Caspase-8 and Apaf-1-independent Caspase-9 Activation in Sendai Virus-infected Cells*

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Michael Bitzer‡§, Sorin Armeanu‡§, Florian Prinz‡, Guy Ungerechts‡, Wolfgang Wybranietz‡, Martin Spiegel‡, Christian Bernlöhr‡, Francesco Cecconi‡, Michael Gregor‡, Wolfgang J. Neubert**, Klaus Schulze-Osthoff‡‡, and Ulrich M. Lauer‡

From the ‡Department of Internal Medicine I, University Clinic Tübingen, D-72076 Tübingen, Germany, ‡§Department of Biology, University of Tor Vergata, I-00133 Roma, Italy, **Max-Planck-Institut für Biochemie, Molekulare Virologie, D-82152 Martinsried, Germany, and the ‡‡Department of Immunology and Cell Biology, University Münster, 48149 Münster, Germany

Apoptotic cell death is of central importance in the pathogenesis of viral infections. Activation of a cascade of cysteine proteases, i.e. caspases, plays a key role in the effector phase of virus-induced apoptosis. However, little is known about pathways leading to the activation of initiator caspases in virus-infected host cells. Recently, we have shown that Sendai virus (SeV) infection triggers apoptotic cell death by activation of the effector caspase-3 and initiator caspase-8. We now investigated mechanisms leading to the activation of another initiator caspase, caspase-9. Unexpectedly we found that caspase-9 cleavage is not dependent on the presence of active caspases-3 or -8. Furthermore, the presence of caspase-9 in mouse embryonic fibroblast (MEF) cells was a prerequisite for Sendai virus-induced apoptotic cell death. Caspase-9 activation occurred without the release of cytochrome c from mitochondria and was not dependent on the presence of Apaf-1 or reactive oxygen intermediates. Our results therefore suggest an alternative mechanism for caspase-9 activation in virally infected cells beside the well characterized pathways via death receptors or mitochondrial cytochrome c release.

Apoptosis, or programmed cell death, is an essential mechanism in development, homeostasis, and the reaction of multicellular organisms to external stimuli such as infectious agents (1–5). The best characterized key effectors of apoptosis are the family of caspases (for cysteine aspartic acid-specific protease), which consists of 14 mammalian members currently identified, participating in a tightly regulated proteolytic cascade (6, 7). According to substrate specificity, prodomain length, and domain composition, they can be grouped in initiators, effectors, and a third, not yet well defined group of caspases, which functionally serve as cytokine processors (6, 7).

Well characterized initiating events of the caspase cascade are (i) the FADD adaptor (Fas-associating protein with death domain)-mediated recruitment of pro-caspase-8 to Fas-ligand-bound, multimerized Fas receptors (CD95, Apo-1), leading to caspase-8 activation consecutively resulting in cleavage of the effector caspases-3 and -7 (3, 9, 10), or via (ii) mitochondria-mediated release of cytochrome c (e.g. triggered by chemotherapeutic agents) that leads to a conformational change of the adaptor molecule Apaf-1 (apoptotic protease activating factor-1) causing multimerization and autocleavage of caspase-9. Furthermore, the presence of caspase-9 in mouse embryonic fibroblast (MEF) cells was a prerequisite for Sendai virus-induced apoptotic cell death. Caspase-9 activation occurred without the release of cytochrome c from mitochondria and was not dependent on the presence of Apaf-1 or reactive oxygen intermediates. Our results therefore suggest an alternative mechanism for caspase-9 activation in virally infected cells beside the well characterized pathways via death receptors or mitochondrial cytochrome c release.

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† Both authors contributed equally to this work.

‡ Both authors contributed equally to this work.

§ To whom correspondence should be addressed. Tel.: 49-7071-2983189; Fax: 49-7071-295692; E-mail: michael.bitzer@uni-tuebingen.de.

1 The abbreviations used are: z-VAD-fmk, benzoyloxycarbonyl-Val-Ala-Asp (Ome) fluoromethyl-ketone (z-VAD-fmk); CrmA, cytokine response modifier protein A; NAC, N-acetylcysteine; HIV, human immunodeficiency virus; FCS, fetal calf serum; MEF, mouse embryonic fibroblast; FACS, fluorescence-activated cell sorter; CARD, caspase recruitment domain.
and mitochondria, were removed by centrifuging twice at 18,000 g. The uninfected control cells is shown in the left lanes and caspase-9 (p37) and caspase-8 (p43), respectively, were detected by Western blot analysis 48 h p.i. A preparation from uninfected control cells are shown with black bars. z-VAD-fmk (40 μM) incubation started immediately after infection; z-VAD-fmk was added in the same amount 24 h p.i.

