Bcl-xL Acts Downstream of Caspase-8 Activation by the CD95
Death-inducing Signaling Complex*

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The Bcl-2 family member Bcl-xL has often been correlated with apoptosis resistance. We have shown recently that in peripheral human T cells resistance to CD95-mediated apoptosis is characterized by a lack of caspase-8 recruitment to the DISC. To test this hypothesis we used a cell line in which caspase-8 signaling was inhibited by overexpression of Bcl-xL. In these MCF7-Fas-bcl-xL cells Bcl-xL had no effect on the recruitment of caspase-8 to the DISC. In contrast, cleavage of a typical substrate for caspase-8, poly(ADP-ribose) polymerase, was inhibited in comparison with the control-transfected CD95-sensitive MCF7-Fas cells. To test whether Bcl-xL would inhibit active caspase-8 subunits in the cytoplasm, a number of immunoprecipitation experiments were performed. Using monoclonal antibodies directed against different domains of caspase-8, anti-Bcl-xL antibodies, or fusion proteins of glutathione S-transferase with different domains of caspase-8, no evidence for a direct or indirect physical interaction between caspase-8 and Bcl-xL was found. Moreover, overexpression of Bcl-xL did not inhibit the activity of the caspase-8 active subunits p18/p10. Therefore, in this cell line that has become resistant to CD95-induced apoptosis due to overexpression of Bcl-xL, Bcl-xL acts independently and downstream of caspase-8.

Apoptosis is essential for development and tissue homeostasis (1). In the nematode Caenorhabditis elegans genetic analysis identified three genes, ced-3, ced-4, and ced-9, that are essential to apoptosis during worm development (2). Genetical ordering of the three genes revealed that ced-9 negatively regulates the induction of apoptosis by ced-3 (3) and that this regulation requires the presence of ced-4 (4). This suggests that ced-9 inhibits ced-3 through ced-4. Mammalian homologues of the C. elegans genes ced-3 and ced-9 have been identified. ced-3 shares high homology with the family of interleukin 1β-converting enzyme-like proteases (caspases) (5), essential mediators of many forms of apoptosis in mammalian cells (reviewed in Ref. 6), while ced-9 has high homology to bcl-2 (bcl-xL) (3).

Cross-linking of CD95 (APO-1/Fas) results in the recruitment of a set of proteins that include FADD/MORT1 (7, 8) and caspase-8 (FLICE/MACH/Mch5; Refs. 9–11) to the receptor forming the death-inducing signaling complex (DISC) (12, 9). Recruitment of caspase-8, which results in its activation (12), requires the presence of FADD and is mediated by an interaction of the death effector domain (DED) of FADD and the first DED of caspase-8 (13, 14). Interestingly, this DED was a region found to share weak homology with two areas in CED-4 (15, 16). In addition, a recent report demonstrated coimmunoprecipitation of Bcl-xL with caspase-8 and vice versa (17). These data suggest a regulatory mechanism in mammalian cells in which the CED-9 homolog Bcl-xL influences the activity of the CED-3 homolog caspase-8 possibly involving the CED-4 homology region of caspase-8.

We have recently reported that in a CD95-resistant peripheral T cell population, FADD was recruited to the activated CD95 receptor, but recruitment and activation of procaspase-8 was not detected. In addition, this apoptosis resistance correlated with the expression of the anti-apoptotic molecule Bcl-xL (18), suggesting that it might directly render cells CD95 apoptosis resistant by interfering with the DISC. Recently, it was shown that overexpression of Bcl-xL inhibited CD95-mediated apoptosis without blocking initial caspase activation (19). We therefore tested whether Bcl-xL might interfere with recruitment of caspase-8 to the DISC or with the activity of caspase-8 using a breast carcinoma cell line MCF7 expressing high levels of CD95 (MCF7-Fas) (20). Overexpression of Bcl-xL in these cells renders them resistant to CD95-induced apoptosis (20). In addition, no DNA fragmentation, morphological changes, or cleavage of poly(ADP-ribose) polymerase (PARP) could be detected in these cells. However, cleavage of caspase-8 was not affected by overexpression of Bcl-xL. No direct or indirect association between Bcl-xL and caspase-8 could be detected, indicating that in these cells Bcl-xL acted independently and downstream of caspase-8.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—The breast carcinoma cell line MCF7 transfected with CD95 and a control plasmid (MCF7-Fas-vector) or CD95 and Bcl-xL (MCF7-Fas-bcl-xL) were a kind gift from M. Jäättelä (Kopenhagen, Denmark). They were cultured as described elsewhere (20). The

