The endothelial monocyte-activating polypeptide II (EMAP II) is a substrate for caspase-7

Heike A. Behrensdorf[a], Marc van de Craen[b], Ulrike E. Knies[a,1], Peter Vandenabeele[b], Matthias Clauss[a,*]

[a] *Department of Molecular Cell Biology, Max-Planck-Institute for Physiological and Clinical Research, Parkstr. 1, 61231 Bad Nauheim, Germany*
[b] *Department of Molecular Biology, Flanders Interuniversity Institute for Biotechnology and University of Ghent, Ledeganckstraat 35, B-9000 Ghent, Belgium*

Received 5 November 1999; received in revised form 16 December 1999

Edited by Marc Van Montagu

Abstract Endothelial monocyte-activating polypeptide II (EMAP II) is a proinflammatory cytokine and a chemoattractant for leukocytes. The mature cytokine is formed in apoptotic cells by cleavage of the precursor proEMAP II. Here we show that caspase-7 is capable of cleaving proEMAP II in vitro. A proEMAP II mutant, in which the ASTD cleavage site was changed to the sequence ASTA, was not processed by caspase-7. The caspase-7-mediated generation and release of mature EMAP II may provide a mechanism for leukocyte recruitment to sites of programmed cell death, and thus may link apoptosis to inflammation.

Key words: Endothelial monocyte-activating polypeptide II; Caspase-7; Apoptosis; Leukocyte recruitment

1. Introduction

Endothelial monocyte-activating polypeptide II (EMAP II) was originally isolated from the supernatant of cultured methylcholanthrene A (Meth A)-transformed fibrosarcoma cells based on its endothelial cell-activating properties [1]. It was shown to be a proinflammatory mediator with the ability to stimulate the chemotactic migration of mononuclear phagocytes and polymorphonuclear granulocytes in vitro, and to induce the expression of tissue factor (the initiator of coagulation) on endothelial cells. In vivo, injection of EMAP II into the footpad of mice leads to an inflammatory swelling response, which is characterized by an infiltration of polymorphonuclear granulocytes [1].

The cloning of the EMAP II-cDNA revealed that the mature 23 kDa form of EMAP II, for which the biological activities have been described, is synthesized as a precursor protein lacking a conventional secretion signal peptide [2]. This precursor (proEMAP II) was recently found to be identical to the p43 subunit of the mammalian tRNA multi-synthetase complex [3]. It is assumed that proEMAP II/p43 functions as a cofactor for the tRNA synthetases in the complex by stabilizing the interaction between these enzymes and the corresponding tRNAs [3,4].

We have recently demonstrated [5] that proEMAP II/p43 is cleaved to the mature, chemotactic EMAP II and released only by apoptotic cells, but not by healthy or necrotic cells. In this respect, proEMAP II resembles the substrates which are hydrolyzed by members of the caspase family of proteases [6]. Caspases are activated in apoptotic cells and subsequently cleave diverse target proteins (like poly(ADP-ribose) polymerase (PARP) or protein kinase Cδ) specifically after aspartate residues. The caspase-mediated cleavage of regulatory, metabolic and structural proteins contributes to the breakdown of cellular functions which finally results in cell death.

The exact position of the cleavage site within the proEMAP II sequence (after the amino acid motif ASTD at position 144) was determined by N-terminal sequencing of the mature murine protein isolated from cellular supernatants [2]. We have shown recently [5] that the cleavage of proEMAP II in cultured cells can be inhibited by the addition of benzyloxycarbonyl-ASTD-fluoromethylketone (Z-ASTD-FMK), a tetrapeptide-based inhibitor mimicking the cleavage site of proEMAP II. The caspase-3 inhibitor benzyloxycarbonyl-DEVD-chloromethylketone (Z-DEVD-CMK) also inhibited the cleavage. In this study, we demonstrate that in vitro translated proEMAP II is cleaved by recombinant caspase-7 and, to a lesser extent, by caspase-3, but not by other known caspases. A proEMAP II mutant in which the ASTD cleavage site was changed to the sequence ASTA was not cleaved by caspase-7. Based on these results, we propose that caspase-7 is the EMAP II-cleaving protease in apoptotic cells.