Results are expressed as the mean ± S.D. of three independent experiments.

**EXPERIMENTAL PROCEDURES**

**Virus and Cells—**Sendai virus (strain Fushimi) and for immunofluorescence studies a recombinant SeV coding for the reporter protein “green fluorescent protein” by an insertion between the two viral genes for P and M in the wild-type genome were grown in 9-day-old embryonated chicken eggs as described previously (29, 30). CV-1 cells (African green monkey kidney cells) were obtained from the American Type Culture Collection (ATCC, Manassas, VA), and MCF7 cells (human breast carcinoma) from the European Collection of Animal Cell Cultures (ETACC, Salisbury, United Kingdom). MCF-vector, -Bcl-2, and -Bcl-X, were a kind gift of M. Jäättela, Copenhagen, Denmark, and have been described previously (31). MCF7-CrmA cells were a kind contribution of C. Vincenz, Ann Arbor, MI. All cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (FCS). Caspase-9 +/+ and −/− MEFs were a kind gift from Richard Flavell, New Haven, CT, and A3AP1 +/+ and −/− MEFs have been described previously (32). Media and supplements were purchased from Invitrogen.

**Infection of Cells—**Cells were seeded out in 35-mm plates and infected when monolayers had reached 85–90% confluency. As standard inoculation procedure monolayers were washed twice with medium lacking FCS (washing medium) and overlaid with washing medium containing SeV at a multiplicity of infection (m.o.i.) of 10. After incubation for 60 min at 37°C, non-adsorbed virus was removed by repeated washing of the cells. Medium containing FCS (growth medium) was added, and the cells were incubated for various periods of time at 37°C.

**Preparation of Cytosol—**5 × 10⁶ cells were harvested, washed once in 0.5 ml of hypotonic buffer (20 mM HEPES, 10 mM KCl, 2 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, pH 7.4), resuspended in 0.2 ml of hypotonic buffer, and incubated on ice for 15 min. Cell membranes were disrupted by shearing 15× through a 21-gauge needle. Debris, nuclei and mitochondria, were removed by centrifuging twice at 18,000 × g for 15 min. Supernatants were boiled at 100°C with one-third volume of 4× RotiLoad (Roth, Karlsruhe, Germany) for 10 min. From each sample 30 μg of cellular protein was electrophoretically separated on 15% SDS-polyacrylamide gels, transferred to polyvinylidene difluoride membranes (Amersham Biosciences), and subjected to immunoblotting.

**Immunoblotting—**To detect proteolytic processing of caspases Western blot assays were done as described previously (25). In brief, 5 × 10⁶ cells (including floating cells) were harvested, washed once in PBS, and resuspended in 0.1 ml of lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl, pH 7.5, containing leupeptin (3 μg/ml), pepstatin (3 μg/ml), aprotinin (3 μg/ml), phenylmethylsulfonylfluoride (2 mM)) and boiled after 15 min of incubation on ice with 4× RotiLoad (Roth) for 10 min. From each sample 30 μg of cellular protein was electrophoretically separated on 12% SDS-polyacrylamide gels under reducing conditions and subsequently transferred to polyvinylidene difluoride membranes. Membranes were blocked in RotiBlock (Roth) for 1 h. Next, membranes were incubated with the respective antibodies for 1 h at room temperature: anti-FLICE C15 1:10, (Biomedia, Baesweiler, Germany); anti-human caspase-9 1:2000 (kind gift of Y. Lazebnik, Cold Spring Harbor, NY, described in Ref. 33); anti-mouse caspase-9 (kind gift of T. Momoi, Tokyo, Japan, described in Ref. 34), anti-Bcl-2 1:2000 (N19, Santa Cruz, Santa Cruz, CA); polyclonal rabbit anti-Bcl-X, 1:1000 (Transduction Labs, San Diego, CA); anti cytochrome c (7H8.2C12, 1:2000, PharMingen, San Diego, CA), anti-tubulin (B521, 1:2000, Sigma), washed three times with TBS-T (150 mM NaCl, 13 mM Tris-HCl, 0.02% Triton X-100, pH 7.5), and incubated with peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:4000, Amersham Biosciences). Membranes were washed for 1 h with TBS-T, and further detection was performed by the ECL Western blotting detection system on Hyperfilm-ECL (Amersham Biosciences).