*The abbreviations used are: DISC, death-inducing signaling complex; FADD, Fas-associated death domain protein; DED, death effector domain; PARP, poly(ADP-ribose) polymerase; mAb, monoclonal antibody; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.
Beads were then washed three times with lysis buffer (30 mM Tris/HCl, pH 7.4, 100 mM NaCl, 0.1% 3-(cyclohexylamino)-1-propanesulfonic acid, 10 mM dithiothreitol, and 10% sucrose) for 4 h at 37 °C. Reactions were stopped by the addition of standard anti-APO-1 for 16 h and then analyzed and photographed at a magnification of × 400. B, MCF7-Fas cells were left untreated (−) or treated with anti-APO-1 (+) for 16 h and subsequently analyzed for their DNA fragmentation using nuclear staining with propidium iodide. The Western blot in B shows the expression of Bcl-xL in MCF7-Fas-vector (left) and MCF7-Fas-bcl-xL cells (right).

**RESULTS**

**MCF7-Fas-bcl-xL Cells Are Resistant to CD95-mediated Apoptosis**—Since the CD95 DISC seemed to determine apoptosis sensitivity in peripheral T cells (18), we investigated whether expression of Bcl-xL would directly affect the composition of the DISC. However, normal T cells can hardly be transfected and overexpression of Bcl-2 or Bcl-xL in lymphoid cells does not necessarily block the CD95 pathway (25–29). Hence, we tested the effect of overexpression of Bcl-xL in a breast carcinoma cell MCF7-Fas, one of the few cell lines that have been reported to become resistant to CD95-mediated apoptosis upon overexpression of Bcl-xL (20). Indeed overexpression of Bcl-xL blocked the morphological changes (i.e. rounding up and detaching) induced by cross-linking of CD95 (Fig. 1A). In addition, fragmentation of chromosomal DNA as compared with control-transfected MCF7-Fas cells was almost completely inhibited in the Bcl-xL transfectants (Fig. 1B). This difference in sensitivity

**FIG. 1. Bcl-xL overexpression blocks apoptosis in MCF7-Fas cells.** A, MCF7-Fas cells were treated with anti-APO-1 for 16 h and then analyzed and photographed at a magnification of × 400. B, MCF7-Fas cells were left untreated (−) or treated with anti-APO-1 (+) for 16 h and subsequently analyzed for their DNA fragmentation using nuclear staining with propidium iodide. The Western blot in B shows the expression of Bcl-xL in MCF7-Fas-vector (left) and MCF7-Fas-bcl-xL cells (right).
toward cross-linking of the CD95 receptor was not due to a difference in expression of the receptor, since both transfectants express similar amounts (data not shown), but due to the expression of Bcl-xL (Fig. 1B).

**Bcl-xL Does Not Associate with Caspase-8 or Block Its Activity**—Recently, Chinnaiyan et al. (17) demonstrated in transient cotransfection experiments that Bcl-xL could be coimmunoprecipitated together with caspase-8 and vice versa. This association was indirect as it required an additional factor believed to be a mammalian CED-4 homolog (17). Most importantly the data suggested that the CED-9 homolog Bcl-xL might act upstream of the CED-3 homolog caspase-8. Since the MCF7- Fas cells apoptosis through CD95 involved activation of caspase-8 and CD95-mediated apoptosis in these cells was blocked by overexpression of Bcl-xL, we could now test this hypothesis in vivo in stably transfected cells. To this end we generated a number of reagents to use in immunoprecipitation experiments. All following experiments were done with untreated and anti-APO-1-treated (+) MCF7-Fas and MCF7-Fas-bcl-xL cells. First we tested whether Bcl-xL could be immunoprecipitated from MCF7 cells using fusion proteins of GST with different functional domains of caspase-8 or FADD (Fig. 2). Using GST-caspase-8 and GST-N-caspase-8 (comprising the first 188 amino acids of caspase-8 containing both DED) we could precipitate FADD from lysates of both transfectants (Fig. 2, B and D, lanes 7–10). Therefore, both DED containing GST-caspase-8 constructs were functionally active in binding the physiological important signaling molecule FADD through their N termini. FADD was not precipitated when GST-C-caspase-8 (amino acids 1–180), and C-caspase-8 (amino acids 181–478).

