- 24 Sato, K. and Ochi, A. (1998) Superclustering of B cell receptor and FcγRIIB1 activates Src homology 2-containing protein tyrosine phosphatase-1. *J. Immunol.* 161, 2716–2722
- 25 Lee, S. *et al.* (1991) Molecular cloning and primary structure of Kell blood group protein. *Proc. Natl. Acad. Sci. U. S. A.* 88, 6353–6357
- 26 Hughes-Jones, N.C. *et al.* (1957) Removal of incompatible cells by the spleen. *Br. J. Haematol.* 3, 125–133
- 27 Mollison, P.L. and Hughes-Jones, N.C. (1967) Clearance of Rh positive cells by low concentration of Rh antibody. *Immunology* 12, 63–73
- 28 Mollison, P.L. (1984) In *Haemolytic disease of the newborn* (Garratty, G., ed.), pp1–32, American Association of Blood Banks
- 29 Minnscoff, S.A. *et al.* (1998) FcγRII-B1 regulates the presentation of B cell receptor-bound antigens. *J. Immunol.* 161, 2079–2083
- 30 Stott, L.M. *et al.* Identification of alloreactive T-cell epitopes on the Rhesus D protein. *Blood* (in press)
- 31 Elson, C.J. *et al.* (1971) Clearance of erythrocytes and the immune response. An experimental study. *Vox Sang.* 20, 201–208
- 32 Woodrow, J.C. *et al.* (1973) Genetic control of the humoral immune response to rabbit erythrocyte isoantigens. *Immunology* 25, 131–139
- 33 Pollack, W. *et al.* (1968) Antibody-mediated immune suppression to the Rh factor: animal models suggesting mechanism of action. *Transfusion* 8, 134–145
- 34 Moise, K.J. *et al.* (1998) An animal model for hemolytic disease of the fetus and newborn in New Zealand White and New Zealand Red rabbits. *Am. J. Obstet. Gynecol.* 179, 1353–1358
- 35 Smith, G.N. and Mollison, P.L. (1974) Suppression of primary immunization to the rabbit red cell alloantigen Hg^A by passively administered anti-Hg^A. *Immunology* 26, 885–892
- 36 Henry, C. and Jerne, N.K. (1968) Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. *J. Exp. Med.* 128, 133–152
- 37 Karlsson, M.C.I. *et al.* (1999) Efficient IgG-mediated suppression of primary antibody responses in Fcγ receptor-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 2244–2249
- 38 Chan, P.L. and Sinclair, N.R., StC. (1973) Regulation of the immune response. VI. Inability of F(ab)₂ antibody to terminate established immune responses and its ability to interfere with IgG antibody-mediated immunosuppression. *Immunology* 24, 289–301
- 39 Takai, T. *et al.* (1996) Augmented humoral and anaphylactic responses in FcγRII-deficient mice. *Nature* 379, 346–349
- 40 Scott, M.D. and Murad, K.L. (1998) Cellular camouflage: fooling the immune system with polymers. *Curr. Pharm. Design* 4, 423–438
- 41 Bouchard, C. *et al.* (1995) A transforming growth factor β-like immunosuppressive factor in immunoglobulin G-binding factor. *J. Exp. Med.* 182, 1717–1726
- 42 MacDonald, I. *et al.* (1996) Transforming growth factor-β1 cooperates with anti-immunoglobulin for the induction of apoptosis in group I (biopsy-like) Burkitt lymphoma cell lines. *Blood* 87, 1147–1154
- 43 Gorgani, N.N. *et al.* (1999) Histidine-rich glycoprotein regulates the binding of monomeric IgG and immune complexes to monocytes. *Int. Immunol.* 11, 1275–1282
- 44 Dong, X. *et al.* (1999) Binding and uptake of agalactosyl IgG by mannose receptor on macrophages and dendritic cells. *J. Immunol.* 163, 5427–5434
- 45 Kumpel, B.M. *et al.* (1994) Galactosylation of human IgG monoclonal anti-D produced by EBV-transformed B-lymphoblastoid cell lines is dependent on culture method and affects Fc receptor-mediated functional activity. *Hum. Antibodies Hybridomas* 5, 143–151
- 46 Raju, T.S. *et al.* (2000) Species-specific variation in glycosylation of IgG: evidence for the species-specific sialylation and branch-specific galactosylation and importance for engineering recombinant glycoprotein therapeutics. *Glycobiology* 10, 477–486
- 47 Woodrow, J.C. (1970) *Rh immunization and its prevention* (*Series Haematologica*) (Vol. 3, Pt 3), Munksgaard
- 48 Rochna, E. and Hughes-Jones, N.C. (1965) The use of purified ¹²⁵I-labelled anti-γ globulin in the determination of the number of D antigen sites on red cells of different phenotypes. *Vox Sang.* 10, 675–686
- 49 Hughes-Jones, N.C. (1967) The estimation of the concentration and equilibrium constant of anti-D. *Immunology* 12, 565–571
- 50 Gunson, H.H. *et al.* (1976) The primary Rh₀(D) immune response in male volunteers. *Br. J. Haematol.* 32, 317–329
- 51 Lee, D. *et al.* (1999) Joint working group of the British Blood Transfusion Society and the RCOG. Recommendations for the use of anti-D immunoglobulin for Rh prophylaxis. *Transfus. Med.* 9, 93–97
- 52 Cohen, C. (1962) Blood groups in rabbits. *Ann. New York Acad. Sci.* 97, 26–36
- 53 Frame, M. and Mollison, P.L. (1972) The rabbit red cell antigen Hg^A and anti-Hg^A. *Immunology* 22, 1037–1042
- 54 Möller, G. (1985) Antibody-mediated suppression of the immune response is determinant specific. *Eur. J. Immunol.* 15, 409–412
- 55 Sinclair, N.R., StC. *et al.* (1968) Role of the Fc fragment in the regulation of the primary immune response. *Nature* 220, 1048–1049
- 56 Heyman, B. and Wigzell, H. (1984) Immunoregulation by monoclonal sheep erythrocyte-specific IgG antibodies: suppression is correlated to level of antigen binding and not to isotype. *J. Immunol.* 132, 1136–1143

