J. Biochem. 120, 699-704 (1996)

Identification of an Interleukin-1β Converting Enzyme-Like Activity That Increases upon Treatment of P19 Cells with Retinoic Acid as the Proteasome[1]

Tatsuyuki Kobayashi,*‡ Ayako Shinozaki,* Takashi Momoi,† Kiichi Arahata,* and Toshifumi Tsukahara*,[2]

* *Department of Neuromuscular Research and* †*Department of Development and Differentiation, National Institute of Neuroscience, NCNP, Ogawahigashi-cho 4-1-1, Kodaira, Tokyo 187; and* ‡*The 3rd Department of Internal Medicine, Tokyo Medical College, Shinjuku-ku, Tokyo 160*

Received for publication, March 4, 1996

We examined changes in proteinase activities in P19 embryonal carcinoma cells during retinoic acid-induced differentiation. The interleukin-1β converting enzyme (ICE)-like Ac-YVAD-MCA hydrolytic activity was increased about 6-fold by treatment with retinoic acid. This activity was inhibited by *N*-ethylmaleimide and Ac-YVAD-H but not by E-64, EDTA, PMSF, or amastatin. The ICE-like activity in P19 cells eluted as a single peak just after the void volume on gel filtration. No ICE-like activity was observed at a molecular mass of 30-50 kDa. Enzymatic purification, Western blot analysis, and an immunoabsorption study demonstrated that the ICE-like activity in P19 cells is caused by the proteasome, and is stimulated during retinoic acid-induced differentiation. The proteasome purified from mouse liver also cleaved Ac-YVAD-MCA. These results strongly suggest that the proteasome is a major ICE-like proteinase in P19 cells and may be involved in the neural differentiation and the apoptotic pathway.

Key words: apoptosis, ICE-like activity, neural differentiation, P19 cells, proteasome.

Genetic analysis of the pathway for apoptosis has been reported for *Caenorhabditis elegans*, and two genes were identified as essential for cell death. One of the two genes, *ced-3*, is similar to a cysteine proteinase, interleukin-1β converting enzyme (ICE) (*1*). Miura *et al.* demonstrated that the overexpression of ICE in cultured mammalian cells causes cell death, and that transfection of viral ICE inhibitory gene *crmA* into cells suppresses ICE-mediated cell death (*2*). Furthermore, Fas-mediated apoptosis is prevented by specific inhibitors of ICE (*3, 4*), and the expression of antisense *Nedd2* (*5*), a member of the ICE family of genes, inhibits the induced apoptosis. These results strongly suggest that ICE-like enzyme activities play crucial roles in the apoptotic process and that the digestion of a (or some) target substrate(s) of ICE-like enzymes causes apoptosis (*6-8*). ICE family members are synthesized and present in cells as inactive precursors (*1, 9*). Therefore, the activation of ICE-like enzymes is thought to trigger apoptosis. However, the activation process of ICE-like enzymes is not yet well-characterized.

Murine embryonal carcinoma cells, P19, can differentiate multipotentially into myogenic, neuronal, and glial cells upon treatment with retinoic acid. During treatment with retinoic acid, cells which do not commit to differentiation die with cytoplasmic contraction and packaging of cellular components (*10, 11*). Here we deal with the changes in proteinase activities in response to the retinoic acid-induced differentiation of P19 cells, and show that an ICE-like activity is stimulated during the induction. We demonstrate that the proteasome, a multicatalytic proteinase, has an ICE-like activity and may play important roles in the neuronal differentiation and apoptosis of P19 cells.

EXPERIMENTAL PROCEDURES

Cell Culture—P19 EC cells were grown in α-minimal essential medium (Gibco BRL) supplemented with 10% fetal calf serum and antibiotics. To induce apoptosis, cells (1×10^6 cells/ml) were aggregated in bacterial-grade dishes with 1 μM retinoic acid for 4 days. The medium was changed every 2 days (*10-12*).