**Flow Cytometry—**Fragmentation of genomic DNA to hypodiploid DNA was assessed by FACS analysis as described previously (25). In brief, 5 × 10⁶ cells (including floating cells) were collected and washed once in PBS (5 min, 1000 × g). Pellets were resuspended in 100 μl of PBS, fixed with 1 ml of aceton-methanol (1:1; −20°C), and subsequently washed with PBS. Next, the pellet was resuspended in 400 μl of PBS containing 1 mg of RNase/ml and incubated on ice for 1 h. After addition of 20 μl of propidium iodide solution (2 mg/ml in PBS, Sigma) and incubation for 30 min on ice, flow cytometry was performed (FACS Calibur, Becton Dickinson, Heidelberg, Germany) using the CellQuest program. Cells to the left of the 2n peak containing hypodiploid DNA were considered as apoptotic.

**Annexin V Staining—**Annexin V staining (Annexin FLUOS staining kit, Roche Molecular Biochemicals) was performed according to the manufacturer. In brief, 5 × 10⁶ cells (including floating cells) were collected, and pellets were resuspended in 100 μl of binding buffer, containing 2 μl of each annexin V and propidium iodide solution and incubated on ice for at least 15 min. Flow cytometry was performed as above.

**Inhibition Experiments—**Apoptosis was blocked by addition of 20–50 μM of the peptide inhibitor z-VAD-fmk (Alexis Biochemicals) every 24 h to the culture supernatant. In other inhibition experiments 1 mM MeSO (Sigma) or up to 10 μM N-acetylcysteine (Merck, Darmstadt, Germany) were used. For inhibition experiments 5 × 10⁶ cells were infected with
SeV (m.o.i. 10) and incubated immediately after infection for various periods of time. Cells were analyzed either by immunoblotting or by flow cytometry.

**Immunofluorescence Staining**—Microscopy was performed as described elsewhere (35). In brief, cells were grown on coverslips until they had reached 70–80% confluency. At 48 h postinfection mitochondria were stained with MitoTracker™ (500 nM, Molecular Probes, Eugene, OR) in Dulbecco’s modified Eagle’s medium with 10% FCS for 30 min at 37 °C, and cells were fixed for 15 min with 4% paraformaldehyde at room temperature. Coverslips were blocked with 10% newborn calf serum in PBS (blocking solution) for 10 min. Cells were incubated with anti-cytochrome c antibody (1 μg/ml, 7H8.2C12, Pharmingen) in blocking solution for 20 min, followed by detection with a secondary Cy™3-conjugated antibody (5 μg/ml, Dianova, Hamburg, Germany). Slides were mounted in a Mowiol 40–88 (Sigma) preparation containing 2.5% DABCO (Sigma) as an antifade reagent. Specimens were examined using a Zeiss LSM 510 confocal microscope.

### RESULTS

**Caspase-9 Cleavage as a Key Event in SeV-induced Apoptotic Cell Death**—Recently, we have demonstrated caspase-dependent apoptosis induction and activation of the effector caspase-3 and the initiator caspase-8 in SeV-infected host cells (25). As neither the contribution of each single caspase on the apoptosis-initiating event nor the involvement of other key caspases after SeV infection have been investigated yet, we set out to further characterize the apoptosis initiator mechanisms in our model system of SeV-induced apoptosis.

It is well known that caspase-3 can be activated in a caspase-8-dependent pathway as a so-called effector or downstream caspase. On the other hand, caspase-3 has been shown to cleave caspase-8 after being activated by another initiator caspase, caspase-9 (12, 36). Therefore, in a starting experiment we chose to exclude the influence of caspase-3 in SeV-infected cells by using the breast carcinoma cell line MCF-7, which is known to be devoid of functional caspase-3 from uninfected controls are shown on the left side. Results are expressed as mean ± S.D. of three independent experiments.

Moreover, Western blot analysis of MCF-7 cells 48 h postinfection (p.i.) showed cleavage of both, caspase-8 and -9, indicating a caspase-3-independent processing of both so-called initiator caspases after SeV infection (data not shown). Starting from this we investigated the process of caspase activation in detail using inhibitors that block different apoptosis signal transducing pathways.