2. Materials and methods

All reagents were purchased from Sigma (Deisenhofen, Germany) unless otherwise indicated. Z-ASTD-FMK, Z-DEVD-CMK, acetyl-ASTD-aminomethylcoumarin (ASTD-AMC) and acetyl-DEVD-aminomethylcoumarin (DEVD-AMC) were obtained from Bachem (Heidelberg, Germany).

2.1. Plasmids

For expression of proEMAP II, the full-length cDNA of mouse proEMAP II [2] cloned in pBluescript II SK$^+$ (Stratagene, La Jolla, CA, USA) was used (insert size: 960 bp). From this, a plasmid for the expression of proEMAP II with a mutated cleavage site (ASTA-proEMAP II) was generated with the 'QuikChange site directed mutagenesis kit' (Stratagene) using the mutagenic sense primer 5′-GCAGTCGGCAGCAGCAAGTACTGCCTCCAAGCCTATCG-3′.

*Corresponding author. Fax: (49)-6032-72259.
E-mail: MClauss@Kerckhoff.mpg.de

[1] Present address: Department of Biochemistry of the Cell Nucleus, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, UK.

Abbreviations: EMAP II, endothelial monocyte-activating polypeptide II; Meth A, methylcholanthrene A; PARP, poly(ADP-ribose) polymerase

PII: S0014-5793(99)01777-9

2.2. In vitro translation of proEMAP II

Coupled transcription/translation reactions were performed using the TNT coupled rabbit reticulocyte lysate system (Promega Biotech, Madison, WI, USA) according to the manufacturers instructions. Murine wild-type proEMAP II and ASTA-proEMAP II were expressed using the plasmids pBluescript II SK^+/proEMAP II or pBluescript II SK^+/ASTA-proEMAP II as templates for T7 RNA polymerase.

2.3. In vitro cleavage assays

Murine caspases were expressed in *Escherichia coli* and purified to homogeneity [7]. During bacterial expression, the caspases are autoprocessed into p20 and p10 subunits. The p20 subunit was used to determine the amount of active enzyme as described by Van de Craen et al. [7]. The caspases were incubated with 2 μl in vitro translated, ^{35}S-methionine labeled proEMAP II for 1.5 h at 37°C in a total volume of 25 μl in CFS buffer [8]. The samples were then analyzed by SDS/PAGE followed by autoradiography. Cleavage of PARP was determined by incubating 100 ng of purified bovine PARP (Biomol, Hamburg, Germany) with recombinant caspases in a total volume of 25 μl in CFS buffer for 1.5 h at 37°C. The samples were analyzed by Western blot.

For fluorometric cleavage assays, recombinant caspases were incubated with 50 μM of the substrates ASTD-AMC or DEVD-AMC in a total volume of 25 μl in CFS buffer for 1 h at 37°C. Then the fluorescence of the samples was measured (excitation wavelength: 360 nm, emission wavelength: 460 nm).

2.4. SDS polyacrylamide gel electrophoresis (SDS/PAGE) and autoradiography

SDS/PAGE was performed as described recently [5]. For the detection of EMAP II, the denatured samples were separated on 15% gels. For the detection of PARP, 8% gels were used. After electrophoresis, the polyacrylamide gels were fixed in 30% methanol/7% acetic acid/0.5% methionine, then incubated in Amplify solution (Amersham, Braunschweig, Germany) for 20 min and dried. Finally the gels were exposed to X-OMAT AR film (Kodak) at −80°C. Alternatively, the dried gel was analyzed using a phosphorimager (Molecular Dynamics, Sunnyvale, CA, USA).

2.5. Western blot analysis

Following the transfer of proteins from SDS/PAGE gels onto nitrocellulose (Schleicher and Schüll, Dassel, Germany) and blocking with PBS/5% non-fat dry milk, the membranes were incubated with the monoclonal PARP-specific antibody C2.10 (Enzyme Systems Products, Livermore, CA, USA; diluted 1:10 000) for 2 h at room temperature. The membranes were then washed and incubated with peroxidase-coupled goat anti-mouse IgG (Jackson, West Grove, USA; diluted 1:3500) for 1 h. After washing, the membranes were developed using the ECL-kit (Amersham).

