Caspase-4 Is Required for Activation of Inflammasomes

Gabriel Sollberger, Gerhard E. Strittmatter, Magdalena Kistowska, Lars E. French and Hans-Dietmar Beer

doi: 10.4049/jimmunol.1101620
http://www.jimmunol.org/content/188/4/1992

**Supplementary Material**
http://www.jimmunol.org/content/suppl/2012/01/13/jimmunol.1101620.DC1

**References**
This article cites 42 articles, 12 of which you can access for free at:
http://www.jimmunol.org/content/188/4/1992.full#ref-list-1

**Subscription**
Information about subscribing to _The Journal of Immunology_ is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Caspase-4 Is Required for Activation of Inflammasomes

Gabriel Sollberger,*† Gerhard E. Strittmatter,† Magdalena Kistowska,† Lars E. French,† and Hans-Dietmar Beer*†

IL-1β and IL-18 are crucial regulators of inflammation and immunity. Both cytokines are initially expressed as inactive precursors, which require processing by the protease caspase-1 for biological activity. Caspase-1 itself is activated in different innate immune complexes called inflammasomes. In addition, caspase-1 activity regulates unconventional protein secretion of many other proteins involved in inflammation and repair. Human caspase-4 is a poorly characterized member of the caspase family, which is supposed to be involved in endoplasmic reticulum stress-induced apoptosis. However, its gene is located on the same locus as the caspase-1 gene, which raises the possibility that caspase-4 plays a role in inflammation. In this study, we show that caspase-4 expression is required for UVB-induced activation of proIL-1β and for unconventional protein secretion by skin-derived keratinocytes. These processes require expression of the nucleotide-binding domain leucine-rich repeat containing, Pyrin domain containing-3 inflammasome, and caspase-4 physically interacts with its central molecule caspase-1. As the active site of caspase-4 is required for activation of caspase-1, the latter most likely represents a substrate of caspase-4. Caspase-4 expression is also essential for efficient nucleotide-binding domain leucine-rich repeat containing, Pyrin domain containing-3 and for absent in melanoma 2 inflammasome-dependent proIL-1β activation in macrophages. These results demonstrate an important role of caspase-4 in inflammation and innate immunity through activation of caspase-1. Therefore, caspase-4 represents a novel target for the treatment of (auto)inflammatory diseases. The Journal of Immunology, 2012, 188: 1992–2000.
11 (26, 22). Mice deficient in caspase-11 seem to resemble the phenotype of those lacking caspase-1 (27).

However, very recently it has been shown that caspase-11 expression is only required for secretion of IL-1β induced by certain stimuli, called noncanonical inflammasome activation (28). Some data suggest that human caspase-4 is the functional ortholog of murine caspase-11 (expression of both is induced by IFN-γ) (22, 27, 29) or of caspase-12 (both are involved in ER stress-induced apoptosis) (30, 31). However, expression of caspase-5 and -11 is induced by LPS (32). In addition, caspase-5 is processed in the NLRP-1 inflammasome, which also suggests that the latter might play a similar role in humans as caspase-11 in mice (33). Because a requirement of caspase-4 during inflammation has never been studied, we addressed this question in this study (22).

In this study, we demonstrate that caspase-4 is secreted from UVB-irradiated keratinocytes. Caspase-4 expression is required for maturation of proIL-1β and proIL-18 and for unconventional protein secretion mediated by the NLRP-3 inflammasome in keratinocytes, but also by the NLRP-3 and AIM2 inflammasomes in activated macrophages. Our results demonstrate an essential role of caspase-4 in activation of caspase-1 in inflammasome complexes and therefore in inflammation.

