Apoptosis: an overview

Andrew H Wyllie

Sir Alastair Currie CRC Laboratories, Western General Hospital, Edinburgh, UK

The term apoptosis first appeared in the biomedical literature in 1972[1]. That cells die had never been in doubt, and that some deaths are part of the enactment of a developmental programme had been recognised at least since the descriptive work of Glucksmann in 1951[2] and put on a firm experimental basis by Saunders[3]. The unique character of this developmentally regulated death had been encapsulated by the term programmed cell death, introduced by Lockshin[4]. The justification for the new term apoptosis was the realisation that cells entering death in development undergo a unique and distinct set of structural changes and that similar or identical changes are also shared by cells dying in a wide variety of circumstances outside of development: T-cell killing, negative selection within the immune system, atrophy induced by endocrine and other essentially physiological stimuli, normal cell turnover in many tissues, and in tumours and normal tissues following exposure to the appropriate (low) doses of ionising radiation, chemotherapy, and even hypoxia. Moreover, this process of death was clearly different from **necrosis**, till then the only mode of death that had been well described in pathological and toxicological literature, and which appears to be the consequence of extreme perturbations of the cellular microenvironment.

Apoptosis represents a stereotyped sequence of structural change

Correspondence to: Prof Andrew H Wyllie, Sir Alastair Currie CRC Laboratories, Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

Briefly, the structural features of apoptosis include the following. The dying cell separates from its neighbours, usually with loss of specialised membrane structures such as microvilli and desmosomes. It undergoes a period of blebbing and contortion that is dramatically visualised in time-lapse cinematography. The blebs are membrane-invested extensions of cytosol that are usually devoid of organelles and are reversibly extruded and resorbed. This is followed by rapid, irreversible condensation of cytoplasm, accompanied by an increase in cell density, compaction of cytoplasmic organelles and condensation of the nuclear chromatin to form dense granular caps or toroidal structures underlying the nuclear membrane. Nuclear pores disappear from the membrane subjacent to

British Medical Bulletin 1997,**53** (No 3) 451–465

these chromatin condensations whilst, within the nucleus, the proteinaceous fibrillar centre of the nucleolus separates from its surrounding shell of osmiophilic transcription complexes. Around this time, the cell splits into a cluster of membrane bounded bodies, each containing a variety of organelles. The organelle structures themselves are largely conserved, although sometimes unusual side-to-side aggregates of cytoskeletal microfilaments and semi-crystalline associations of ribosomes can be identified. The endoplasmic reticulum dilates and connects with the cell surface by a series of gaping pits. Apoptotic cells within tissues, or even *in vitro*, are the objects of phagocytosis by their viable neighbours or by specialist phagocytes. They are swiftly digested within heterophagasomes, and this underlies one of the most striking features of apoptosis as a whole: the dying cells disappear rapidly from the tissue which seems to telescope into itself without generation of any inflammatory reaction. Various estimates of the time for which an apoptotic cell is visible by conventional microscopy have been made, a matter of a few hours at most, the majority of which is spent undergoing degradation within the phagocytic cell.

All of this contrasts with the described features of necrosis. Here, the dying cell swells, cytosolic as well as nuclear structures alter, but the general disposition of hetero- and euchromatin is maintained, as are the nuclear pores. The plasma membrane ruptures and internal materials reach the extracellular space, where some of them (notably some mitochondrial proteins) induce an inflammatory reaction including chemotaxis of neutrophil polymorphs.

Some of the features of necrosis, however, can become superimposed on those of apoptosis if the dead cell for some reason fails to be recognised and engulfed by a phagocyte. Thus apoptotic cells *in vitro*, after the initial brisk rise in buoyant density, show a progressive fall in density to subnormal levels over the ensuing few hours. Their membranes become permeable to dyes that had previously been excluded, such as trypan blue or propidium iodide, and their ultrastructure is a series of distended cytosolic membrane profiles, although the conspicuous blocks of condensed chromatin remain in the nucleus. Similar structural features can be seen *in vivo* in apoptotic cells that have, for example, been shed from an epithelium into a duct lumen. This has been called **secondary necrosis**.