3. Results

3.1. Cleavage of proEMAP II by caspase-7 and caspase-3

In order to investigate whether proEMAP II serves as a substrate for any of the known caspases, we employed a cleavage assay in which in vitro translated proEMAP II was incubated with recombinant caspases. In vitro translation of the proEMAP II cDNA generated two translation products (Fig. 1, control). The apparent molecular mass of the upper band (43 kDa) corresponds to the size of the natural proEMAP II, while the lower band might be due to the usage of an internal ribosome entry site. We found that caspase-1, -2, -6, -8 and -11 at concentrations of 500 nM (referring to the p20 subunit of the active caspases) were not able to process in vitro translated proEMAP II (Fig. 1). In contrast, complete cleavage of the substrate was seen within 90 min with 500 nM caspase-7, and partial processing was found after incubation with caspase-3 (Fig. 1). The apparent molecular mass of the cleavage product (23 kDa) corresponds to the size of mature EMAP II as reported by Kao et al. [1]. After longer exposure of the film, an additional faint band of 20 kDa could be observed (data not shown). This band corresponds to the N-terminal cleavage fragment of proEMAP II, which contains only two methionine residues.

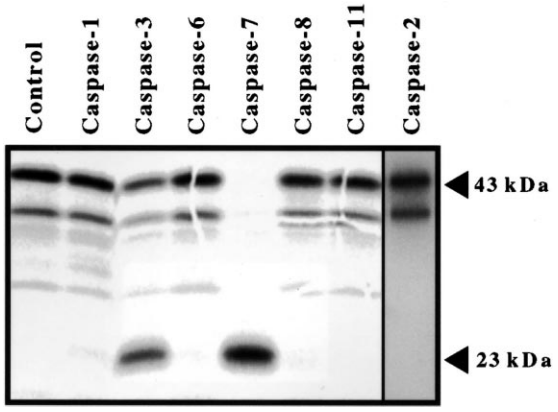

Fig. 1. Cleavage of proEMAP II by recombinant caspases in vitro. In vitro translated proEMAP II labeled with ^{35}S-methionine was incubated with recombinant caspases (at concentrations of 500 nM) for 90 min at 37°C. The samples were then subjected to SDS/PAGE and analyzed using a phosphorimager or, in the case of caspase-2, by autoradiography. Indicated are the positions of proEMAP II (upper arrow) and the cleaved form (lower arrow).

3.2. ProEMAP II is cleaved more efficiently by caspase-7 than by caspase-3

Having demonstrated that caspase-3 and -7 are able to cleave proEMAP II, we wanted to compare the affinities of both enzymes for this substrate. Therefore, we incubated in vitro translated proEMAP II with increasing concentrations of caspase-3 (Fig. 2A) or caspase-7 (Fig. 2B). Our results demonstrate that proEMAP II is cleaved more efficiently by caspase-7 than by caspase-3. About 50% of the substrate was processed when caspase-7 concentrations between 30 and 60 nM were used (Fig. 2B). In contrast, approximately 10-fold higher concentrations of caspase-3 were needed to obtain comparable amounts of the cleaved product (Fig. 2A; Fig. 1). We further tested the cleavage of PARP by increasing concentrations of caspase-3 and -7. As PARP is reported to be cleaved by both enzymes with almost identical affinities [9], these proteolytic reactions served as a control for the activities of the two recombinant caspases. Western blot analysis demonstrated that caspase-3 (Fig. 2C) and caspase-7 (Fig. 2D) were equally active with regard to the cleavage of PARP, indicating that the differential cleavage of proEMAP II is not due to different activities of the recombinant caspases.

3.3. Cleavage of proEMAP II by caspase-7 is time-dependent and can be inhibited by Z-ASTD-FMK

To obtain further information about the time course of the cleavage, in vitro translated proEMAP II was incubated with 125 nM caspase-7 for differing lengths of time. Already after 5 min of co-incubation, the mature 23 kDa EMAP II could be detected (Fig. 3A). After 90 min, almost complete cleavage of the substrate was obtained. We then examined whether the proteolytic reaction can be inhibited using the compounds Z-ASTD-FMK (a competitive inhibitor mimicking the EMAP II cleavage site) and Z-DEVD-CMK (an inhibitor for caspase-3 and -7), which have been shown to inhibit the