Caspases: more than just killers?

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Proteases of the caspase family constitute the central executioners of apoptosis. Several recent observations suggest that caspases and apoptosis-regulatory molecules exert important functions beyond that of cell death, including the control of T-cell proliferation and cell-cycle progression. Here, Los and colleagues propose a model that directly connects cell suicide mechanisms to the regulation of cell-cycle progression.

Since the discovery that the *Caenorhabditis elegans* *Ced-3* gene encodes a protein related to mammalian interleukin 1β (IL-1β)-converting enzyme (ICE; caspase-1), proteolytic activation of the caspase

cascade has emerged as the most central step of apoptosis. To date, 14 mammalian caspases have been identified and implicated in different aspects of cell death, although the exact function of each individual caspase is still largely unknown. Caspases cleave precursors to produce mature cytokines (caspase-1 and -11), initiate the propagation of apoptotic signals (caspase-8 and -9) and execute the apoptotic program through cleavage of several vital proteins (caspase-3, -6 and -7)¹. Surprisingly, growing evidence now indicates a participation of caspases and other apoptosis regulators not only in cell death, but also in the control of the cell cycle.

Apoptosis and cell proliferation

Although the processes of cell proliferation and cell death appear to be opposing and mutually contradictory, some evidence suggests that the two events are linked. The maintenance of genomic stability is essential for the survival of organisms. To ensure this stability, checkpoints exist to interrupt cell-cycle progression when damage to the genome is detected. In multicellular organisms, a further