**Fig. 2.** Bcl-xL does not coprecipitate with GST-caspase-8. GST-fusion proteins (20 μg) were incubated with cellular lysates from unstimulated (−) or 2-h anti-APO-1-treated (+) 10⁷ MCF7-Fas (A, B) or MCF7-Fas-bcl-xL (C, D) and analyzed by Western blotting for coprecipitation of Bcl-xL (A and C) or FADD (B and D). Lysates of 5 × 10⁷ cells were loaded on the same gel to determine the quantity and migration position of Bcl-xL and FADD. The following fusion proteins with GST were used for immunoprecipitation: caspase-8 (CASP-8), N-caspase-8 (amino acids 1–180), and C-caspase-8 (amino acids 181–478).

Since the GST domain might have prevented interaction of caspase-8 and CD95-mediated apoptosis in these cells was blocked by overexpression of Bcl-xL, we could now test this hypothesis in vivo in stably transfected cells. To this end we generated a number of reagents to use in immunoprecipitation experiments. All following experiments were done with untreated and anti-APO-1-treated (+) MCF7-Fas and MCF7-Fas-bcl-xL cells. First we tested whether Bcl-xL could be immunoprecipitated from MCF7 cells using fusion proteins of GST with different functional domains of caspase-8 or FADD (Fig. 2). Using GST-caspase-8 and GST-N-caspase-8 (comprising the first 188 amino acids of caspase-8 containing both DED), we could precipitate FADD from lysates of both transfectants (Fig. 2, B and D, lanes 7–10). Therefore, both DED containing GST-caspase-8 constructs were functionally active in binding the physiological important signaling molecule FADD through their N termini. FADD was not precipitated when GST-C-caspase-8 was used (lacking the two N-terminal DED) (Fig. 2, B and D, lanes 11 and 12). We then tested whether cytoplasmic Bcl-xL from anti-APO-1-treated and untreated cells would coimmunoprecipitate with GST-caspase-8 or GST-C-caspase-8. As a control for specificity, we used GST, GST-FADD, or GST-C-FADD (lacking the DED). Bcl-xL was clearly detectable by Western blot analysis in Triton X-100 lysates from 5 × 10⁷ MCF7-Fas-bcl-xL cells (Fig. 2C, lanes 13 and 14). However, no associated Bcl-xL could be detected with any of the GST fusion proteins tested, although 20-fold more lysate (10⁷ cells) was used for precipitation (Fig. 2C, lanes 1–12).

Since the GST domain might have prevented interaction of caspase-8 with Bcl-xL, specific anti-caspase-8 and anti-Bcl-xL antibodies were used for immunoprecipitation experiments (Figs. 3 and 4). The two expressed caspase-8 isoforms (21) and their cleavage products (p43/p41, their prodomains p26/p24, and the caspase-8 active subunit p18) (21) were clearly detected in similar amounts in cellular lysates from unstimulated and anti-APO-1-stimulated cells (−/+ in Fig. 3). Immunoprecipitation with a rabbit anti-Bcl-xL serum detected the complex formation between p10 and p18. However, no associated Bcl-xL was immunoprecipitated from 10⁷ untreated (−) or 2-h anti-APO-1-treated (+) MCF7-Fas or MCF7-Fas-bcl-xL. As a control, cellular lysates were incubated with rabbit IgG. Immunoprecipitates were analyzed for the presence of Bcl-xL (C) and for coimmunoprecipitation of endogenous full sized caspase-8 (A) or the active caspase-8 subunit p18 (B). Full-length caspase-8 and the cleaved subunits were detected in cellular lysates from 5 × 10⁷ cells (lanes 7–10). Immunoblots were done with the mAb N2 directed against the N terminus (A), with the p18 mAb C15 (B), and with a polyclonal anti-Bcl-xL antibody (C). Positions of rabbit (rBlG14) (detected due to some cross-reactivity of the secondary anti-mIgG1 Ab with the heavy chain of the anti-Bcl-xL anti-serum), the two expressed caspase-8 isoforms, their cleavage intermediates p43/p41, their prodomains p26/p24, and the caspase-8 active subunit p18 and Bcl-xL are indicated.