The co-ordinated structural changes that make up the process of apoptosis are driven by a set of molecular interactions, here called the **terminal effector events**. Perhaps surprisingly, it transpires that most, if not all, living cells contain the molecules that participate in these events, but in a form that requires activation. The process of death is the result of an interaction between initiating stimuli – which can be physiological or the result of injury in various parts of the cell – and factors that

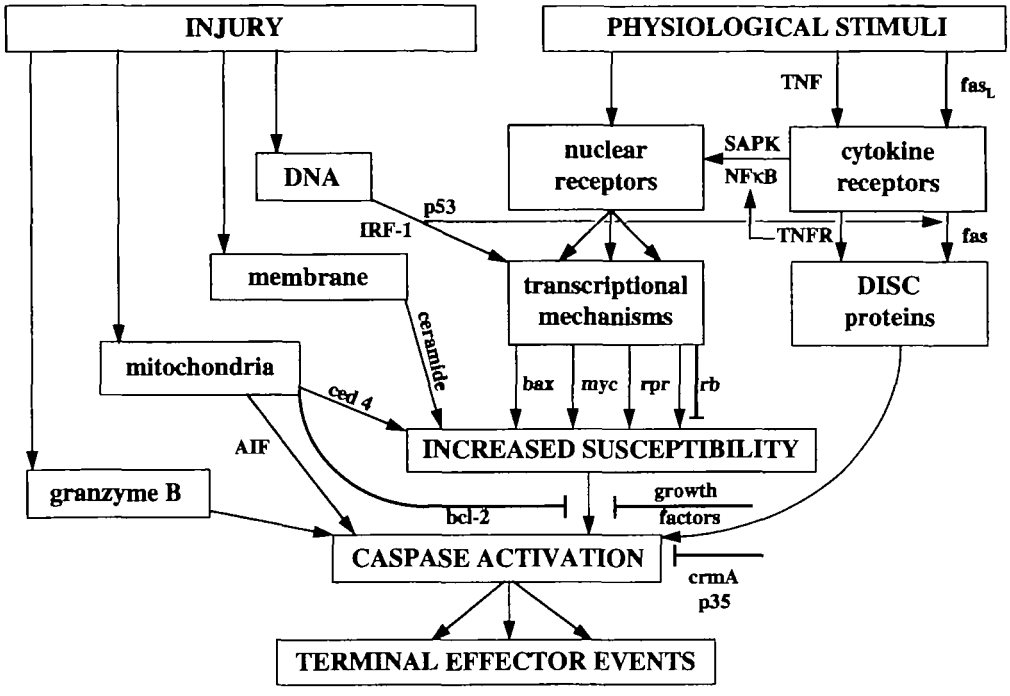

Fig. 1 Scheme of cellular events in apoptosis (reproduced by permission from the *European Journal of Cell Biology*).

determine the susceptibility of the cell to activation of the terminal effector events. Successive chapters in this volume enlarge upon the terminal effector events, the physiological and injury-related initiative stimuli, the factors controlling cellular susceptibility to death, and the role of all of these in normal tissue homeostasis and pathology. What follows in this chapter is a brief overview of the process of apoptosis as a whole (Fig. 1).

Specific genes regulate apoptosis

The basic design of the genetic regulation of apoptosis was identified through painstaking studies in the tiny nematode *Caenorhabditis elegans*[5]. During the development of *C. elegans*, the position, division and fate of each cell is predetermined precisely by a genetically-defined programme. Moreover, even the adult organism is sufficiently small and translucent that every cell can be visualised by Nomarski interference

optics. There are 1090 cell births and 131 cell deaths in the construction of the adult worm (excluding the germ cells). This fixed pattern can be disturbed by mutagenesis and, by this means, it proved possible to identify around a dozen genes directly involved in the cell death pathway. Mutations in these genes generate abnormalities of various different characters in the process of death. Thus, defective function of one gene (*nuc 1*) inhibits the destruction and digestion of the nuclear material of dead cells, but in no way influences the decision to die. Loss of function in another set of genes cripples the recognition of the dying cell by neighbouring phagocytes. But, most significantly, two genes (*ced 3*, *ced 4*) are necessary and sufficient for the initiation of death itself, whilst a third (*ced 9*) is required to sustain life, through inhibition of the action of the previous two. All these genes are relevant to any of the cell deaths in nematode development, but further sets regulate them in a site-specific way. These observations thus established the existence – albeit in a small invertebrate organism – of a genetic basis for the cell death programme. The enormous general significance of this programme became evident when structural and functional homology was identified between two of the core genes – *ced 9* and *ced 3* – with the mammalian genes *bcl-2*[6] and *ICE*[7].

ICE-like proteases drive the terminal effector events

The gene *ced 3* encodes a protease with a cysteine-containing active site and a predilection for cutting peptides on the carboxy-terminal side of an aspartic acid residue in a 4-amino acid motif. Although this single gene is required for all programmed deaths in *C. elegans*, a family of over a dozen related genes is present in the mammalian genome, collectively called caspases (for cysteine-containing asp-ases)[8]. The founder member of this mammalian family is ICE, the interleukin 1β converting enzyme, which is known to be responsible for cleavage and activation of the inflammatory cytokine IL-1β. There remains some doubt over the role of ICE itself in mammalian cell death, although it can restore normal cell death when expressed as a result of genetic engineering in *ced 3*-deficient nematode cells. Other members of the caspase family, however, are clearly central to mammalian apoptosis: they are activated during apoptosis, death is forstalled by specific inhibitors, and germ-line knock-out of their genes produces abnormal development with retention of cells which normally are deleted.