Proteinase Assay—Proteinase activities were determined by measuring the fluorescence of aminomethylcoumarin liberated from peptide substrates (Peptide Institute) (*13*). We used the following substrates in this study: acetyl (Ac)-YVAD-methylcoumarinamide (MCA) for ICE-like activity (*14*), succinyl (Suc)-LLVY-MCA for chymotrypsin-like activity and the proteasome, a multicatalytic proteinase (*13*), carbobenzoxy(Z)-FR-MCA for trypsin-like activity and cathepsins B+L, Suc-GPLGP-MCA for prolyl endopeptidase (*15*), and D-MCA for aminopeptidase A. The reaction mixtures (100 μl) comprised 0.1 M buffers and 0.1 mM substrates. After incubation at 37°C, the reaction was terminated by the addition of 100 μl of 10% SDS and 1.3 ml of 0.2 M Tris-HCl, pH 9.0.

Protein concentration was determined by DC protein

[1] This work was supported in part by research grants from the Ministry of Education, Science, Sports and Culture and in part by a grant from the National Center of Neurology and Psychiatry of the Ministry of Health and Welfare of Japan.

[2] To whom correspondence should be addressed. Tel: +81-423-46-1712, Fax: +81-423-46-1742

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

assay (Bio-Rad).

Electrophoresis and Western Blotting—Proteins were analyzed by denaturing and non-denaturing polyacrylamide gel electrophoresis, and visualized by silver staining.

For Western blot analysis, proteins were transferred to a nitrocellulose membrane after electrophoresis and then detected with antibody, using peroxidase-conjugated avidin-biotin as described previously (*13*).

Immunoabsorption Study—Gel-filtered proteinase was mixed with 0.5 mg/ml of anti-proteasome IgG or anti-maltose binding protein IgG as a control. After incubation at 4°C overnight, sample mixtures were centrifuged and the Ac-YVAD-MCA hydrolytic activities of the supernatant were assayed.

Purification of the Proteasome from Mouse Liver—The purification of the proteasome from mouse liver was achieved by the method of Tsukahara *et al.* and Tanaka *et al.* (*16-18*), involving successive chromatographies on a 30 ml DE52 column, a hydroxyapatite column, a HiLoad Superdex 200 column (Pharmacia), a DEAE-2SW column (Tosoh), and a poly-L-Lys agarose column. The purified proteasome appeared homogeneous in native polyacrylamide gel electrophoresis.

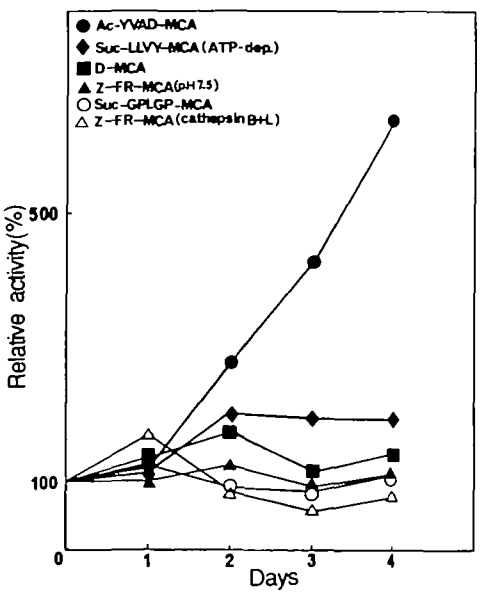

Fig. 1. **Chronological changes in proteinase activities during RA-treatment.** After 1, 2, 3, and 4 days of culture with RA, P19 cells were collected and lysed in PBS. Proteinase activities and protein concentrations were determined. The activities of proteinases were determined with the following buffers and supplements: endopeptidase, Tris-HCl, pH 7.0, containing 1 mM 2-mercaptoethanol; cathepsins B+L, sodium acetate, pH 5.5, containing 1 mM EDTA and 2-mercaptoethanol; trypsin-like activity, Tris-HCl, pH 7.5, containing 10 μg/ml E64-c; ICE-like activity, Tris-HCl, pH 7.5, containing 1 mM EDTA, 1 mM 2-mercaptoethanol, 1 mM amidinophenylmethanesulfonyl fluoride (APMSF) and 10 μg/ml E64-c; D-MCA-hydrolytic activity, Tris-HCl, pH 7.5, containing 1 mM EDTA, 1 mM 2-mercaptoethanol and 1 mM APMSF; ATP-dependent proteasome activity, Tris-HCl, pH 9.0, with and without 1 mM ATP. The figure shows the results as specific activities relative to untreated cells as a control.