Fig. 1. Model for the involvement of the Fas-associated death domain protein (FADD) and caspases in the control of cell-cycle progression. Caspases may be selectively triggered during the G₂/M phase or other steps of cell-cycle progression, such as G₁/S transition. Their activation could be regulated by the phosphorylation (P) of FADD or a hypothetical mitotic death-inducing signaling complex (DISC)-forming protein (MDP). Caspase activity might then be required for the removal of S/G₂-phase-specific proteins that function as mitosis entry blockers. Alternatively, caspase activation could lead to the maturation of proteins promoting cell-cycle progression. According to this model, the fine-tuning of cell-cycle-specific caspase activation is regulated by proteins antagonizing caspase action.

checkpoint is the induction of apoptosis to eliminate cells with irreparable DNA damage that otherwise might become deleterious. Apoptosis is also induced when cell division has gone astray and cell-cycle progression is unscheduled. The signaling system that communicates between DNA lesions, the cell cycle and the cell death machinery is currently the subject of intense research. Previously, apoptosis has often been simply considered as a mitotic catastrophe. Some recent findings, however, suggest rather the opposite: that apoptosis-like processes may essentially control cell-cycle progression.

In the past few years, several caspase-knockout mice have been generated. Although the phenotypes of most of them confirm an essential role in cell death, non-apoptotic functions in development and differentiation have also become apparent. For instance, caspase-3^{-/-} mice are born at a lower frequency and are smaller than their littermates².

Whether this reduced size is an indirect result of disturbed development and differentiation patterns, or is mediated by reduced cell proliferation or apoptosis, is unknown. Gene targeting of the death-receptor-associated caspase-8 and its adaptor molecule, the Fas-associated death domain protein (FADD), has provided even more striking data. Caspase-8^{-/-} and FADD^{-/-} mice exhibit impaired heart muscle development with thin trabeculae and ventricular musculature^{3,4}. In the immune system, hematopoietic precursor cells from these knockout mice reveal a strongly impaired colony-forming activity and a defect in maintaining sufficient numbers of T-cell progenitors entering thymic development.

Proliferation of primary T cells is inhibited by cell-permeable caspase inhibitors

In FADD^{-/-} mice or animals expressing a dominant-negative FADD protein, activation-induced proliferation is impaired in T cells despite normal IL-2 production⁵⁻⁷. T cells lacking FADD function arrest at the G₀/G₁ transition of the cell cycle. The role of FADD in proliferation is supported by data obtained from chimeric mice that are transgenic for dominant-negative FADD and are deficient for the recombinase-activating gene *RAG-1*. Pro-T cells from these animals fail to proliferate in response to CD3ε ligation. Concomitant signaling through the pre-T-cell receptor (TCR) and death receptors appears to trigger cell survival, proliferation and differentiation, whereas death-receptor signaling in thymocytes that lack a pre-TCR induces apoptosis⁸. Interestingly, FADD has been recently shown to become phosphorylated by an unknown serine protein kinase during the G₂/M phase transition, whereas it is not phosphorylated in cells arrested in G₁/S of the cell cycle⁹. Together, these events could link FADD and possibly caspase-8 to the cell cycle. Indeed, it has been observed that caspase-8 is cleaved in non-apoptotic cells after TCR stimulation^{10,11}. Other supportive, yet indirect, evidence for a role of the FADD/caspase-8 pathway in cell growth is the observation that proliferation of primary T cells is inhibited by cell-permeable caspase inhibitors^{10,11}. However, it should also be noted that the ability of FADD to activate cell proliferation may be mediated by interaction with a signal transducer other than caspase-8. In this respect, it has been shown that expression of CrmA, a caspase-8 inhibitor, in T cells completely blocks CD95-mediated apoptosis, whereas T-cell proliferation is not affected⁶. Nevertheless, there is considerable evidence involving FADD in T-cell proliferation. Whether the proliferative effect of FADD requires caspase-8 activation or unrelated signaling events is not fully established.