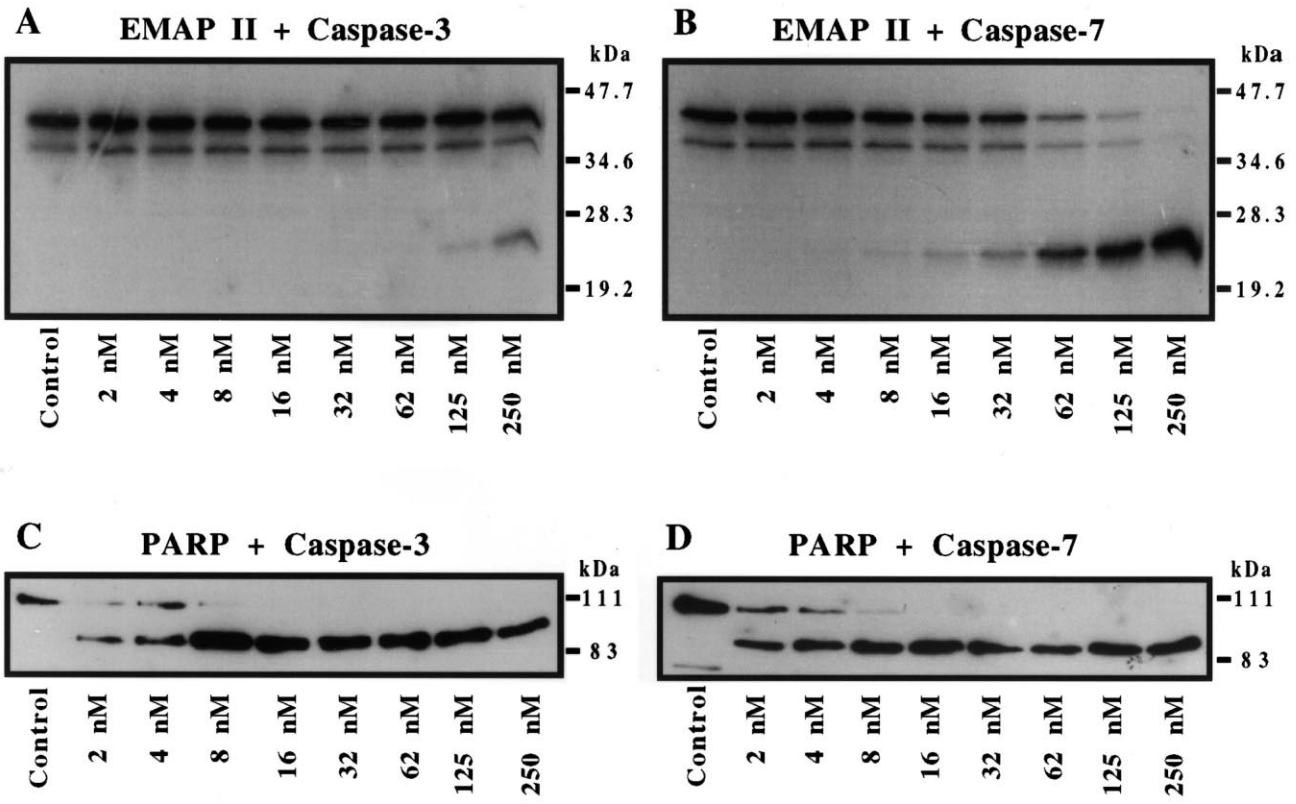

Fig. 2. Comparison of proEMAP II cleavage by caspase-7 and caspase-3. In vitro translated proEMAP II was incubated with increasing concentrations of caspase-3 (A) or caspase-7 (B) for 1.5 h at 37°C. Indicated are the concentrations of the p20 caspase subunit. Control incubations were carried out in the absence of caspases. The samples were analyzed by SDS/PAGE followed by autoradiography. Cleavage of PARP (4 μg/ml) by increasing concentrations of caspase-3 (C) and caspase-7 (D) was determined by Western blot analysis.

cleavage of the EMAP II precursor in cultured apoptotic cells [5]. Indeed, the processing of in vitro translated proEMAP II by caspase-7 was completely inhibited by 10 μM Z-ASTD-FMK and also by 10 μM Z-DEVD-CMK (Fig. 3B).