RESULTS

Increase in the ICE-Like Activity of P19 Cells by Retinoic Acid Treatment—As described previously, the neuronal differentiation of P19 cell differentiation was induced by plating the cells in suspension in the presence of retinoic acid (RA) for 4 days, then reseeding in a tissue culture dish (*12*). In suspension culture with RA, P19 cells formed aggregates, embryoid bodies (*10, 11*). This is a necessary step in neuronal differentiation. During RA-treatment, morphological changes in some P19 cells which contained small vacuoles or displayed large translucent swellings of delicate and fragile appearance are observed. Intracellular proteinase activities during the progress of apoptosis were investigated. We measured six proteinase activities, prolyl endopeptidase, cathepsins B+L, trypsin-like activity, ICE-like activity, D-MCA hydrolytic activity, and ATP-dependent proteasome activity, on each day of RA-treatment. The ICE-like, Ac-YVAD-MCA-hydrolytic, activity was notably stimulated by RA-treatment and increased more than 6-fold by the fourth day as shown in Fig. 1. The ATP-dependent proteasome activity was also increased about twofold. On the other hand, there was no significant change in the other proteinase activities during retinoic acid treatment.

The D-MCA-hydrolytic activity did not change during RA-treatment. However, it is possible that the Ac-YVAD-MCA-hydrolytic activity of P19 cells is due to a combination of chymotrypsin-like activity and aminopeptidase activities. To eliminate this possibility, we studied the time-dependent release of fluorogenic substances. If substrate hydrolysis is the result of a two-step process, then AMC fluorescence should be released exponentially. Figure 2 clearly shows an almost linear increase in fluorescence, and inhibition by the cysteine-modifying reagent, *N*-ethylmaleimide (NEM). We conclude that the Ac-YVAD-MCA-hydrolytic activity in P19 cells is caused by a single enzyme with ICE-like activity.

The Sensitivity of the ICE-Like Activity in P19 Cells to Various Reagents—To elucidate the enzymatic properties of the ICE-like activity in RA-treated P19 cells, we investigated the effects of various proteinase inhibitors and reagents (Table I). The ICE-like activity was inhibited by

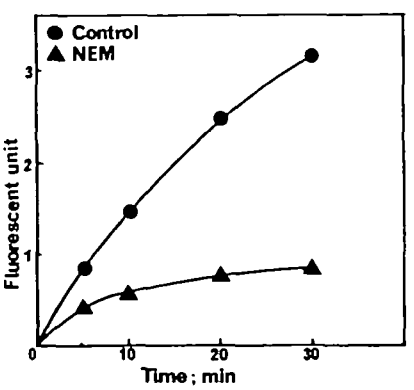

Fig. 2. **Time-dependent increase in fluorescence.** Ac-YVAD-MCA hydrolysis was monitored at the indicated time, with or without 1 mM *N*-ethylmaleimide as described in the legend to Fig. 1 without 2-mercaptoethanol.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

NEM and iodoacetic acid but not by E64-c. Both ICE-specific inhibitors, Ac-YVAD-H and Ac-YVKD-H, significantly inhibited the activity. The K_i values of these two inhibitors for the ICE-like activity were 29 and 14 μM, respectively. We generated a fusion protein of crmA, cowpox virus-derived ICE inhibitor protein, with maltose binding protein. The fusion protein did not inhibit the ICE-like activity (data not shown). The metalloproteinase inhibitor, EDTA, and the serine proteinase inhibitors, PMSF and DFP, had little effect on the ICE-like activity, and the aminopeptidase inhibitors amastatin and bestatin did not affect the activity at all.