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The role of caspases

Why should caspases be required for cell-cycle progression? One attractive hypothesis is that caspases could confer additional checkpoints, assuring that only healthy cells will complete the cell cycle. During apoptosis, FADD together with caspase-8 forms the death-inducing signaling complex (DISC). During mitosis, FADD could also associate with caspase-8 or with another hypothetical 'mitotic DISC-forming protein' (MDP), an event that might be facilitated by the phosphorylation of FADD at the G2/M transition. In the proposed model (Fig. 1), caspase activation could then be necessary either to proteolytically remove a block of entry into mitosis, such as a hypothetical 'mitosis entry blocker' (MEB), or to activate other cell-cycle-regulatory proteins. The MEB function could be fulfilled by proteins such as topoisomerase-1 or nuclear replication factor MCM3, which are indeed targeted by caspases¹². Their elimination might be necessary to secure cell-cycle progression. Other proteins cleaved by caspases include several negative regulators such as Wee1, an inhibitor of the cell-cycle-regulatory kinases CDK2 and CDC2, and CDC27, which is a component of the anaphase-promoting complex. Wee1 is a critical factor of the G2/M cell-cycle checkpoint machinery and mediates cell-cycle arrest by phosphorylation of CDC2. Therefore, cleavage of Wee1 in proliferating lymphocytes could lead to its inactivation, thus allowing cell-cycle progression. Of note, Wee1 processing by caspases during apoptosis in Jurkat cells correlated with a 20-fold decrease in Wee1 activity and an increase in CDC2 activity¹³. Moreover, the cyclin inhibitors p21^{Waf1} and p27^{Kip1} are targeted by caspases, resulting in increased CDK2 activity that could allow cell-cycle progression¹⁴.

When caspases are activated during mitosis, a critical question is how caspase cleavage could be restricted to just these cell-cycle regulators, while leaving other vital proteins intact. The answer could lie in a specific subcellular compartmentalization of caspases, the existence of scaffold proteins, or a different accessibility of cleavable substrates. Some caspases are translocated to a certain organelle during activation and, in some cell types, certain caspases have been localized in the nucleus. Interestingly, it has been found that, although caspases were activated and Wee1 was cleaved after TCR triggering, neither DNA replication factor RFC140 nor DFF45, the inhibitor of caspase-activated DNase, were cleaved in proliferating T cells¹¹. Cleavage of RFC140 and DFF45 would lead to inhibition of DNA replication and fragmentation of genomic DNA, events that are not compatible with T-cell proliferation. Thus, selective substrate processing in non-apoptotic cells could explain why T cells survive and proliferate despite caspases being activated.

Apoptosis inhibitors and cell-cycle regulation

To protect cell-cycle-regulatory and other vital structures, the activity of caspases must be tightly

regulated. This could be achieved by various apoptosis inhibitors. It has been observed that Bcl-2 (which, like FADD, is phosphorylated at the G2/M transition) delays the re-entry of resting NIH-3T3 cells into the cell cycle¹⁵. Moreover, Bcl-2-transgenic mice have delayed T-cell proliferation, whereas transgenic overexpression of Bax accelerates cell-cycle progression and apoptosis^{15,16}. Cells overexpressing Bcl-2 also contain decreased levels of phosphorylated retinoblastoma protein, which is a key regulator of cell-cycle progression¹⁷. Finally, downregulation of Bcl-2 by antisense approaches enhances proliferation of acute myeloid leukemic cells¹⁸. Overexpression of the Bcl-2-related protein Mcl-1 has also recently been shown to inhibit cell-cycle progression through the S phase¹⁹. Mcl-1, but no other Bcl-2 member, associates with the cell-cycle regulator PCNA (proliferating cell nuclear antigen). Interestingly, an Mcl-1 mutant that lacks PCNA binding still retains its anti-apoptotic function. It has also been observed that the anti-apoptotic function of Bcl-2 can be genetically separated from its inhibitory effect on cell-cycle entry²⁰. However, as all mutations that suppress the anti-apoptotic activity of Bcl-2 also abolish the inhibitory effect on cell-cycle transition, these two activities of Bcl-2 may be not entirely independent^{21,22}. Thus, these findings would lend support to the idea that inactivation of pathways leading to caspase activity results in reduced cell-cycle progression.

Selective substrate processing in non-apoptotic cells could explain why T cells survive and proliferate despite caspases being activated.