To exclude that the antibodies against Bcl-xL interfered with the binding to caspase-8, we performed a reverse immunoprecipitation with different mAbs against either the C terminus or the N terminus of caspase-8 and determined whether coimmunoprecipitation of Bcl-xL could be detected. Using the C1 mAb, which recognizes the active caspase-8 subunit p18, we could immunoprecipitate full sized caspase-8 (Fig. 4A, lanes 5, 6, 13, and 14) and the p18 and the p10 subunits (Fig. 4B, C, lanes 6 and 14), indicating that the mAb did not interfere with the complex formation between p10 and p18. However, no coimmunoprecipitation of Bcl-xL could be detected using this antibody (Fig. 4D, lanes 5, 6, 13, and 14). The N2 antibody, which reacted with the caspase-8 prodomain, immunoprecipitated full sized caspase-8 (Fig. 4A, lane 7, 8, 15, and 16), yet did not precipitate any Bcl-xL (Fig. 4D, lanes 7, 8, 15, and 16). Since the reported interaction between Bcl-xL and caspase-8
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Fig. 4. Bcl-x<sub>L</sub> does not communoprecipitate with caspase-8. Cellular lysates from 10<sup>7</sup> untreated or anti-APO-1 treated MCF7-Fas or MCF7-Fas-bcl-x<sub>L</sub> were incubated with Sepharose-coupled mAb against the caspase-8 p10 subunit (C5), the p18 subunit (C1), the N-terminal part (N2), or as a control mouse IgG. Immunoprecipitates were analyzed for the precipitated caspase-8 subunits using the C1 mAb to detect full-length caspase-8 and the p18 subunit (A and B) or the C5 mAb to detect the p10 subunit (C). Immunoprecipitates were analyzed for co-immunoprecipitation of Bcl-x<sub>L</sub> by Western blotting with a polyclonal anti-Bcl-x<sub>L</sub> antibody. As a control, cellular lysates of 5 × 10<sup>5</sup> MCF7-Fas-vector or MCF7-Fas-bcl-x<sub>L</sub> cells were analyzed (lanes 17–20). E, the active caspase-8 subunits were immunoprecipitated using the C5 mAb, and after immunoprecipitation complexes were incubated for 4 h at 37 °C with recombinant PARP. Resulting PARP cleavage was determined by immunoblotting with the C-II-10 mAb. Cells were stimulated as in Fig. 3. Immunoprecipitations were performed with 10 μg for C1, N2, and mlgG and with 25 μg for C5. Indicated are the positions of heavy and light chain (mainly visible in C5 immunoprecipitations due to aspecific cross-reaction of the secondary antibodies with the high amount of heavy and light chain present), caspase-8, p18, p10, Bcl-x<sub>L</sub>, and the p116 and p85 PARP fragments.

was shown to involve both the N terminus and the p18 subunit of caspase-8 (17), both mAbs against caspase-8 used could still interfere with the interaction of Bcl-x<sub>L</sub> with caspase-8. Therefore, we made use of a third caspase-8 mAb, C5, that is directed against the p10 subunit. This region was reported by Chinnaiyan et al. (17) not to be involved in the binding of Bcl-x<sub>L</sub>. The C5 mAb immunoprecipitated both the full-length caspase-8 isoforms (Fig. 4A, lanes 3, 4, 11, and 12) and the active subunits p18 and p10 (Fig. 4, B and C, lanes 4 and 12), indicating that the mAb did not interfere with complex formation between p10 and p18. However, also C5 did not communoprecipitate any Bcl-x<sub>L</sub> (Fig. 4D, lanes 3, 4, 11, and 12). Taken together in all experiments, no caspase-8/Bcl-x<sub>L</sub> association was detected even when the Western blots were highly overexposed (data not shown).