Purification of the ICE-Like Activity in P19 Cells and Its Identification as a Multicatalytic Proteinase, the Proteasome—Next, we attempted to purify the ICE-like activity from RA-treated P19 cells. P19 cells treated for four days with RA were collected and lysed by sonication with phosphate-buffered saline, then centrifuged. The supernatant was applied to a DEAE-2SW column (Tosoh) equilibrated with 20 mM Tris-HCl, pH 7.5, and eluted with an NaCl gradient. The activity eluted as a single peak at 0.24 M NaCl, as shown in Fig. 3. This appears to correspond to the ICE-like activity that increased during RA-treatment. The active fractions were collected and applied to a Superdex 200 column (Pharmacia) equilibrated with 50 mM Tris-HCl containing 0.15 M NaCl. The activity eluted just after the void volume. The molecular mass was estimated at about 700 kDa (Fig. 4). No significant activity eluted at the position corresponding to a molecular mass of 30–50 kDa, where ICE-family enzymes are generally eluted. The active fractions were applied to a polylysine-agarose column equilibrated with 5 mM phosphate buffer and eluted with a step-wise NaCl gradient. The activity was eluted at 0.4 M NaCl. The K_m values with Ac-YVAD-MCA were the same for the crude extract and gel-filtered fraction: 0.73 and 0.79 mM, respectively.

The purified proteinase appeared almost homogeneous in native polyacrylamide gel electrophoresis, while the results of SDS-polyacrylamide gel electrophoresis demonstrated a multisubunit structure (Fig. 5). The unusually high molecular mass and the multisubunit structure are similar to those of the proteasome, the multicatalytic proteinase (*16-18*).

TABLE I. **Effects of various reagents on ICE-like and proteasome activities.** After 4 days of culture with RA, P19 cells were collected and lysed with PBS, and then centrifuged. The Ac-YVAD-MCA-hydrolytic activity of the lysate was assayed in the presence of the indicated reagents as described in "EXPERIMENTAL PROCEDURES." The values are relative activities compared with reactions carried out without additions.

Inhibitor	Conc.	Relative activity (%)
PMSF	1 mM	87
DFP	1 mM	76
EDTA	5 mM	90
NEM	1 mM	26
IAA	1 mM	56
ATP	1 mM	36
SDS	0.04%	52
SDS	0.01%	266
Amastatin	5 μM	94
Bestatin	5 μM	98
E64-c	20 μg/ml	90
TLCK	200 μM	66
Ac-YVAD-H	50 μM	36
Ac-YVKD-H	50 μM	21

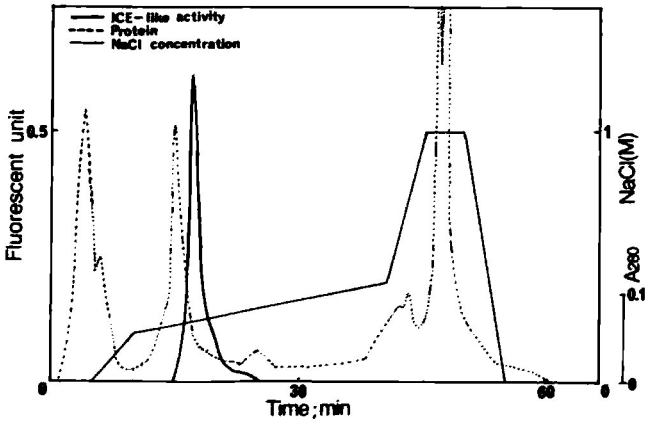

Fig. 3. **Elution of the ICE-like activity from a TSK DEAE-2SW column.** P19 cells treated with RA for 4 days were collected and lysed with PBS, and then centrifuged. The supernatant was applied to a DEAE-2SW column equilibrated with 20 mM Tris-HCl, pH 7.5. Proteins were eluted with an NaCl gradient.