Caspases activated during initiation of apoptosis can be assayed in a variety of broken-cell systems, by exposing cytosolic extracts from

apoptotic or pre-apoptotic cells to substrates prepared from normal viable cells[9–11]. In this way, it is possible to prove that caspase activation is responsible for driving all the structural changes in the nucleus that accompany apoptosis, and to explore the range of proteins that undergo caspase-induced cleavage. Such proteins include several involved in the cytoskeleton and its organisation (actin, fodrin, and the familial polyposis protein APC), in DNA repair (DNA-PK, PARP), in the integrity of the nuclear envelope (the lamins) and in the cell cycle (the retinoblastoma protein). In all these there is evidence that the cleavage site selectively disables the normal function of the protein substrate, so providing a logic for the rapid near-simultaneous changes in many parts of the cell in apoptosis. Although the identity of the nucleases responsible for chromatin cleavage in apoptosis is still somewhat controversial, one candidate is activated by proteolytic cleavage[12]. There is also very good evidence that some caspases activate others by proteolytic cleavage and many are autocatalytic. Hence different stimuli – perhaps initially triggering different caspases – may result in the same set of terminal proteolytic events, accounting for the remarkable stereotypy of apoptosis, regardless of the reason for its initiation.

Caspase activation is held under restraint in living cells

The fact that most, or all, cells contain caspases presupposes that powerful mechanisms must exist to suppress their inappropriate activation. Such suppression could act as an integral part of the regulation of death: it may be more effective to turn on apoptosis by modifying the level of suppression than by activating the effectors directly. Alternatively, suppression may function as a means of aborting the death process at the last possible step prior to the self-amplifying cascade of caspase activation. In the nematode, suppression is supplied by the product of the gene *ced-9*. In mammals, it is effected by structurally and functionally homologous molecules, bcl-2 and bcl-x_L.

Three routes of action are currently known for these molecules, all mutually compatible. First, they bind to structurally related but pro-apoptotic molecules (for example bax) and so apparently titrate down the lethal activity of these[13]. Second, they bind to structurally different molecules which are also lethal effectors. The nematode prototype is *ced 4*, and this also binds and is inactivated by mammalian bcl-2[14], but the mammalian homologue(s) of *ced 4* have still to be described. Finally, they insert into intracellular membranes – the outer membranes of the mitochondria and the nuclear envelope, and the ER membranes. Their

cytoprotective action is heavily dependent on this insertion and elegant experiments using engineered chimaeric molecules have demonstrated that correct location in the cell may be essential in response to particular types of injury[15]. Interestingly, the three dimensional structure of bcl-x closely resembles that of bacterial cytocidins, killer molecules which also insert into their target membranes to form highly permeable 'mega channels'[16]. Quite how this might relate to the cytoprotective function of the bcl-2 proteins is obscure. Mitochondrial transmembrane potential is known to discharge during apoptosis and bcl-2 may regulate the probability of this[17].

A striking feature of bcl-2 action is its extreme 'downsteam' point of action in the death pathway. Only one commonly studied death stimulus is consistently unaffected by bcl-2 protection, the broad-spectrum protein kinase inhibitor staurosporine[18] (there is controversy over the extent to which bcl-2 protects cells from killing via the CD 95/fas/apo-1 pathway). Indeed, contrived over-expression of bcl-2 can rescue cells from some circumstances that would otherwise be associated with necrosis. It is relatively easy to rationalise the usefulness of such a late revoke of death signals. The possibility remains, however, that there are other families of molecules, unrelated to bcl-2, involved in restraint of the death process, and perhaps with different points of action. Such molecules ought to be relatively easy to screen for and identify, as their over-expression should select for survivors in cell populations exposed to the appropriate lethal stimuli. So far, however, only one strong candidate outwith the bcl-2 family (DAD-1) has been reported[19].

A variety of signalling systems activate apoptosis

In mammalian cells as in the nematode, the central elements of apoptosis are thus envisaged as a ced3/caspase-driven engine, held in check by survival proteins of which the best known are members of the ced9/bcl-2 family, and released by killer proteins that include the ced4 homologue(s) and other bcl-2 binding partners (bax, bik, bad). How does this respond appropriately to the presence of cell injury and to physiological death signals? And what are the critical signals that set this engine running? This is probably the most imperfectly understood part of the apoptotic mechanism, but it is already clear, firstly, that a great variety of signals exist, both transcriptional and non-transcriptional, and secondly that they can interact with each other in interesting ways.