3.4. ASTA-proEMAP II is not processed by caspase-7

The ASTD cleavage site of proEMAP II does not display a close similarity to described target sequences for caspase-7, such as the motif DEVD [10]. Therefore, we investigated whether the cleavage of proEMAP II by caspase-7 occurs at the ASTD motif or at an alternative aspartate residue. We generated a site-specific proEMAP II mutant, in which the aspartate of the ASTD sequence was replaced by an alanine. After in vitro translation, the resulting ASTA-proEMAP II was incubated with recombinant caspases. Indeed, this mutant protein was neither processed by caspase-7 nor by caspase-3 (Fig. 4A), demonstrating that the ASTD site is essential for the substrate recognition. To confirm that caspase-7 and caspase-3 are able to recognize the ASTD motif, we performed fluorometric cleavage assays using the tetrapeptide-based substrate ASTD-AMC (Fig. 4B). This substrate was cleaved by both caspases with almost identical affinities. However, DEVD-AMC (which is the prototype substrate for caspase-7) was more efficiently processed by caspase-7 than ASTD-AMC (Fig. 4B).

4. Discussion

Several lines of evidence suggest that a caspase might be responsible for the processing of proEMAP II: Firstly, the cleavage takes place after an aspartate residue. Secondly, the cleaved, mature EMAP II is only generated by apoptotic, but not by healthy or necrotic cells [5]. It is known that most caspases are activated during apoptotic, but not necrotic cell death [11,12]. Finally, cleavage of proEMAP II in apoptotic cells can be inhibited by Z-ASTD-FMK and by the caspase-3 inhibitor Z-DEVD-CMK [5], suggesting that the EMAP II-cleaving enzyme shows structural similarities to caspases.

In an in vitro assay, we could show that only caspase-7 and (to a lesser degree) caspase-3, but not other caspases, are able to cleave proEMAP II to a fragment corresponding to the size of mature EMAP II [1,5]. Surprisingly, caspase-2, which is reported to have a similar substrate specificity as caspase-3 and -7 [10], did not cleave proEMAP II. Several reports claim that caspase-3 and -7 display almost indistinguishable cleavage activities and substrate specificities [9,10]. In contrast, we found clear differences between both enzymes with regard to their ability to cleave proEMAP II (although PARP was processed by both caspases with very similar affinities). In support of this finding is a report showing that kinectin (a protein involved in microtubule-based vesicle transport) is also processed by caspase-7 and not by caspase-3 [13]. In contrast to the proEMAP II protein, a small substrate comprising only the ASTD motif (ASTD-AMC) was cleaved by caspase-3 and -7 with comparable affinities. This indicates that the different cleavage activities of both enzymes towards proEMAP II are not based on differences in cleavage site recognition between the two caspases, but are rather due to steric factors.

According to a study in which the substrate specificities of several caspases were defined [10], we did not expect the ASTD site of proEMAP II to be recognized by caspase-3 or caspase-7, as both enzymes reportedly display a strong preference for an aspartate residue at the P4 position of the cleavage site with DEVD being the optimal recognition motif. As we have shown that proEMAP II is efficiently processed by caspase-7 in vitro, it was important to identify the site at which this cleavage occurs. The sequence of proEMAP II contains several potential recognition sites for caspases. Of special interest was an EEVD motif (at amino acid position 178 of proEMAP II), which closely resembles the optimal target sequence DEVD. However, a proEMAP II mutant in which the ASTD motif (at position 144) was changed to ASTA could not be cleaved by caspase-7 or caspase-3, demonstrating that the ASTD site is essential for proEMAP II recognition by both caspases. Furthermore, using the substrate ASTD-AMC, we could confirm that both enzymes are in principle capable of recognizing the ASTD motif. This finding is further supported by a report [14] showing that caspase-3 can convert pro-interleukin-16 into mature interleukin-16 by cleaving at a very similar site (SSTD).