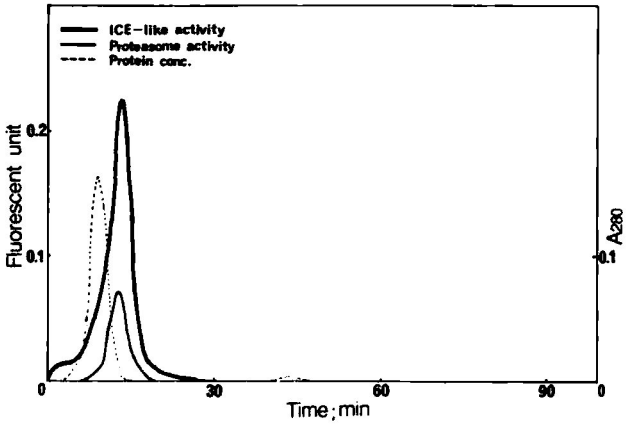

Fig. 4. **Elution profile of the ICE-like activity from a HiLoad 16/60 Superdex 200 column.** The active fractions were concentrated and then applied to a HiLoad 16/60 Superdex 200 column. The column was equilibrated with 50 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl and eluted at 1 ml/min.

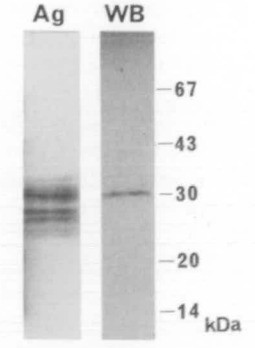

Fig. 5. **Identification of the protein showing ICE-like activity in RA-treated P19 cells as proteasome.** The purified enzyme fraction (0.4 μg of protein) was subjected to SDS polyacrylamide electrophoresis, and visualized by silver-staining or Western blotting with anti-proteasome polyclonal antiserum.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

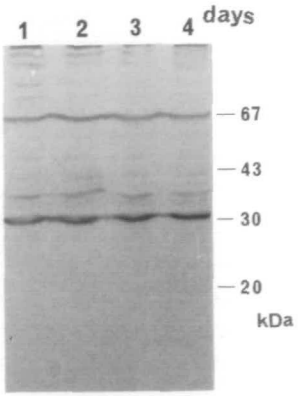

Fig. 6. **Western blot analysis of proteasome in RA-treated P19 cells.** After 1, 2, 3, and 4 days of culture with RA, P19 cells were collected and lysed with PBS. Twenty-five micrograms of protein was subjected to Western blot analysis with anti-proteasome polyclonal antiserum.

The sensitivity to inhibitors is also consistent with this (Table I). Furthermore, throughout the purification procedure, all fractions showing ICE-like activity had Suc-LLVY-MCA-hydrolytic activity (data not shown). We employed an immunological method to identify the ICE-like enzyme. The purified enzyme was detected by anti-proteasome antibody (Fig. 5). We further confirmed the enzymatic identification by an immunoabsorption study. The ICE-like activity was markedly decreased (27% of untreated activity) by treatment with anti-proteasome IgG, but not with control IgG. However, the ICE-like activity was not absorbed with anti-ICE IgG (data not shown). Therefore, we conclude that the ICE-like activity that increases during RA-treatment is identical to the proteasome.

To see whether the increase in the ICE-like activity is due to enhanced expression or to activation of the proteasome, Western blot analysis with anti-proteasome antiserum was employed. The results (Fig. 6) demonstrate that there is no significant change in the amount of the proteasome during RA-treatment. We found no difference during RA-treatment by Northern blot analysis using the rat proteasome L subunit as a probe (data not shown). Therefore, the increase in the ICE-like activity during RA-treatment seems to reflect stimulation of the activity.

Cleavage of an ICE Substrate by the Proteasome from Mouse Liver—As described above, the ICE-like activity that increases during RA-treatment in P19 cells is identical to the proteasome. To clarify whether proteasome purified from other tissue can cleave substrates of ICE-family enzymes, we purified the proteasome from mouse liver. As shown in Table II, the mouse liver proteasome cleaved Ac-YVAD-MCA, but not D-MCA, a substrate of aminopeptidase A. Further, the relative activity of the purified liver proteasome for Ac-YVAD-MCA *versus* that for Suc-LLVY-MCA at pH 7.5 was not different from that of the enzyme in P19 cells; 4.0 and 3.4, respectively.