Surface receptors

Early in the history of research in apoptosis a receptor that signalled death was identified on the surface of activated lymphocytes[20]. This receptor – CD95/apo-1/fas – is now understood in great detail[21,22], much of which is discussed in a later chapter. In summary, CD95 is a member of the TNF receptor family and binds a TNF-family ligand. Ligand binding initiates trimerisation of the receptor and this permits the immediate recruitment of several proteins that form a complex around the cytoplasmic moiety of the receptor – the death initiating signalling complex or DISC. DISC proteins bind to each other and CD95 through a series of homologous domains. Thus the C-terminus of CD95 contains an amino acid sequence (called the **death domain**) that binds a similar sequence on the C-terminus of a DISC protein called FADD, whilst FADD contains a distinct N-terminal domain (called the **death effector domain**) that binds to a homologous region in the N-terminus of a third protein FLICE. FLICE is so named (fas-activated protein like ICE) because its C-terminus has structural and functional homology to the caspases, and can activate them. Thus, a cytokine receptor on the cell surface is coupled to the heart of the caspase engine by a remarkable, direct, non-transcriptional pathway[23].

Other members of the TNF receptor family also signal death – p75NTR, DR3, TNFR1 and TNFR2, and some of these are known to engage similar mechanisms. Thus TNFR1 possesses a death domain that binds a protein called TRADD. TRADD probably couples via FADD to FLICE. However, the cytosolic moiety of TNFR1 also includes a second domain that activates transcription through NFκB, and this may signal for survival[21]. There are further proteins that have the capacity to interact simultaneously with several of these signalling molecules, and hence are sometimes called 'adaptors'. Some of these adaptors are known to be recruited to the DISC. These include RIP, a protein containing a death domain coupled to a kinase domain, and RAIDD (or CRADD) in which an N-terminal domain that interacts with caspase 2 is coupled to a C-terminal death domain that interacts with RIP. These molecules are still of somewhat uncertain function, but they have the capacity to modify the death signal positively. Other adaptors exist which may exert a negative effect on the death signal, and some may contribute to 'cross talk' between the different receptors.

In summary, there is direct, nontranscriptional, coupling between cytokine receptors of the TNF receptor family and caspase activation, but even this system has many additional elements permitting modulation of the death signal. Much has still to be learned about these, and also the more basic question of how cytokine receptor expression itself is regulated in various cell types.

Transcriptional mechanisms

Members of many transcriptional modifying pathways have been implicated in the regulation of apoptosis. Thus there are examples of positive or negative regulation of death by the stress-activated JNK/SAPK pathway, by ras and rho, by the tyrosine kinase abl, by NFκB and by members of the steroid receptor and JAK-STAT families. Some of these are dealt with in later chapters in this volume. Here, however, attention is focused on new transcripts that are directly implicated in the initiation of apoptosis.

Reaper, *grim* and *hid* are three *Drosophila* genes whose function is required for normal sensitivity of embryonic cells to death induced in development or in response to injury stimuli[24]. The genes are transcribed a few hours before the morphology of apoptosis appears, and embryos deficient in *reaper* are some thousand fold more resistant to ionising radiation than the wild type. Intriguingly, *reaper* has regions of homology to the CD95 death domain[25]. It is, therefore, possible that these genes represent a transcriptionally activated death pathway that taps into the same terminal effector events as the cytokine-receptor-mediated system described above. No mammalian homologue for *reaper* has yet been discovered.

Expression of the proto-oncogene *c-myc* also increases susceptibility to apoptosis[26]. This observation was initially surprising, as *c-myc* is well known as an immediate early response gene in growth factor dependent entry to the cell cycle. The role of *c-myc* in apoptosis was demonstrated by artificially inducing its expression in cells deprived of growth factor, circumstances in which it is normally transcriptionally silent in cultured cells. One interpretation, therefore, was that the unusual combination of growth-associated and growth-inhibitory signals had created a 'conflict' within the cell which somehow led to initiation of apoptosis. It is difficult to completely exclude this hypothesis, as the notion of resolution of conflict is itself anthropomorphic, but there is a more elegant interpretation equally compatible with the observations. Transcriptional activation of *c-myc* is conceived of as initiating a state of susceptibility to both apoptosis and proliferation. A second signal is required to determine which is selected. In the presence of cytokine survival factors (which need not be mitogens), such as IGF-1, apoptosis is abrogated and proliferation ensues[27]. In this way, the potentially dangerous process of cell proliferation is made dependent on dual controls. This interpretation is supported by the observation that apoptosis frequently co-localises with proliferation in tissues, with and without injury. It is also suggested by the fact that, although *c-myc* dysregulation is a feature of most cancers, dysregulation of a single oncogene cannot be sufficient to

permit unrestrained cellular proliferation, as background levels of proto-oncogene mutation would then render cancer a much more common phenomenon than it is in the tissues of large, long-lived mammals.

