected asymptomatic individuals. J Exp Med 175: 331
26. Haecker G, Vaux DL (1994) Viral, worm and radical implications for apoptosis. Trends Biochem Sci 19: 99
27. Haupt Y, Rowan S, Oren M (1995) P53-mediated apoptosis in HeLa cells can be overcome by excess pRb. Oncogene 10: 1563
28. Haupt Y, Maya R, Kazaz A, Oren M (1997) Mdm2 promotes the rapid degradation of p53. Nature 387: 296
29. Irmler M, Hofmann K, Vaux DL, Tschopp J (1997) Direct physical interaction between the *Caenorhabditis elegans* death proteins Ced-3 and Ced-4. FEBS Lett 406: 189
30. Irmler M, Thome M, Hahne M, Schneider P, Hofmann B, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE, Tschopp J (1997) Inhibition of death receptor signals by cellular FLIP. Nature 388: 190
31. Kagi D, Vignaux F, Ledermann B, Burki K, Depraetere V, Nagata S, Hengartner H, Golstein P (1994) Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science 265: 528
32. Kumar S (1995) ICE-like proteases in apoptosis. Trends Biochem Sci 20: 198
33. Laster SM, Wood JG, Gooding LR (1988) Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. J Immunol 141: 2629
34. Manji GA, Hozak RR, Lacount DJ, Friesen PD (1997) Baculovirus inhibitor of apoptosis functions at or upstream of the apoptotic suppressor p35 to prevent programmed cell death. J Virol 71: 4509
35. Maraskovsky E, O'Reilly LA, Teepe M, Corcoran LM, Peschon JJ, Strasser A (1997) Bcl-2 can rescue T lymphocyte development in interleukin-7 receptor-deficient mice but not in mutant rag-1(–/–) mice. Cell 89: 1011
36. McDonnell TJ, Korsmeyer SJ (1991) Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14; 18). Nature 349: 254
37. Nicholson DW, Thornberry NA (1997) Caspases – Killer proteases. Trends Biochem Sci 22: 299
38. Pickup DJ, Ink BS, Hu W, Ray CA, Joklik WK (1986) Hemorrhage in lesions caused by cowpox virus is induced by a viral protein that is related to plasma protein inhibitors of serine proteases. Proc Natl Acad Sci USA 83: 7698
39. Raff MC (1992) Social controls on cell survival and cell death. Nature 356: 397
40. Rieux-Laucat F, Le Deist F, Hivroz C, Roberts IAG, Debatin KM, Fischer A, Villartay JP de (1995) Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science 268: 1347

41. Russell JH, Masakovski VR, Dubois CB (1980) Mechanisms of immune lysis. I. Physiological distinction between target cell death mediated by cytotoxic T lymphocytes and antibody plus complement. J Immunol 124: 1100
42. Sentman CL, Shutter JR, Hockenbery D, Kanagawa O, Korsmeyer SJ (1991) Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell 67: 879
43. Seshagiri S, Miller LK (1997) *Caenorhabditis elegans* Ced-4 stimulates Ced-3 processing and Ced-3-induced apoptosis. Curr Biol 7: 455
44. Spector MS, Desnoyers S, Hoeppner,DJ, Hengartner MO (1997) Interaction between the *C. elegans* cell-death regulators Ced-9 and Ced-4. Nature 385: 653
45. Strasser A, Harris AW, Cory S (1991) Bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell 67: 889
46. Strasser A, Whittingham S, Vaux DL, Bath ML, Adams JM, Cory S, Harris AW (1991) Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. Proc Natl Acad Sci USA 88: 8661
47. Strasser A, Harris AW, Cory S (1993) E mu-bcl-2 transgene facilitates spontaneous transformation of early pre-B and immunoglobulin-secreting cells but not T cells. Oncogene 8: 1
48. Strasser A, Harris AW, Corcoran LM, Cory S (1994) Bcl-2 expression promotes B- but not T-lymphoid development in SCID mice. Nature 368: 457
49. Strasser A, Harris AW, Jacks T, Cory S (1994) DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. Cell 79: 329
50. Strasser A, Huang DCS, Vaux DL (1997) The significance of defects in cell death control for tumourigenesis and resistance to chemotherapy. Biochim Biophys Acta (in press)
51. Surh CD, Sprent J (1994) T-cell apoptosis detected in situ during positive and negative selection in the thymus. Nature 372: 100
52. Thomas WS, Oltvai ZN, Yang E, Wang K, Boise LH, Thompson CB, Korsmeyer SJ (1995) Multiple bcl-2 family members demonstrate selective dimerizations with bax. Proc Natl Acad Sci USA 92: 7834
53. Thornberry NA, Ranon TA, Pieterson EP, Rasper DM, Timkey T, Garciacalvo M, Houtzager VM, Nordstrom PA, Roy S, Vaillancourt JP, Chapman KT, Nicholson DW (1997) A combinatorial approach defines specificities of members of the caspase family and granzyme B – functional relationships established for key mediators of apoptosis. J Biol Chem 272: 17907
54. Tsujimoto Y, Croce CM (1986) Analysis of the structure, transcripts and protein products of bcl-2, the gene involved in human follicular lymphoma. Proc Natl Acad Sci USA 83: 5214
55. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM (1984) Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 226: 1097
56. Vandenbroek MF, Kagi D, Ossendorp F, Toes R, Vamvakas S, Lutz WK, Melief C, Zinkernagel RM, Hengartner H (1996) Decreased tumor surveillance in perforin-deficient mice. J Exp Med 184: 1781
57. Vaux DL, Hacker G (1995) Hypothesis – apoptosis caused by cytotoxins represents a defensive response that evolved to combat intracellular pathogens. Clin Exp Pharmacol Physiol 22: 861
58. Vaux DL, Cory S, Adams JM (1988) Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335: 440
59. Vaux DL, Haecker G, Strasser A (1994) An evolutionary perspective on apoptosis. Cell 76: 777
60. Vogelstein B, Kinzler KW (1992) p53 function and dysfunction. Cell 70: 523
61. Wang K, Yin XM, Chao DT, Milliman CL, Korsmeyer SJ (1996) Bid – a novel BH3 domain-only death agonist. Genes Dev 10: 2859
62. Wu DY, Wallen HD, Nunez G (1997) Interaction and regulation of subcellular localization of Ced-4 by Ced-9. Science 275: 1126
63. Wyllie AH (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284: 555
64. Xue D, Horvitz HR (1995) Inhibition of the *Caenorhabditis elegans* cell-death protease Ced-3 by a Ced-3 cleavage site in baculovirus p35 protein. Nature 377: 248
65. Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ (1995) Bad, a heterodimeric partner for bcl-xl and bcl-2, displaces bax and promotes cell death. Cell 80: 285
66. Yonish RE, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M (1991) Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 353: 345
67. Zou H, Henzel WJ, Liu XS, Lutschg A, Wang XD (1997) Apaf-1, a human protein homologous to *C. elegans* Ced-4, participates in cytochrome *c*-dependent activation of caspase-3. Cell 90: 405