TABLE II. **Substrate specificities of proteasome from mouse liver and P19 cells.** The activity of the purified proteasome was determined as described in the legend to Fig. 1 without any supplement. The values are relative activities, with Suc-LLVY-MCA hydrolysis at pH 7.5 being taken as 100.

Substrate	pH	Relative activity (%)	
		Mouse liver	P19 cells
Suc-LLVY-MCA	7.5	100	100
Suc-LLVY-MCA	9.0	73.1	66.7
Ac-YVAD-MCA	7.5	4.0	3.4
D-MCA	7.5	<1	<1

DISCUSSION

P19 embryonal carcinoma cells can differentiate multipotentially into many types of cells, including myogenic, neuronal, and glial cells, upon treatment with RA. Swollen and shrunken P19 cells were observed after RA-treatment. Sionov and Gallily demonstrated that some RA-treated P19 cells were sensitive to killing by nonactivated macrophages (*19*). This may be related to RA-induced apoptosis.

Accompanying the morphological change, the ICE-like activity was stimulated more than 6-fold. Although the fusion protein of crmA did not inhibit the ICE-like activity, we do not know whether the fusion protein retains inhibitory activity or not. Therefore, these results are not in conflict with the reported properties of ICE (*14*). However, the activity was considered to be due to the proteasome, based on the following results. First, there was no significant peak except for that of the proteasome on either ion-exchange or gel-filtration chromatography (Fig. 4). When the crude lysate was applied to a gel-filtration column, the Ac-YVAD-MCA-hydrolytic activity was not observed at the position corresponding to a molecular mass of 30-50 kDa (*1, 14*), even in the first chromatography (data not shown). Therefore, it is unlikely that ICE was coeluted with the proteasome. In fact, the ICE-like activity was not absorbed with anti-ICE antibody. Second, the substrate Ac-YVAD-MCA appeared to be hydrolyzed in a one-step reaction (Fig. 2). Third, the purified ICE-like activity presented similar patterns on electrophoresis to the proteasome in terms of protein staining and Western blot (Fig. 5). Furthermore, the ICE-like activity was absorbed with anti-proteasome IgG, but not with anti-ICE IgG. Thus, we conclude that the ICE-like activity that increased during RA-treatment is due to the proteasome, and the proteasome is a major ICE-like proteinase, at least in P19 cells. Indeed, the purified mouse liver proteasome had an ICE-like activity (Table II). Kinetic constants of the ICE-like proteasome activity were quite different from those of ICE. The K_m values of the ICE-like activity from P19 cells and purified human ICE for Ac-YVAD-MCA were 0.7 mM and 14 μM, and the K_i values for Ac-YVAD-H were 30 μM and 0.8 nM, respectively [this study and (*14*)]. Therefore, Ac-YVAD-MCA seems to be a poor substrate of the proteasome. Because the proteasome is an abundant proteinase, it acts as a major Ac-YVAD-MCA hydrolytic activity in P19 cells in spite of the lower specificity.

The proteasome is a ubiquitous proteinase found in many tissues and cells and participates in important biological processes, including antigen presentation and the degradation of ubiquitinated proteins (*20, 21*). There are no previous reports of an ICE-like activity of the proteasome. However, the proteasome is a multicatalytic proteinase with many proteolytic activities, including trypsin-like and chymotrypsin-like activities. In addition, granzyme B also

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

has an ICE-like activity (*22*). Therefore, the present finding is not particularly surprising.

Since there was no significant change in the amount of the proteasome as determined by Western blot analysis, the proteasome seems to be activated during RA-treatment. The extents of stimulation of the ICE-like activity and the proteasome activity during RA-treatment were different (Fig. 1). It is possible that the Ac-YVAD-MCA-hydrolytic activity of the proteasome is more important than the Suc-LLVY-MCA-hydrolytic activity for the induced apoptosis. The proteasome from mouse liver cleaved Ac-YVAD-MCA in the same ratio as that from P19 cells (Table II). Apoptotic cell death is obviously observed in liver, so the ICE-like activity of the proteasome may also be activated in liver. It was demonstrated that interferon gamma induced subunit replacements of X and Y by LMP7 and LMP2, which alter the proteolytic specificity of proteasomes (*23*). We could not find any difference in subunits between the proteasomes from the apoptosis-induced and uninduced cells. However, it is possible that some subunits are replaced and the substrate specificity was changed during RA-treatment. Further studies are needed to clarify the molecular mechanism of proteasome activation.