Forced expression of the transcriptional transactivator E2F-1, and knock-out of its inhibitory binding protein, the oncosuppressor protein rb-1, also initiate susceptibility to apoptosis[28,29]. Indeed, since both E2F-1 expression and rb-1 inactivation normally lead to *c-myc* expression and entry to the cell cycle, the role of E2F-1 and rb-1 in apoptosis may be mediated by *c-myc*.

Apoptosis is a critical component of the cellular response to injury

DNA injury

DNA injury can initiate apoptosis by a powerful, early-activated mechanism dependent on the nuclear phosphoprotein p53 (discussed in detail later in this issue by Bellamy and Hickman & Boyle). The p53 protein is activated by both transcriptional and post-translational means and is a critical element in the cellular response to double-strand DNA breaks. Such breaks appear, for example, following damage inflicted by ionising radiation: p53 is also critical, however, in the response to damage by UV light, where the initial events do not involve strand breakage but the generation of nucleotide dimers. Similarly, we have shown that p53 is essential for the apoptosis of some cells responding to drugs that cause DNA alkylation (Toft N, Clarke AR, Margison G and Wyllie AH, unpublished results). In these cases, DNA strand breaks may appear in the course of repair reactions. In a manner that is not completely understood, p53 forms part of a decision fork in which the cell is directed either towards the completion of repair or to apoptosis. It is also clear that cells possess pathways that couple DNA injury to apoptosis in the complete absence of p53[30].

Injury to cell membranes

Injury to cell membranes, and in particular the plasma membrane, activates acid sphingomyelinase and so generates the second messenger ceramide from membrane lipids[31,32]. Ceramide, perhaps through modification of the usage of MAP kinase *versus* JUN kinase signalling pathways, alters cellular susceptibility to apoptosis[33,34].

Mitochondrial injury

Mitochondrial injury can lead to depolarisation of the mitochondrial membrane, a frequent (although not inevitable) early event in apoptosis. The depolarisation is linked to release of pro-apoptotic factors from the mitochondrion, some of which are familiar players in the central apoptosis mechanism (e.g. ced 4), whilst others are still poorly defined apoptosis initiating factors (AIF) – perhaps including cytochrome c – that activate the caspases[17].

Cytotoxic T cell killing

Cytotoxic T cell killing is effected by at least two major pathways. Following recognition of the target cell by the cytotoxic T lymphocyte (CTL), fas is activated through binding of the fas ligand, expressed on the CTL surface[35]. The CTL also releases the contents of its granules, amongst them perforin (which effects increased permeability of the target cell membrane) and a group of proteases of which the best defined is the serine protease granzyme B. Granzyme B directly activates the target cell caspases[36].

Viruses

Many viruses interact with the apoptosis pathway of their target cells and these are discussed in detail later in this issue by Young, Dawson & Eliopoulos. Viruses with lytic cycles – somewhat unexpectedly – encode anti-apoptotic proteins. Amongst these are the IAPs (inhibitors of apoptosis proteins) of baculovirus, which appear to interfere with TNF cytokine signalling, and baculovirus p35 and the cowpox serpin protein crmA, both of which directly inhibit caspases[37]. Presumably this represents one aspect of the complexity of the viral-host cell relationship. Amongst the host cell reactions to the presence of viral infection is activation of apoptosis, but viral genesis of anti-apoptotic proteins may forestall host cell death for long enough to permit initiation of viral replication, the synthesis of coat proteins and hence the completion of a viral infective cycle. A more extreme situation, however, is engendered by transforming DNA viruses, most if not all of which possess genes whose products can protect from apoptosis in a long-lasting way. The Epstein-Barr virus codes for 2 anti-apoptotic proteins, BHRF-1, a bcl-2 analogue, and LMP-1, which appears to block signalling by the TNF receptor pathway[38]. The papilloma virus early gene product HPV16 E6

and the adenoviral E1A55kDa protein inactivate p53, whilst the adenoviral E1A19kDa protein enhances the intracellular effectiveness of members of the bcl-2 family, through binding their inhibitors[13]. Interestingly, such anti-apoptotic genes are closely linked to others equally essential for cell transformation which initiate host cell replication. Thus the HPV16 E7 protein, and the adenoviral protein E1B initiate replication through inactivation of rb-1. The papovavirus SV40 transforming protein T-antigen combines both the replicative and anti-apoptotic functions in a single polyvalent molecule. This close linkage forms part of the evidence for the view expressed earlier, that initiation of replication (with activation of *c-myc*) co-ordinately increases susceptibility to apoptosis, which must then be inhibited by a separate mechanism if the cell population is to increase.