Both caspases-8 and -9 have been shown to be capable of indirectly activating the other one (i.e. caspase-9-dependent caspase-8 cleavage and vice versa), depending on the apoptotic stimulus (6, 38). To further investigate the role of active caspase-8 for caspase-9 cleavage, we used MCF-7 cells overexpressing the cytokine response modifier protein A (CrmA) from cowpox virus, which has been described to predominantly bind to and inhibit caspases-8 and -1 (39). As a result, overexpression of CrmA did not block SeV-triggered cleavage of caspase-9 (Fig. 1A) but did inhibit caspase-8 cleavage (Fig. 1B). Moreover, looking at annexin V staining, CrmA overexpression in MCF-7 cells could not reduce the percentage of SeV-induced apoptotic cells compared with MCF-7 control cells (Fig. 1C). As a further control demonstrating the dependence of apoptosis on the presence of activated caspases, the broad caspase-inhibitor z-VAD-fmk nearly completely blocked SeV-induced apoptosis (Fig. 1C). These results suggested a key role of caspase-9 in SeV-induced apoptotic cell death, being independent of the presence of active caspases-3 or -8. Therefore we next used MEF cells prepared from mice lacking caspase-9 (caspase-9 -/- ) (40) to further determine the role of this caspase in the SeV-mediated death program. Surprisingly, compared with caspase-9 +/+ MEFs SeV-mediated apoptosis was completely absent in caspase-9 -/- cells 48 h p.i. (Fig. 2). These results stimulated us to further investigate the mechanisms of caspase-9 activation in SeV-infected host cells.

**Bcl-2 or Bcl-X<sub>L</sub> Do Not Inhibit SeV-mediated Caspase-9 Cleavage**—Cytochrome c release from mitochondria has been
described as a prerequisite for caspase-9 activation in numerous settings, including certain viral infections (e.g. Ref. 41). After the release of cytochrome c into the cytosol an interaction with the adaptor protein apoptosis-inducing factor 1 (Apaf-1) subsequently leads to an oligomerization of caspase-9 monomers resulting in a self-activating process by an intrinsic autocatalytic activity (42, 43). Caspase-9 activation in this pathway can be blocked by antiapoptotic members of the Bcl-2 family like Bcl-2 or Bcl-XL (44, 45) by inhibiting the mitochondrial release of intermembrane proteins including cytochrome c (46, 47). Therefore, we next investigated the effects of SeV infection in MCF-7 cell lines overexpressing either Bcl-XL or Bcl-2 (Fig. 3B). Unexpectedly we found caspase-8 cleavage both in Bcl-2 family like Bcl-2 or Bcl-XL (44, 45) by inhibiting the mitochondrial release of intermembrane proteins including cytochrome c (46, 47). Therefore, we next investigated the effects of SeV infection in MCF-7 cell lines overexpressing either Bcl-XL or Bcl-2 (Fig. 3B). Unexpectedly we found caspase-8 cleavage both in Bcl-2 family (Fig. 3A, left side) and Bcl-2 (data not shown) as well as caspase-9 cleavage in Bcl-2 (Fig. 3A, right side) and Bcl-XL-overexpressing cell lines (data not shown). Most strikingly, MCF-7 cells overexpressing Bcl-XL (Fig. 3C) showed a percentage of annexin V staining quite similar to SeV-infected MCF-7 control cells (Fig. 1C). The functionality of overexpressed Bcl-2 and Bcl-XL proteins in MCF-7 cells was confirmed by an inhibition of apoptosis after the incubation for 24 h with 50 μM cisplatin; the mean percentage of living cells was determined by trypan blue staining to be 1.57, and 95% for MCF-7 control, MCF-7 Bcl-2, and MCF-7 Bcl-XL cells, respectively. Taken together, these results suggested a SeV-triggered caspase-9 activation not being dependent on cytochrome c release in host cells. However, an inactivation of Bcl-2 proteins during the viral replication cycle of Sindbis virus leading to proapoptotic Bcl-2 fragments has been described recently (48) and therefore could not be ruled out in our setting. Consequently, we further investigated a possible SeV-mediated cytochrome c release into the cytosol of infected cells.