It was demonstrated that the ubiquitin-mediated protein degradation system was required for programmed cell death in insect skeletal muscles (*24*). The proteasome is a main proteinase in the ubiquitin pathway (*21*). Therefore, the proteasome may play a crucial role in apoptosis. We demonstrate here that the activation of the proteasome occurs during RA-treatment.

Several proteins, including poly ADP-ribose polymerase (PARP), p70 U1 small nuclear ribonucleoprotein (U1snRNP), and lamin, have been reported to be degraded during apoptosis (*25-28*). In particular, PARP is cleaved in a restricted manner at the P1 Asp position, and this cleavage is inhibited by the ICE-inhibitor YVAD-CMK (*28*). Therefore, PARP (and probably other proteins) is considered to be a target protein whose digestion marks a critical point for apoptosis. As demonstrated in this study, the proteasome can also cleave the substrate of ICE. It is possible that the proteasome digests substrates of the ICE-like enzyme, as well as PARP, U1snRNP, and so on. The activation mechanism of the proteasome during RA-treatment should be investigated.

We are grateful to Drs. Yukiko K. Hayashi, Asako Hase, and Yuichi Tsuchiya, National Institute of Neuroscience, for discussions and advice.

REFERENCES

1. Yuan, J., Shaham, S., Ledoux, S., Ellis, H.M., and Horvitz, H.R. (1993) The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell* **75**, 641-652
2. Miura, M., Zhu, H., Rotello, R., Hartwieg, E.A., and Yuan, J. (1993) Induction of apoptosis in fibroblasts by IL-1β-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell* **75**, 653-660
3. Enari, M., Hug, H., and Nagata, S. (1995) Involvement of an ICE-like protease in Fas-mediated apoptosis. *Nature* **375**, 78-81
4. Los, M., Vande Crean, M., Penning, L.C., Schenk, H., Westendrop, M., Baeuerle, P.A., Droge, W., Krammer, P.H., Flers, W., and Schulze-Osthoff, K. (1995) Requirement of an ICE/CED-3 protease for Fas/APO-1-mediated apoptosis. *Nature* **375**, 81-83
5. Kumar, S. (1995) Inhibition of apoptosis by the expression of antisense *Nedd-2*. *FEBS Lett.* **368**, 69-72
6. Osborne, B.A. and Schwartz, L.M. (1994) Essential genes that regulate apoptosis. *Trends Cell Biol.* **4**, 394-399
7. Steller, H. (1995) Mechanisms and genes of cellular suicide. *Science* **267**, 1445-1462
8. Martin, S.J. and Green, D.R. (1995) Protease activation during apoptosis: Death by a thousand cuts? *Cell* **82**, 349-352
9. Ayala, J.M., Yamin, T.-T., Egger, L.A., Chin, J., Kostura, M.J., and Miller, D.K. (1994) IL-1β-converting enzyme is present in monocytic cells as an inactive 45-kDa precursor. *J. Immunol.* **153**, 2592-2599
10. McBurney, M.W., Jones-Villeneuve, E.M., Edwards, M.K., and Anderson, P.J. (1982) Control of muscle and neuronal differentiation in a cultured embryonal carcinoma cell line. *Nature* **299**, 165-167
11. Campione-Piccardo, J., Sun, J.J., Craig, J., and McBurney, M.W. (1985) Cell-cell interaction can influence drug-induced differentiation of murine embryonal carcinoma cells. *Dev. Biol.* **109**, 25-31
12. Tsukahara, T., Kunika, N., Momoi, T., and Arahata, K. (1995) Regulation of alternative splicing in the amyloid precursor protein (APP) mRNA during neuronal and glial differentiation of P19 embryonal carcinoma cells. *Brain Res.* **679**, 178-183