To test whether Bcl-x<sub>L</sub> overexpression would inhibit the activity of caspase-8, we immunoprecipitated the active subunits p18/p10 and determined the <i>in vitro</i> PARP cleavage activity of the immunoprecipitated complex. The immunoprecipitate of the active subunits from anti-APO-1-treated cells using the C5 mAb displayed clear PARP cleavage <i>in vitro</i> (Fig. 4E, lanes 4 and 12), while no cleavage of PARP was observed when caspase-8 was immunoprecipitated from untreated cells (Fig. 4E, lanes 3 and 11) or when mlgG control immunoprecipitates were used (Fig. 4E, lanes 1, 2, 8, and 10). Importantly, again no difference could be detected <i>in vitro</i> PARP cleavage when the active subunits were immunoprecipitated from MCF7-Fas or MCF7-Fas-bcl-x<sub>L</sub> cells, indicating that overexpression of Bcl-x<sub>L</sub> did not affect the enzymatic activity of caspase-8.

Formation of the DISC and Activation of Caspase-8 Are Unaffected in MCF7-Fas-bcl-x<sub>L</sub> Cells—The above data indicate that Bcl-x<sub>L</sub> likely does not exert its inhibitory activity by physically interacting with procaspase-8 or active caspase-8 subunits in the cytoplasm, regardless whether anti-APO-1-treated or untreated cells were tested. We have shown recently that <i>in vivo</i> caspase-8 is activated by recruitment to the CD95 DISC (13). If Bcl-x<sub>L</sub> functioned upstream of caspase-8, it should interfere with caspase-8 cleavage by the DISC. The first step in DISC formation is recruitment of FADD (12). This can be visualized by immunoprecipitation of CD95 and subsequent immunoblotting with a FADD-specific mAb. Treatment of SKW6.4 B cell lymphomas with anti-APO-1 clearly resulted in an association of FADD with CD95 (Fig. 5A, lanes 7 and 8). Similarly, both MCF7-Fas-vector and MCF7-Fas-bcl-x<sub>L</sub> cells, which express comparable amounts of FADD (Fig. 5A, lanes 1 and 2), efficiently recruited FADD to CD95 after treatment with anti-APO-1 (Fig. 5A, lanes 3–6). We next tested whether the activity of the DISC to process caspase-8 was affected by the Bcl-x<sub>L</sub> overexpression. We have recently shown that this activity can be determined <i>in vitro</i> by incubation of immunoprecipitated DISC with <i>in vitro</i> translated <sup>35</sup>S-labeled caspase-8 (13). Testing the MCF7-Fas transfectants in this assay demonstrated that Bcl-x<sub>L</sub> overexpression did also not alter the activity of the DISC to activate caspase-8 <i>in vitro</i> (Fig. 5B).

In Vivo Bcl-x<sub>L</sub> Acts Downstream of Caspase-8 and Upstream of a PARP Cleaving Caspase—To test whether Bcl-x<sub>L</sub> would inhibit cleavage of caspase-8 <i>in vivo</i>, the C15 mAb recognizing the p18 subunit of caspase-8 was used. This antibody allowed to follow caspase-8 activation <i>in vivo</i> by detecting both the full-length caspase-8 isoforms as well as the active caspase-8 subunit p18 (Fig. 6A). Importantly, in both control transfected and Bcl-x<sub>L</sub> overexpressing MCF7-Fas cells the p18 subunit was formed to a similar extent and with identical kinetics (Fig. 6A, lower panel). Furthermore, as has been shown previously for...
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We have recently shown that CD95 resistance in short term activated peripheral T cells is characterized by a lack of caspase-8 recruitment to the DISC despite high expression of caspase-8 in the cytoplasm (18). In addition, we found that the expression of Bcl-x<sub>L</sub> correlated with CD95 apoptosis sensitivity in these T cells, making it possible that Bcl-x<sub>L</sub> directly prevented caspase-8 recruitment to the DISC. However, for caspase-8 being the main target for Bcl-x<sub>L</sub> is in contradiction to the reported effects of Bcl-2 or Bcl-x<sub>L</sub> on the CD95 signaling pathway in lymphoid cells. Reports range from no inhibition (27–29, 40) to partial inhibition (25) to substantial inhibition of CD95-mediated apoptosis (19, 34, 38). One of the few cell lines that have been reported to be completely resistant to CD95 apoptosis after overexpression of Bcl-x<sub>L</sub> is the breast carcinoma cell line MCF7-Fas (20). Using these cells we did not find any association of Bcl-x<sub>L</sub> with pro-caspase-8 or caspase-8 active subunits or any effect of Bcl-x<sub>L</sub> on caspase-8 activation. However, our experiments do not exclude that Bcl-x<sub>L</sub> could influence the action of caspase-8 by preventing a possible translocation of the caspase-8 active subunits within the cell from one location to their physiological target(s) without blocking their enzymatic activity.