It is probable that the transforming genes of RNA viruses also combine replicative and anti-apoptotic functions. Thus v-src, v-abl[39] and probably the transforming ras genes[40,41], inhibit apoptosis in addition to activating replication. Recently, the proto-oncogene tyrosine kinase abl has been shown to play a specific role in cells that have undergone DNA injury, binding to and apparently being phosphorylated by the DNA damage recognition protein ATM[42]. Although the precise role of abl in the cellular response to injury is not yet clear it seems probable that this is part of a survival response in the injured cell.

Apoptosis and pathology

Many articles in this volume highlight the role of apoptosis, or the implications of failure of its regulation, in pathological processes. There is substantial evidence that failure to activate apoptosis after DNA injury may be one route to **carcinogenesis**[43,44]. In the chapter by Lyons & Clarke, the theme is developed that oncogene activation and oncosuppressor deficiency may abrogate the apoptosis of cells bearing DNA damage, which then become the founders of tumours. Certainly, this would provide a credible explanation for the frequency with which pro-apoptotic regulatory genes such as p53 are deleted in common human cancers. The reported incidence in human cancer, however, of deficiency of genes inside the apoptosis effector pathway is quite low and, in particular, there is little published evidence for mutation in fas or the caspases. Nor is the incidence of tumours high in animals genetically deficient in fas or fas ligand. It may be that the inappropriate survival of injured cells is more readily achieved by alterations in the genes regulating susceptibility to death (and such genes also regulate other processes, such as replication and repair) than by those within the

effector mechanism of apoptosis itself. One immediate if naive explanation for this could be that the genes in the effector mechanism are often members of parallel, partially redundant pathways.

In early tumour growth, **angiogenesis** plays a critical role. Such angiogenesis occurs simultaneously with the step-down in the intrinsic level of apoptosis within the early tumour mass[45,46]. The mechanisms underlying this interesting transition are not clear, but it is not difficult to speculate that the angiogenesis permits growth factor supply above limiting levels, or more subtle effects contingent on the ingress of stromal cells, including the provision of new cytokines and extracellular matrix. It is probable that this relationship between angiogenesis and apoptosis has much to do with the establishment of metastasis. It is also clear that tumour angiogenesis is only an extension of regulatory processes which match vasculature to the presence of parenchymal cells, and there is already evidence in non-neoplastic systems for the regulation of apoptosis in microvascular endothelium[47,48].

The many roles of apoptosis in the normal function and pathology of **immune reactions** are discussed in the chapters by Ekert & Vaux and by Peter, Ehret, Berndt & Krammer. Detailed knowledge of the receptor signalling pathways related to apoptosis has clarified major issues underlying the deficiency of CD4+ lymphocytes in AIDS and the generation of auto-immune disorders in animals in which there are congenital defects in fas or fas ligand. Very recently, evidence has accumulated that some tumour cells express fas ligand on their surface and so activate apoptosis in fas-bearing cytotoxic T-cells, a potential mechanism for immune evasion[49].

Evidence is also accumulating that apoptosis is a significant part of the pathology of processes which have long been regarded as dependent on necrosis alone, such as **stroke, myocardial infarction** and **heart failure**[50]. The chapter by Clutton in this volume examines the potential role of reactive oxygen species, including those generated during reperfusion injury, in initiation of apoptosis. Apoptosis probably plays a role in the loss of cells in **chronic neurodegenerative conditions** such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.

Apoptosis also provides a critical regulatory element in the inflammatory process. The chapter by Haslett deals with the outcome of disordered phagocytosis of neutrophils in the conclusion of acute inflammation. Interestingly, one mechanism of bacterial pathogenesis also appears to be through subversion of the process of apoptosis in the host defenses[51]. Pathogenic *Shigella* species possess plasmid-borne aggression factors which permit them to enter macrophages and there to activate the macrophage endogenous apoptosis terminal cascade. This strategy apparently permits the bacteria to evade phagocytosis. At the same time there is activation of macrophage IL-1β (presumably through

the caspase mechanism) which has the effect of engendering a continuing, tissue-destructive inflammatory process. These mechanisms probably underlie the characteristic mucosal ulceration and rapid bacterial proliferation of *Shigella* dysentery. It will be extremely interesting to see to what extent other micro-organisms exploit the apoptosis process of the host defence cells.