**Caspase-9 Activation in SeV-infected Cells**—To determine whether a cellular redistribution of cytochrome c plays any role in the signal transduction of SeV-triggered apoptotic cell death, cytochrome c detection in infected cells was performed by immunochemistry and confocal microscopy. Thereby the infection of individual cells could be confirmed by using recombinant SeV particles coding for the reporter protein green fluorescent protein (GFP). Our results demonstrate that cytochrome c (Fig. 4, B and E, displaying blue staining of cytochrome c) colocalizes with mitochondria (Fig. 4, A and D, displaying red staining of mitochondria with MitoTracker) for up to 48 h after SeV-GFP infection (Fig. 4C, infection was verified by displaying green staining due to SeV-mediated GFP expression) indicating a lack of cytochrome c release to the cytosol. As a control, cytochrome c release could be demonstrated by a missing colocalization with mitochondria (MitoTracker) for up to 48 h after SeV-GFP infection (Fig. 4C). SeV usually is propagated and replicates in caspase-3-competent cells and activation of caspase-3 might be involved in generating proapoptotic cytochrome c-releasing protein fragments (48), we subsequently performed a Western blot analysis of cytosolic extracts from SeV-infected CV-1 cells, employing primary antibodies to cytochrome c. In accordance with the immunofluorescence staining results in MCF-7 cells, we did not detect any cytosolic cytochrome c up to 48 h after SeV infection (Fig. 5). In conclusion, the experiments using overexpression of Bcl-2 or Bcl-XL, confocal microscopy, and cytochrome c Western blot analysis clearly demonstrate a cytochrome c-independent...
Caspase-9 cleavage after SeV infection of host cells.

Apaf-1-independent Caspase-9 Processing—Currently, Apaf-1 is the best characterized adaptor molecule being involved in caspase-9 activation. The interaction between both proteins has been described to be highly specific, and it could not be shown for any other caspase so far (36, 42). Theoretically, caspase-9 cleavage could still be mediated by Apaf-1 through a cytochrome c-independent mechanism. Therefore we investigated caspase-9 processing in Apaf-1-deficient SeV-infected embryonic fibroblasts (Apaf-1−/− cells) (32). First, looking at the amount of SeV-infected apoptotic cells 48 h p.i., we found a similar percentage of apoptotic cells in Apaf-1−/− and Apaf-1+/+ cultures (Fig. 6A, column pair on the right side). Cisplatin and staurosporine were used for control experiments, indicating that cisplatin-induced apoptosis involves Apaf-1 (decreased apoptosis rate in Apaf-1−/− cells), whereas for cell death following prolonged staurosporine treatment over 24 h the presence of Apaf-1 seemed to be dispensable. Furthermore, looking at caspase-9 cleavage products after SeV infection, a Western blot analysis could detect cleavage products p38 and p23 of mouse caspase-9 in both Apaf-1−/− and Apaf-1+/+ cultures (Fig. 6B, lane 2) as well as in Apaf-1−/− cells (Fig. 6B, lane 5). The antibody used in this study has been described to detect caspase-9 cleavage products only but not the uncleaved caspase-9 proform (anti-m9D368) (34), therefore equal loading was confirmed by tubulin staining. Staurosporine, used as a positive control, also demonstrated that ROI production does not contribute substantially to the apoptotic process or to the cleavage of caspase-9 after SeV infection. In conclusion, our results demonstrate that SeV infection triggers a so-far-unknown pathway of caspase-9 cleavage that does not depend on caspase-8, cytochrome c, and Apaf-1.

DISCUSSION

The activation of caspases has been shown to be an important component of the pathogenesis of viral infections (1, 2, 4, 19). However, neither the exact order of virus-triggered caspase cascades during different viral infections nor the molecular mechanisms that lead to a caspase activation following viral entry have been elucidated in detail yet. In a recent study investigating SeV infection, we found cleavage of caspases-3 and -8 following SeV replication in host cells (25). Caspase-3 is thought to be an important effector caspase, being able to cleave other members of the caspase family, including initiator caspase-8 and in a feedback loop initiator caspase-9 (38). Here we show that (i) both initiator caspases are cleaved in the absence of active caspase-3 after SeV infection, (ii) caspase-9 cleavage could be detected in the absence of active caspase-8, and (iii) using caspase-9-deficient cells (40) we could demonstrate that SeV-mediated apoptosis induction is dependent on the presence of caspase-9.