13. Tsukahara, T., Ishiura, S., and Sugita, H. (1990) Changes in proteinase activities during the differentiation of murine erythroleukemia cells. *Exp. Cell Res.* **188**, 111-116
14. Thornberry, N.A. (1994) Interleukin-1β converting enzyme in *Methods in Enzymology* (Barrett, A.J., ed.) Vol. 244, pp. 615-631, Academic Press, New York
15. Tsukahara, T., Ishiura, S., and Sugita, H. (1990) Regulation of prolyl endopeptidase activity by the intracellular redox state. *J. Biol. Chem.* **265**, 21448-21453
16. Tsukahara, T., Ishiura, S., and Sugita, H. (1988) An ATP-dependent protease and ingensin, the multicatalytic proteinase, in K562 cells. *Eur. J. Biochem.* **177**, 261-266
17. Tanaka, K., Ii, K., Ichihara, A., Waxman, L., and Goldberg, A.L. (1986) A high molecular weight protease in the cytosol of rat liver. *J. Biol. Chem.* **261**, 15197-15203
18. Yamamoto, T., Nojima, M., Ishiura, S., and Sugita, H. (1986) Purification of the two forms of the high-molecular-weight neutral proteinase ingensin from rat liver. *Biochim. Biophys. Acta* **882**, 297-304
19. Sionov, R.V. and Gallily, R. (1992) The unique killing of embryo-derived teratocarcinoma cells by nonactivated murine macrophages is not due to a lack of H-2 antigen expression. *Cell. Immunol.* **142**, 416-425
20. Peters, J.M. (1994) Proteasomes: Protein degradation machines of the cell. *Trends Biochem. Sci.* **19**, 377-382
21. Hochstrasser, M. (1995) Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. *Curr. Opin. Cell Biol.* **7**, 215-223
22. Irmler, M., Hertig, S., MacDonald, H.R., Sadoul, R., Becherer, J.D., Proudfoot, A., Solari, R., and Tschopp, J. (1995) Granzyme A is an interleukin 1β-converting enzyme. *J. Exp. Med.* **181**, 1917-1922
23. Akiyama, K., Yokota, K., Kagawa, S., Shimbara, N., Tamura, T., Akioka, H., Nothwang, H.G., Noda, C., Tanaka, K., and Ichihara, A. (1994) cDNA cloning and interferon gamma down-regulation of proteasomal subunits X and Y. *Science* **265**, 1231-1234
24. Haas, A.L., Baboshina, O., Williams, B., and Schwartz, L.M. (1995) Coordinated induction of the ubiquitin conjugation pathway accompanies the developmentally programmed death of insect skeletal muscle. *J. Biol. Chem.* **270**, 9407-9412
25. Kaufmann, S.H., Desnoyers, S., Ottaviano, Y., Davidson, N.E., and Poirier, G.G. (1993) Specific proteolytic cleavage of poly(ADP-ribose)polymerase: An early marker of chemotherapy-induced apoptosis. *Cancer Res.* **53**, 3976-3985
26. Lazebnik, Y.A., Kaufmann, S.H., Desnoyers, S., Poirier, G.G., and Earnshaw, W.C. (1994) Cleavage of poly(ADP-ribose)polymerase by a proteinase with properties like ICE. *Nature* **371**,

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

346-347
27. Casciola-Rosen, L.A., Miller, D.K., Anhalt, G.J., and Rosen, A. (1994) Specific cleavage of the 70-kDa protein component of the U1 small nuclear ribonucleoprotein is a characteristic biochemical feature of apoptotic cell death. *J. Biol. Chem.* **269**, 30757-30760
28. Lazebnik, Y.A., Takahashi, A., Moir, R.D., Goldman, R.D., Poirier, G.G., Kaufmann, S.H., and Earnshaw, W.C. (1995) Studies of the lamin proteinase reveal multiple parallel biochemical pathways during apoptotic execution. *Proc. Natl. Acad. Sci. USA* **92**, 9042-9046

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012