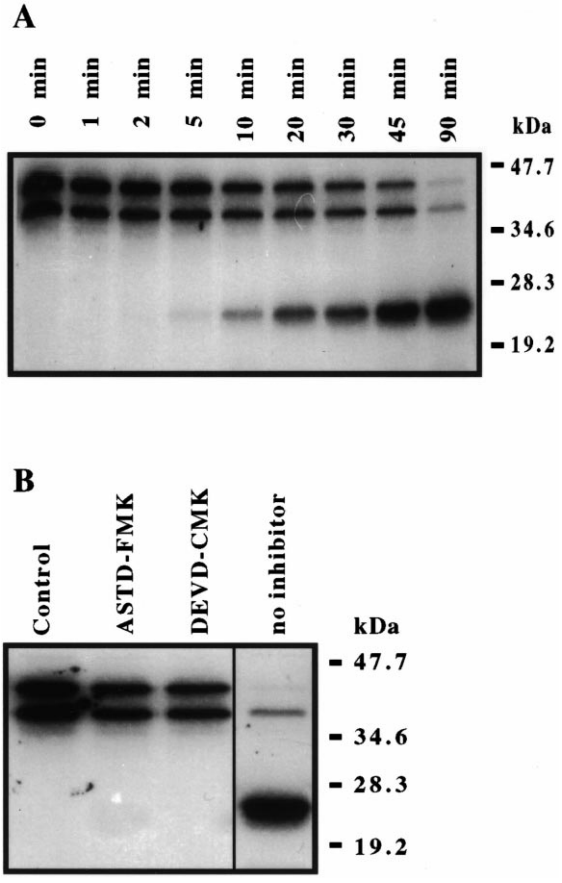

Fig. 3. A: Time course of proEMAP II cleavage by caspase-7. ^{35}S-labeled proEMAP II was incubated with 125 nM recombinant caspase-7 at 37°C for the times indicated. The samples were then analyzed by SDS/PAGE and autoradiography. B: Inhibition of proEMAP II cleavage in vitro by Z-ASTD-FMK. In vitro translated proEMAP II was incubated with 125 nM recombinant caspase-7 for 1.5 h at 37°C in the absence or presence of 10 μM Z-ASTD-FMK or Z-DEVD-CMK. The samples were then subjected to SDS/PAGE followed by autoradiography. The control lane shows in vitro translated proEMAP II incubated in the absence of caspases.

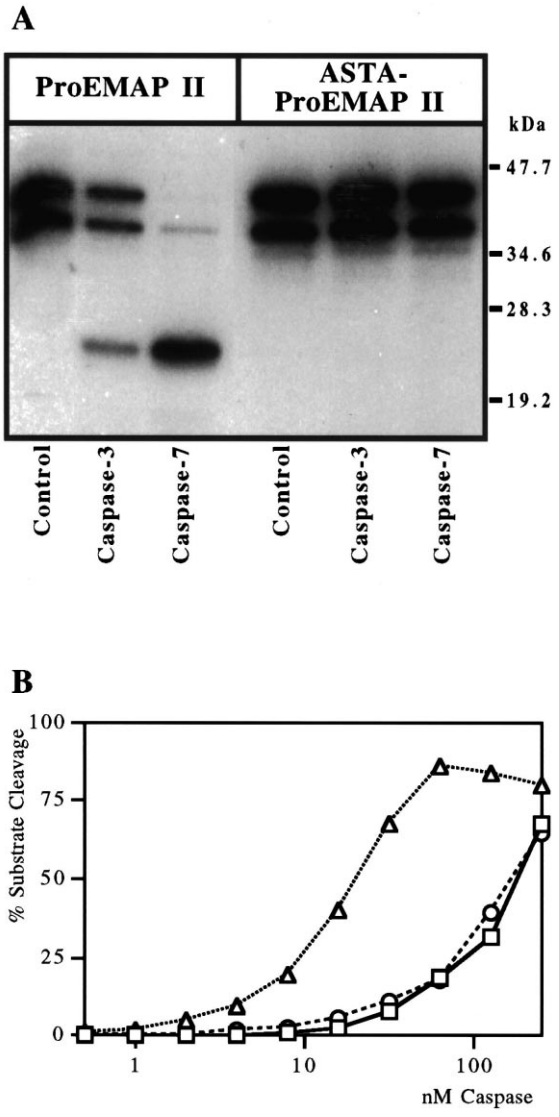

Fig. 4. A: ASTA-proEMAP II is not processed by caspase-7. In vitro translated proEMAP II or ASTA-proEMAP II was incubated without caspases (control) or with recombinant caspase-7 or caspase-3 (at concentrations of 125 nM p20) for 1.5 h at 37°C. The samples were then analyzed by SDS/PAGE and autoradiography. B: Cleavage of ASTD-AMC by caspase-7 and caspase-3. 50 μM ASTD-AMC or DEVD-AMC was incubated with the indicated concentrations of caspase-7 or caspase-3 for 1 h at 37°C. Fluorescence was measured, and the concentration of the released AMC group was determined using an AMC standard curve. □: cleavage of ASTD-AMC by caspase-7; ○: cleavage of ASTD-AMC by caspase-3; △: DEVD-AMC cleavage by caspase-7.