Much attention is being paid to the role of the inhibitor of apoptosis proteins (IAPs), in particular survivin, in the control of apoptosis and the cell cycle. Survivin is specifically induced in the G2/M phase and associates with microtubules of the spindle apparatus at the beginning of mitosis. Earlier work had suggested that survivin directly binds to and inhibits caspase 3 (Ref. 23). This led to the initial idea that survivin might function both as a cell cycle regulator and apoptosis suppressor. Because caspase 3 and the CDK inhibitor p21Waf1 colocalize with survivin at the centrosomes, it had been speculated that caspase activity and survivin could act together as a central part of the G2/M checkpoint²⁴. Indeed, inhibition of survivin function induced caspase 3 activity and apoptosis and produced a defect characterized by hyperploidy and supernumerary centrosomes.

More recent experiments, however, raise serious doubts that survivin is implicated in apoptosis at all. Survivin homologs have been found in yeast, worm and *Drosophila*^{25–28}. In these organisms, survivin

proteins also localize to spindle zones, where they are required for efficient mitotic cell division. Because yeast genomes, however, do not encode caspases, the survivin of lower organisms might have a primary role in cell division rather than in inhibition of cell death. Moreover, also in *C. elegans*, survivin is presumably not involved in apoptosis but required for chromosome separation and cytokinesis. Overexpression of the survivin homolog Bir-1 was unable to inhibit developmentally occurring cell death in *C. elegans* and inhibition of Bir-1 expression did not increase cell death²⁷. Instead, embryos lacking Bir-1 did not complete cytokinesis and became multinucleate. A similar phenotype has very recently been also found in survivin-deficient mice that showed disrupted microtubule formation and polyploidy, and failed to survive beyond 4.5 days post coitum²⁹. Thus, these experiments in knockout mice, yeast and knockout worms suggest that there are two classes of IAP: the ancient forms (including mammalian survivin and the worm and yeast homologs) that control mitotic division but have no obvious role in apoptosis, and the other forms (for example, X-IAP) that do control caspase activation and thereby influence apoptosis, but have no influence on cell-cycle control.

Concluding remarks

Clearly, much remains to be learned about a potential dual role of caspases in apoptosis and cell

proliferation. Although the model presented in Fig. 1 is hypothetical, it may provide an explanation for the proliferative abnormalities and developmental defects that are observed in caspase-8- and FADD-deficient mice, as well as in animals overexpressing an inactive FADD mutant. Likewise, it could explain why caspases are generally not deleted or silenced in most tumors. In contrast to earlier models suggesting that apoptosis is simply an aberrant form of mitosis, we would rather propose an inverse scenario in which a subset of apoptotic molecules may play a role in mitosis.

Certainly, many questions pertaining to the exact contribution of caspases to proliferation remain unanswered: how are the dual functions of caspases regulated and what are their cell-cycle-relevant targets? Is activation of caspases a normal part of the movement through the cell cycle, and which checkpoints do they control? Furthermore, is the cell-cycle-specific phosphorylation of Bcl-2 and FADD important for the regulation of M-phase events? Finally, does survivin suppress a default apoptotic pathway that is required for a certain step of the cell cycle or spindle assembly in mammals? Answering these questions might provide new insights into lymphocyte proliferation and the biological roles of caspases. The generation of conditional and cell-type-specific caspase-knockout mice will certainly shed more light on this issue.