References

1 Kerr JFR, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972; 26. 239–57
2 Glücksmann A. Cell deaths in normal vertebrate development. *Biol Rev* 1951; 26 59–86
3 Saunders JW, Fallon JS. Cell death in morphogenesis. In: Lock M. ed. *Major problems in developmental biology*. 25th Symposium of the Society for Developmental Biology. New York: Academic Press, 1966; 289–314
4 Lockshin RA, Beaulaton J. Programmed cell death. *Life Sci* 1974; 15 1549–65
5 Hengartner MO, Horvitz HR. The ins and outs of programmed cell death during *C. elegans* development *Phil Trans R Soc Lond B* 1994; 345. 243–8
6 Hengartner MO, Horvitz HR. *C. elegans* survival gene ced-9 encodes a functional homologue of the mammalian proto-oncogene bcl-2 *Cell* 1994; 76: 665–76
7 Yuan JY, Shaham S, Ledoux S, Ellis HM, Horvitz HR. The *C elegans* cell death gene ced-3 encodes apoptosis similar to mammalian interleukin-1β-converting enzyme. *Cell* 1993, 75: 641–52
8 Whyte M. ICE/CED-3 proteases in apoptosis. *Trends Cell Biol* 1996; 6: 245–8
9 Lazebnik YA, Cole S, Cooke CA, Nelson WG, Earnshaw WC. Nuclear events of apoptosis *in vitro* in cell-free mitotic extracts: a model system for analysis of the active phase of apoptosis *J Cell Biol* 1993; 123: 7–22
10 Martin SJ, O'Brian GA, Nishioka WK *et al.* Proteolysis of fodrin (nonerythroid spectrin) during apoptosis. *J Biol Chem 1995,* 270: *6425–8*
11 Ehari M, Talanian RV, Wong WW, Nagata S Sequential activation of ICE-like and CPP32-like proteases during fas-mediated apoptosis. *Nature* 1996; 380: 723–6
12 Wright SC, Wei QS, Zhong J, Zheng H, Kinder DH, Larrick JW. Purification of a 24-kD protease from apoptosis tumor-cells that activates DNA fragmentation. *J Exp Med* 1994; 180· 2113–23
13 White E. Life, death and the pursuit of apoptosis. *Genes Dev* 1996, 10 1–15
14 Spector MS, Desnoyers S, Hoeppner DJ, Hengartner MO. Interaction between the *C. elegans* cell-death regulators CED-9 and CED-4. *Nature* 1997; 385: 653–6
15 Zhu WJ, Cowie A, Wasfy GW, Penn LZ, Leber B, Andrews DW. Bcl-2 mutants with restricted subcellular location reveal spatially distinct pathways for apoptosis in different cell types *EMBO J* 1996; 15: 4130–41
16 Muchmore SW, Sattler M, Liang H *et al.* X-ray and NMR structure of human bcl-xl, an inhibitor of programmed cell death. *Nature* 1996, 381: 335–41
17 Kroemer G. The proto-oncogene Bcl-2 and its role in regulating apoptosis. *Nature Med* 1997; 3: 614–20
18 Jacobson MD, Raff MC. Programmed cell death and bcl-2 protection in very low oxygen *Nature* 1995; 374: 814–6
19 Sugimoto A, Hozak RR, Nakashima T, Nishimoto T, Rothman JH. *dad-1*, an endogenous programmed cell death suppressor in *Caenorhabditis elegans* and vertebrates. *EMBO J* 1995; 14: 4434–41
20 Trauth BC, Klas C, Paters AMJ *et al.* Monoclonal antibody mediated tumor regression by induction of apoptosis. *Science* 1989; 245: 301–5
21 Wallach D Placing death under control *Nature* 1997; 338: 123–6