The presence of homologous functionally interchangeable proteins in <i>C. elegans</i> and mammals suggests a universal principle in apoptosis regulation conserved throughout evolution. The data presented here demonstrate that the CED-9 homolog...
Bcl-xL does not act by interfering with the activation of the CED-3 homolog caspase-8. Therefore, apoptosis sensitivity is at least regulated at two separate levels: at the level of the DISC and independently further downstream at the level of Bcl-xL, likely regulating the activity of other CED-3 homologs. In contrast to C. elegans mammalian cells seem to have developed at least two levels at which caspases act. First level caspases, such as caspase-8, may couple the intracellular death machinery to death receptors and may not be regulated by CED-9-like molecules. Second level caspases likely representing caspase-3-like proteases located downstream of mitochondria may represent the CED-3 homologs likely to be regulated by the CED-9 homolog Bcl-xL (Bcl-2).

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Addendum—While this manuscript was being reviewed cloning of a human CED-4 homolog Apaf-1 was reported (41). Upon binding of cytochrome c Apaf-1 was demonstrated to activate caspase-3. This finding is in complete agreement with our observation that Bcl-xL acts downstream of caspase-8, since it also places the action of a human CED-4 homolog downstream of caspase-8.

REFERENCES
1. Steller, H. (1995) Science 267, 1445–1449
2. Ellis, R. E., Yuan, J., and Horvitz, H. R. (1991) Annu. Rev. Cell Biol. 7, 663–698
3. Hengartner, M. O., Ellis, R. E., and Horvitz, H. R. (1992) Nature 356, 494–499
4. Shaham, S., and Horvitz, H. R. (1996) Genes Dev. 10, 578–591
5. Yuan, J., Shaham, S., Ledoux, S., Ellis, H. M., and Horvitz, H. R. (1993) Cell 75, 641–652
6. Henkart, P. A. (1996) Immunity 4, 195–201
7. Chinnaiyan, A. M., O’Rourke, K., Tewari, M., and Dixit, V. M. (1995) Cell 81, 505–512
8. Boldin, M. P., Varfolomeev, E. E., Pancer, Z., Mett, I. L., Camonis, J. H., and Wallach, D. (1995) J. Biol. Chem. 270, 7795–7798
9. Muzio, M., Chinnaiyan, A. M., Kischkel, F. C., O’Rourke, K., Shevchenko, A., Scaffidi, C., Zhang, M. N. J., Gentz, R., Mann, M., Krammer, P. H., Peter, M. E., and Dixit, V. M. (1996) Cell 85, 817–827
10. Boldin, M. P., Goncharov, T. M., Geltsev, Y. V., and Wallach, D. (1996) Cell 85, 803–815
11. Fernandes-Alnemri, T., Armstrong, R. C., Krebs, J., Srinivasula, S. M., Wang, L., Bullrich, F., Fritz, L. C., Trapani, J. A., Tomasselli, K. J., Litwack, G., and Alnemri, E. S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7464–7469
12. Kischkel, F. C., Hellbardt, S., Behrmann, I., Germer, M., Pavilia, M., Krammer, P. H., and Peter, M. E. (1995) EMBO J. 14, 5579–5588
13. Medema, J. P., Scaffidi, C., Kischkel, F. C., Shevchenko, A., Mann, M., Krammer, P. H., and Peter, M. E. (1997) EMBO J. 16, 2794–2804
14. Chinnaiyan, A. M., Tepper, C. G., Seldin, M. F., O’Rourke, K., Kischkel, F. C., Hellbardt, S., Krammer, P. H., Peter, M. E., and Dixit, V. M. (1996) J. Biol. Chem. 271, 4961–4965