References

- Los, M. *et al.* (1999) The role of caspases in development, immunity, and apoptotic signal transduction: lessons from knockout mice. *Immunity* 10, 629–639
- Kuida, K. *et al.* (1996) Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* 384, 368–372
- Varfolomeev, E.E. *et al.* (1998) Targeted disruption of the mouse caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. *Immunity* 9, 267–276
- Yeh, W.C. *et al.* (1998) FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. *Science* 279, 1954–1958
- Zhang, J. *et al.* (1998) Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. *Nature* 392, 296–300
- Newton, K. *et al.* (1998) A dominant interfering mutant of FADD/MORT1 enhances deletion of autoreactive thymocytes and inhibits proliferation of mature T lymphocytes. *EMBO J.* 17, 706–718
- Zörnig, M. *et al.* (1998) p53-dependent impairment of T-cell proliferation in FADD dominant-negative transgenic mice. *Curr. Biol.* 8, 467–470
- Newton, K. *et al.* (2000) FADD/MORT1 regulates the pre-TCR checkpoint and can function as a tumour suppressor. *EMBO J.* 19, 931–941
- Scaffidi, C. *et al.* (2000) Phosphorylation of FADD/MORT1 at serine 194 and association with a 70-kDa cell cycle-regulated protein kinase. *J. Immunol.* 164, 1236–1242
- Kennedy, N.J. *et al.* (1999) Caspase activation is required for T cell proliferation. *J. Exp. Med.* 190, 1891–1896
- Alam, A. *et al.* (1999) Early activation of caspases during T lymphocyte stimulation results in selective substrate cleavage in nonapoptotic cells. *J. Exp. Med.* 190, 1879–1890
- Stroh, C. and Schulze-Osthoff, K. (1998) Death by a thousand cuts: an ever increasing list of caspase substrates. *Cell Death Differ.* 5, 997–1000
- Zhou, B.B. *et al.* (1998) Caspase-dependent activation of cyclin-dependent kinases during Fas-induced apoptosis in Jurkat cells. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6785–6790
- Levkau, B. *et al.* (1998) Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. *Mol. Cell* 1, 553–563
- O'Reilly, L.A. *et al.* (1996) The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry. *EMBO J.* 15, 6979–6990
- Brady, H.J.M. *et al.* (1996) Bax α perturbs T cell development and affects cell cycle entry of T cells. *EMBO J.* 15, 6991–7001
- Mazel, S. *et al.* (1996) Regulation of cell division cycle progression by bcl-2 expression: a potential mechanism for inhibition of programmed cell death. *J. Exp. Med.* 183, 2219–2226
- Konopleva, M. *et al.* (2000) Liposomal bcl-2 antisense oligonucleotides enhance proliferation, sensitize acute myeloid leukemia to cytosine-arabinoside, and induce apoptosis independent of other antiapoptotic proteins. *Blood* 95, 3929–3938
- Fujise, K. *et al.* (2000) Regulation of apoptosis and cell cycle progression by MCL1: differential role of PCNA. *J. Biol. Chem.* published September 7, 2000 as 10.1074/jbc.M006626200
- Huang, D.C. *et al.* (1997) The anti-apoptosis function of Bcl-2 can be genetically separated from its inhibitory effect on cell cycle entry. *EMBO J.* 16, 4628–4638
- O'Reilly, L.A. *et al.* (1996) The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry. *EMBO J.* 15, 6979–6990
- O'Connor, L. *et al.* (2000) Apoptosis and cell division. *Curr. Opin. Cell Biol.* 12, 257–263
- Tamm, I. *et al.* (1998) IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res.* 58, 5315–5320
- Li, F. *et al.* (1999) Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. *Nat. Cell Biol.* 1, 461–466
- Uren, A.G. *et al.* (1998) Conservation of baculovirus inhibitor of apoptosis repeat proteins (BIRPs) in viruses, nematodes, vertebrates and yeasts. *Trends Biochem. Sci.* 23, 159–162
- Uren, A.G. *et al.* (1999) Role for yeast inhibitor of apoptosis (IAP)-like proteins in cell division. *Proc. Natl. Acad. Sci. U. S. A.* 96, 10170–10175
- Fraser, A.G. *et al.* (1999) Caenorhabditis elegans inhibitor of apoptosis protein (IAP) homologue BIR-1 plays a conserved role in cytokinesis. *Curr. Biol.* 9, 292–301
- Jones, G. *et al.* (2000) Deterin, a new inhibitor of apoptosis from *Drosophila melanogaster*. *J. Biol. Chem.* 275, 22157–22165
- Vren, A.G. *et al.* (2000) Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype. *Curr. Biol.* 10, 1319–1328