In conclusion, caspase-7 and (to a lesser extent) caspase-3 process proEMAP II at the ASTD motif, and thus generate a cleavage product with an identical N-terminus as the originally described mature EMAP II [2]. The inability of caspase-7 to cleave the EEVD motif might be due to the three-dimensional structure of proEMAP II. Our suggestion that caspase-7 is the EMAP II-cleaving enzyme under physiological conditions is further supported by the observation that both the processing of proEMAP II by caspase-7 in vitro and the generation of mature EMAP II in apoptotic cells

can be inhibited by the same compounds (Z-ASTD-FMK or Z-DEVD-CMK).

We have demonstrated recently [5] that proEMAP II is processed at a late time point during apoptotic cell death, while the activation of caspase-7 is known to be an early event in apoptotic cells [15]. In accordance with these findings, we observed that the appearance of active caspase-7 in lysates of apoptotic Meth A fibrosarcoma cells precedes the appearance of mature EMAP II by 4–6 h (data not shown). One possible explanation for this discrepancy is that in healthy cells, p43/proEMAP II is enclosed in the tRNA multi-synthetase complex and could thereby be protected from proteolysis. During progression of the apoptosis program, the complex may disassemble or change its conformation and proEMAP II may then become accessible for caspase-7. Secondly, following its activation, caspase-7 has been reported to be located in microsomes and mitochondria [16,17], where it cannot interact with the cytosolic proEMAP II. Only later during apoptosis, when the membranes of these organelles become permeable, the active enzyme might come into contact with its substrate proEMAP II. In favor of this hypothesis is the finding that the protein PARP, although cleaved equally by both caspase-3 and -7 in vitro, is predominantly cleaved by caspase-3 in vivo [18]. Therefore, it is likely that during early apoptosis, active caspase-7 is enclosed in subcellular organelles and cannot interact with cytoplasmic or nuclear substrates.

The finding that mature EMAP II is generated late during apoptosis has several implications for the potential role of EMAP II. In most in vivo settings, apoptotic cells are phagocytosed at an early stage, before the plasma membrane ruptures [19]. This rapid clearance avoids the leakage of proinflammatory factors from the dying cells. In such cases, where only a few cells undergo apoptosis, the dying cells are likely to be cleared before proEMAP II is processed. However, a recent report [20] has demonstrated that severe apoptosis can induce inflammatory events. At sites of massive apoptosis, the neighboring cells may fail to phagocytose all of the dying cells. Subsequently, some cells reach a late stage of apoptosis. We propose that the generation of mature EMAP II in vivo is particularly important at sites where extensive cell death occurs. The late apoptotic cells found in such areas could release mature EMAP II, which then attracts leukocytes. The recruited mononuclear phagocytes may contribute to the clearance of dying cells, while the polymorphonuclear granulocytes could induce an inflammatory response. Therefore, we suggest that EMAP II functions as a link between severe apoptosis and inflammation.

In conclusion, the processing of EMAP II by caspase-7 can contribute to the events of apoptosis by two distinct mechanisms. Firstly, the generated mature EMAP II can recruit leukocytes to the site of apoptotic cell death. Secondly, after cleavage, proEMAP II/p43 should no longer function as a tRNA synthetase cofactor, and this may contribute to the breakdown of cellular protein synthesis.

Acknowledgements: We are grateful to Dr. Simon Bamforth for critically reading the manuscript. This work was supported by the DFG (Sonderforschungsbereich 547 'Kardiopulmonales Gefäßsystem'). P.V. is research leader with the FWO (Fonds voor Wetenschappelijk Onderzoek Vlaanderen).