Concerning the activation of caspases-8 and -9, either of these initiator caspases can be cleaved downstream of the other one in the caspase cascade: (i) in receptor mediated apoptosis, caspase-8 is able to cleave the proapoptotic Bcl-2-family member BID, thereby generating a proteolytic fragment that leads to mitochondrial cytochrome c release and subsequent caspase-9 activation (51–53); (ii) caspase-independent mitochondrial cytochrome c release (e.g. triggered by chemothera-
peutic agents) leads to caspase-9 cleavage, activating effector caspases that cleave caspase-8 as a downstream event (12, 36). In both scenarios cytochrome c release has been described to be a prerequisite for caspase-9 activation. In contrast to these models, our results show a cytochrome c-independent caspase-9 cleavage following SeV infection. Indirect evidence that cytochrome c might not be involved in SeV-mediated caspase-9 cleavage came from our SeV infection studies using Bcl-2- and Bcl-XL-overexpressing cell lines. These two members of the Bcl-2 family are able to prevent cytochrome c efflux from mitochondria to the cytosol, thereby abrogating apoptosis mediated via this pathway (44, 45, 54). Therefore, a possible cytochrome c release after SeV infection was investigated by using two further approaches: confocal microscopy and immunoblot techniques. Surprisingly, no cytochrome c efflux to the cytosol was detected. Considering the question that small amounts of cytochrome c could be missed by these investigations, a recent study found that once the cytochrome c release is initiated, cytochrome c release continues until it is completely released from all mitochondria in individual cells within a 5-min time range (55). Moreover it has been demonstrated that complete cytochrome c release occurs regardless of the type or strength of stimulus or the time elapsed since the stimulus was applied (55). In conclusion, our results clearly show that caspase-9 activation is not mediated via cytochrome c release from mitochondria after SeV infection of host cells.

A general principle for the activation of initiator caspases is the involvement of adaptor molecules that link death sensors such as death receptors or mitochondria to long prodomain initiator caspases (6). The best characterized adaptor molecule being involved in caspase-9 activation is Apaf-1 (56), which usually oligomerizes after cytochrome c release from mitochondria in the presence of dATP or ATP. This complex recognizes procaspase-9 via interactions between the caspase recruitment...
domain (CARD) of Apaf-1 and the caspase-9 domain, leading to autocatalytic processing of caspase-9 (36, 42, 43, 57). This interaction is highly specific and could not be shown for any other known caspase so far (36, 42).

Theoretically, caspase-9 cleavage could still be mediated by Apaf-1 through non-cytochrome c-dependent mechanisms. This could be shown recently for an amino acid exchange at residue 368 or for the deletion of the WD-40 repeats in the Apaf-1 molecule (43, 58). These mutants have been found to make Apaf-1 constitutively active and therefore independent of cytochrome c binding. However, these proteins have been generated in vitro and could not be detected in vivo. Furthermore, using Apaf-1-deficient embryonic fibroblasts we could demonstrate cleavage of caspase-9 in the absence of Apaf-1. Similar results could be gained for the prolonged treatment of these cells with staurosporine as a positive control, which is thought to mediate apoptotic cell death by multiple pathways including downstream events of Apaf-1 (59, 60).

Apaf-1-independent caspase-9 activation has been described recently for cytotoxic proteins like Bcl-10/mE10 (61, 62), DIVA/Boo (63, 64), and Nod1 (65). These proteins share a so-called CARD (66), which binds and triggers activation of caspase-9 when overexpressed in cells. It is tempting to speculate that caspase-9 could be used in vitro but not in vivo. Furthermore, using Apaf-1-deficient embryonic fibroblasts we could demonstrate cleavage of caspase-9 in the absence of Apaf-1. Similar results could be gained for the prolonged treatment of these cells with staurosporine as a positive control, which is thought to mediate apoptotic cell death by multiple pathways including downstream events of Apaf-1 (59, 60).

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Possible further mechanisms involved in SeV-induced apoptosis could be (i) the cleavage of caspase-9 by another caspase, (ii) the generation of ROI, or (iii) the activation of a cellular protease-like cathepsin D (18) or calpain (6, 8), which is not a caspase itself but able to cleave caspase-9. Despite numerous studies in which cleavage of caspses by other members of the same protease family have been investigated, cleavage of caspase-9 by another caspase so far has not been detected with the exception of a feedback loop involving caspase-3 (36, 38). Concerning ROI generation after SeV infection we could not demonstrate any influence on caspase-8 or caspase-9 cleavage by incubating SeV-infected cells with antioxidants. Finally, by incubating infected cells with specific inhibitors of cathepsin D or calpain we did not alter the rate of SeV-induced apoptosis 48 or 72 h p.i. (data not shown).

Taken together these results suggest the existence of an alternative caspase-9-activating pathway in SeV-infected host cells in addition to well characterized caspase-9 cleavage events mediated by death receptors or Apaf-1. The understanding of molecular events being involved in virally triggered apoptosis transduction pathways are important steps for the development of innovative tools to minimize viral pathogenicity.

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