15. Nagata, S. (1996) Curr. Biol. 6, 1241–1243
16. Bauer, M. K. A., Wesselborg, S., and Schulze-Osthoff, K. (1997) FEBS Lett. 402, 256–258
17. Chinnaiyan, A. M., O’Rourke, K., Lane, B. R., and Dixit, V. M. (1997) Science 275, 1122–1126
18. Peter, M. E., Kischkel, F. C., Schuergsflug, C. G., Medema, J. P., Debatin, K.-M., and Krammer, P. H. (1997) Eur. J. Immunol. 27, 1207–1212
19. Boise, L. H., and Thompson, C. B. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3759–3764
20. Ja¨a¨ttela ¨, M., Benediet, M., Tewari, M., Shayman, J. A., and Dixit, V. M. (1995) Oncogene 10, 2297–2305
21. Scaffidi, C., Medema, J. P., Krammer, P. H., and Peter, M. E. (1997) J. Biol. Chem. 272, 26953–26958
22. Trauth, B. C., Klas, C., Peters, A. M. J., Matzku, S., Hollen, P., Falk, W., Debatin, K.-M., and Krammer, P. H. (1989) Science 245, 301–305
23. Peter, M. E., Hellbardt, S., Schwartz-Albiez, R., Westendorp, M. O., Moldenauer, G., Grell, M., and Krammer, P. H. (1995) Cell Death Differ. 2, 163–171
24. Nicotelli, I., Migliorati, G., Pagliacci, M. C., Grignani, F., and Riccardi, C. (1991) J. Immunol. Methods 139, 271–279
25. Itoh, N., Tsujimoto, Y., and Nagata, S. (1993) J. Immunol. 151, 621–627
26. Enari, M., Hase, A., and Nagata, S. (1995) EMBO J. 14, 5201–5208
27. Strasser, A., Harris, A. W., Huang, D. C. S., Krammer, P. H., and Cory, S. (1995) EMBO J. 14, 6136–6147
28. Chinnaiyan, A. M., Orth, K., O’Rourke, K., Duan, H., Poirier, G. G., and Dixit, V. M. (1996) J. Biol. Chem. 271, 4573–4576
29. Huang, D. C. S, Cory, S., and Strasser, A. (1997) Oncogene 14, 405–414
30. Tewari, M., Quan, L. T., O’Rourke, K., Desnoyers, S., Zeng, Z., Seidler, D. R., Poirier, G. G., Salvesen, G. S., and Dixit, V. M. (1996) Cell 81, 801–809
31. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lazebnik, Y. A., Manday, N. A., Raju, S. M., Smulson, M. E., Yamin, T.-T., Yu, V. L., and Miller, D. K. (1996) Science 275, 37–43
32. Spector, M. S., Desnoyers, S., Hoeppner, D. J., and Hengartner, M. O. (1997) Nature 385, 653–656
33. Wu, D., Wallen, H. D., and Nuñez, G. (1997) Science 275, 1126–1129
34. Armstrong, R. C., Aja, T., Xiang, J., Gaur, S., Krebs, J. F., Hoang, K., Bai, X., Korsmeyer, S. J., Karanewsky, D. S., Fritz, L. C., and Tomaselli, K. J. (1996) J. Biol. Chem. 271, 16850–16855
35. Shimizu, S., Kuguchi, Y., Kamikawa, W., Matsuda, H., and Tsujimoto, Y. (1996) Oncogene 12, 2251–2257
36. Perry, D. K., Smyth, M. J., Wang, H. G., Reed, J. C., Duriez, P., Poirier, G. G., Obeid, L. M., and Hannun, Y. A. (1997) Cell Death Differ. 4, 29–33
37. Estoppey, S., Rodriguez, I., Sodupe, R., and Martinou, J. C. (1997) Cell Death Differ. 4, 34–38
38. Armstrong, R. C., Aja, T., Xiang, J., Gaur, S., Krebs, J. F., Hoang, K., Bai, X., Korsmeyer, S. J., Karanewsky, D. S., Fritz, L. C., and Tomaselli, K. J. (1996) J. Biol. Chem. 271, 16850–16855
39. Golstein, P. (1997) Science 275, 1081–1082
40. Menon, S. D., Guy, G. R., and Tan, Y. H. (1995) J. Biol. Chem. 270, 18881–18887
41. Zou, H., Hengzel, W. J., Liu, X., Lutschg, A., and Wang, X. (1997) Cell 90, 405–413
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