22 Yuan JY. Transducing signals of life and death. *Curr Opin Cell Biol* 1997; 2: 247–51
23 Muzio M, Chinnaiyan AM, Kischkel FL *et al* FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signalling complex. *Cell* 1996; 85: 817–27
24 White K, Tahaoglu E, Steller H Cell killing by the *Drosophila* gene *reaper*. *Science* 1996; 271: 805–7
25 Golstein P, Marguet D, Depraetere V. Homology between *reaper* and the cell-death domains of fas and TNFR1. *Cell* 1995; 81 185–6
26 Evan GI, Wyllie AH, Gilbert CS *et al*. Induction of apoptosis in fibroblasts by c-myc protein. *Cell* 1992; 69: 119–28
27 Harrington EA, Bennett MR, Fanidi A, Evan GI. C-myc induced apoptosis in fibroblasts is inhibited by specific cytokines *EMBO J* 1994; 13: 3286–95
28 Wu X, Levine AJ. P53 and E2F cooperate to mediate apoptosis. *Proc Natl Acad Sci USA* 1994; 91: 3602–6
29 Clarke AR, Maandag ER, van Room M *et al*. Requirements for a functional Rb-1 gene in murine development. *Nature* 1992; 359: 328–30
30 Clarke AR, Purdie CA, Harrison DJ *et al*. Thymocyte apoptosis induced by p53-dependent and independent pathways *Nature* 1993; 362: 849–51
31 Haimovitz-Friedman A, Kan C-C, Enleiter D *et al*. Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. *J Exp Med* 1994 180: 525–35
32 Santana P, Peña LA, Haimovitz-Friedman A *et al*. Acid sphingomyelinase deficient lymphoblasts and mice are defective in radiation-induced apoptosis. *Cell* 1996; 86: 189–99
33 Westwick JK, Bielawska AE, Dbaibo G, Hanun YA, Brenner DA. Ceramide activates the stress-activated protein kinases *J Biol Chem* 1995, 270: 22689–92
34 Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg M. Opposing effects of SEK and JNK-p38 MAP Kinases on apoptosis. *Science* 1995; 270: 1326–31
35 Rouvier E, Luciani MF, Golstein P. Fas involvement in Ca^{2+}-independent T cell-mediated cytotoxicity. *J Exp Med* 1993; 177: 195–200
36 Greenberg AH. Activation of apoptosis pathways by granzyme B. *Cell Death Differ* 1996; 3: 269–74
37 Clem RJ, Hardwick JM, Miller LK. Anti-apoptotic genes of baculoviruses. *Cell Death Differ* 1996; 3 9–16
38 Mosialos G, Birkenbach M, Yalamanchili R, Van Ardale T, Ware C, Kieff E. The Epstein-Barr virus transforming protein LMP-1 engages signalling proteins for the tumor necrosis factor receptor family. *Cell* 1995; 80: 389–99
39 Chapman RS, Whetton AD, Dive C. The suppression of drug-induced apoptosis by activation of v-abl protein-tyrosine kinase. *Cancer Res* 1994; 54: 5131–7
40 Kauffmann-Zeh A, Rodriguez-Viciani P, Ulrich E, Gilbert C, Coffer P, Evan G. Suppression of c-myc-induced apoptosis by ras signalling through PI 3-kinase and PKB. *Nature* 1997; 385: 781–4
41 Arends MS, McGregor AH, Toft NJ, Brown EJH, Wyllie AH. Susceptibility to apoptosis is differentially regulated by c-myc and mutated Ha-ras oncogenes and is associated with endonuclease availability. *Br J Cancer* 1993; 68: 1127–33
42 Brown L, McCarthy N, Evan GI. DNA repair – a sense-abl response? *Nature* 1997; 387 450–1
43 Lowe SW, Jacks T, Housman DE, Ruley HE Abrogation of oncogene-associated apoptosis allows transformation of p53-deficient cells. *Proc Natl Acad Sci USA* 1994; 91: 2026–30
44 Griffiths SD, Clarke AR, Healy LE *et al* Absence of p53 promotes propagation of mutant cells following genotoxic damage. *Oncogene* 1997; 9 603–9
45 Naik P, Karrim J, Hanahan D. The rise and fall of apoptosis during multistage tumorigenesis: down-modulation contributes to tumor progression from angiogenic progenitors. *Genes Dev* 1996; 10: 2105–6
46 O'Reilly MS, Holmgren L, Chew C, Folkman J Angiostatin induces and sustains dormancy of human tumors in mice. *Nature Med* 1996; 2: 689–92
47 Meeson A, Palmer M, Calfon M, Lang R. A relationship between apoptosis and flow during programmed capillary regression is revealed by vital analysis. *Development* 1996; 122: 3929–38

48 Lang RA, Bishop JM. Macrophages are required for cell death and tissue remodelling in the developing mouse eye. *Cell* 1993, 74. 453–62
49 Strand S, Hoffmann WJ, Hug H *et al.* Lymphocyte apoptosis induced by CD 45 (APO-1/fas) ligand-expressing tumour cells–a mechanism of immune-evasion? *Nature Med* 1996; 2: 1361–6
50 Olivetti G, Abbi R, Quaini F *et al.* Apoptosis and the failing human heart. *N Engl J Med* 1996, 336: 1131–41
51 Zychlinsky A, Sansonetti P. Apoptosis as a proinflammatory event: What can we learn from bacteria-induced cell death? *Trends Microbiol* 1997; 5: 201–204