References

[1] Kao, J., Ryan, J., Brett, G., Chen, J., Shen, H., Fan, Y.G., Godman, G., Familletti, P.C., Wang, F., Pan, Y.C., Stern, D. and Clauss, M. (1992) J. Biol. Chem. 267, 20239–20247.
[2] Kao, J., Houck, K., Fan, Y., Haehnel, I., Libutti, S.K., Kayton, M.L., Grikscheit, T., Chabot, J., Nowygrod, R., Greenberg, S., Kuang, W.J., Leung, D.W., Hayward, J.R., Kisiel, W., Heath, M., Brett, J. and Stern, D.M. (1994) J. Biol. Chem. 269, 25106–25119.
[3] Quevillon, S., Agou, F., Robinson, J.C. and Mirande, M. (1997) J. Biol. Chem. 272, 32573–32579.
[4] Park, S.G., Jung, K.H., Lee, J.S., Jo, Y.J., Motegi, H., Kim, S. and Shiba, K. (1999) J. Biol. Chem. 274, 16673–16676.
[5] Knies, U.E., Behrensdorf, H.A., Mitchell, C.A., Deutsch, U., Risau, W., Drexler, H.C. and Clauss, M. (1998) Proc. Natl. Acad. Sci. USA 95, 12322–12327.
[6] Cryns, V. and Yuan, J. (1998) Genes Dev. 12, 1551–1570.
[7] Van de Craen, M., Declercq, W., Van den Brande, I., Fiers, W. and Vandenabeele, P. (1999) Cell Death Differ. 6, 1117–1124.
[8] Susin, S.A., Zamzami, N., Castedo, M., Daugas, E., Wang, H.G., Geley, S., Fassy, F., Reed, J.C. and Kroemer, G. (1997) J. Exp. Med. 186, 25–37.
[9] Fernandes-Alnemri, T., Takahashi, A., Armstrong, R., Krebs, J., Fritz, L., Tomaselli, K.J., Wang, L., Yu, Z., Croce, C.M., Salveson, G., Earnshaw, W.C., Litwack, G. and Alnemri, E.S. (1995) Cancer Res. 55, 6045–6052.
[10] Thornberry, N.A., Rano, T.A., Peterson, E.P., Rasper, D.M., Timkey, T., Garcia-Calvo, M., Houtzager, V.M., Nordstrom, P.A., Roy, S., Vaillancourt, J.P., Chapman, K.T. and Nicholson, D.W. (1997) J. Biol. Chem. 272, 17907–17911.
[11] Vercammen, D., Brouckaert, G., Denecker, G., Van de Craen, M., Declercq, W., Fiers, W. and Vandenabeele, P. (1998) J. Exp. Med. 188, 919–930.
[12] Kawahara, A., Ohsawa, Y., Matsumura, H., Uchiyama, Y. and Nagata, S. (1998) J. Cell Biol. 143, 1353–1360.
[13] Machleidt, T., Geller, P., Schwandner, R., Scherer, G. and Kronke, M. (1998) FEBS Lett. 436, 51–54.
[14] Zhang, Y., Center, D.M., Wu, D.M.H., Cruikshank, W.W., Yuan, J., Andrews, D.W. and Kornfeld, H. (1998) J. Biol. Chem. 273, 1144–1149.
[15] Levkau, B., Koyama, H., Raines, E.W., Clurman, B.E., Herren, B., Orth, K., Roberts, J.M. and Ross, R. (1998) Mol. Cell 1, 553–563.
[16] Zhivotovsky, B., Samali, A., Gahm, A. and Orrenius, S. (1999) Cell Death Differ. 6, 644–651.
[17] Chandler, J.M., Cohen, G.M. and MacFarlane, M. (1998) J. Biol. Chem. 273, 10815–10818.
[18] Van de Craen, M., Vandenabeele, P., Declercq, W., Van den Brande, I., Van Loo, G., Molemans, F., Schotte, P., Van Criekinge, W., Beyaert, R. and Fiers, W. (1997) FEBS Lett. 403, 61–69.
[19] Kerr, J.F., Wyllie, A.H. and Currie, A.R. (1972) Br. J. Cancer 26, 239–257.
[20] Daemen, M.A., Van 't Veer, C., Denecker, G., Heemskerk, V.H., Wolfs, T.G., Clauss, M., Vandenabeele, P. and Buurman, W.A. (1999) J. Clin. Invest. 104, 541–549.