Materials and Methods

Material and Abs

The following reagents were used: small interfering RNA (siRNA) from Sigma-Aldrich (11, 19); caspase-4 S1, 5'-GUUGAAGUAGAGAAGAA- GAATT-3' and caspase-4 S2, 5'-CUCAUGAGAAGUUGUUTT-3'; primers from Microsynth (Balgach, Switzerland); caspase-1 inhibitor Ac- YVAD-CMK and caspase-4 inhibitor Z-LEVDMK from Axxora (Lausen, Switzerland); lactate dehydrogenase (LDH) assay from Promega (Madison, WI); and IL-1β DuoSet ELISA from R&D Systems (Minneapolis, MN). For Western blotting, the following Abs were used: anti-caspase-4 (goat, sc-1229) (Figs. 1B, 2A, 4, Supplemental Fig. 1); anti-caspase-1 (rabbit, sc-622); anti-Bid (rabbit, sc-11423); anti-HA (rabbit, sc-805) (Santa Cruz Biotechnology, Santa Cruz, CA); anti-thioredoxin-1 (rabbit, ab16835) (Abcam, Cambridge, U.K.); anti-β-actin (mouse, A5411); anti-Flag (mouse, F3165) and anti-gelsolin (mouse, G4896) (Sigma-Aldrich); anti-apolipoprotein A1 (rabbit); rabbit, sc-622); anti-peroxiredoxin-1 (rabbit, sc-1229) (Figs. 1B-1D). Abs were either activated with ATP (Axxora, Lausen, Switzerland) or mock treated and harvested after 1.5, 3, and 4.5 h. Western blots of lysates and of supernatants; the latter were concentrated by acetone precipitation. Unspecific bands are marked by an asterisk.

Cell culture and induction of unconventional secretion

Cultivation of human primary keratinocytes has been described (34). Cells were incubated with UVB (Medison HF-54, Schulze & Böhm or UV802L, Waldmann; 0.26 mW/cm² for 6 min) in keratinocyte-serum-free medium (Invitrogen). TPH-1 cells were grown in RPMI 1640 (Sigma-Aldrich) with 10% FBS (Invitrogen) and 1% penicillin/streptomycin. A total of 1 × 10⁷ cells/ml in a 12-well or 6-well was differentiated with 25 ng/ml 12-O-tetradecanoylphorbol-13-acetate for 3 d. Differentiated cells were primed with LPS (Sigma-Aldrich; 1 μg/ml) overnight, if indicated. Then they were either activated with ATP (Axxora, Lausen, Switzerland; 5 mM, 30 min) and monosodium urate (MSU; 300 μg/ml), which was prepared from uric acid (Sigma-Aldrich), or transfected with poly(dA:dT) (Sigma-Aldrich; 1 μg/ml) after the medium was replaced by OptiMEM (Invitrogen). COS-1 cells were cultivated in DMEM (Sigma-Aldrich) with 10% FBS and 1% penicillin/streptomycin and transfected in OptiMEM (Invitrogen). The measurement of protein release from activated cells has been described recently (34).

Comunoprecipitation

Comunoprecipitation (CoIP) of transfected COS-1 cells has been described recently (35). Immunoprecipitation of endogenous caspase-1 and proIL-1β was performed with lystate of UVB-irradiated keratinocytes. Briefly, keratinocytes were grown on 14-cm dishes to 70-90% confluency and harvested in 400 μl CoIP buffer per dish containing Complete protease inhibitor (Roche, Rotkreuz, Switzerland) 3 h After irradiation with UVB, as described above. Cells were lysed with a douncer, treated with a single-pulse ultrasound, and centrifugated for 20 min at 17,000 × g. The supernatant was dialuted with the same volume of CoIP buffer containing aprotinin (1:100) and 0.5 mM 4-(2-aminoethyl)benzenesulfonylfluoride (both from Sigma-Aldrich) and incubated with 20 μg Ab for 3 h at 4°C on a rocker. Abs used were directed against caspase-1 (Santa Cruz Biotechnology; rabbit), hemagglutinin (HA; Santa Cruz Biotechnology; rabbit), IL-1β (R&D Systems; mouse), or Flag (Sigma-Aldrich; mouse). After centrifugation, 150 μl (50 mg/ml) protein A-Sepharose (GE Health Care) was added. After 1.5 h, the beads were washed four times with CoIP buffer and suspended in 70 μl 2× SDS buffer.

Analysis of caspase-4 expression in human skin

Psoriatic biopsies, human skin wounds, and human belly skin were a gift of S. Werner (ETH Zurich). Freshly isolated belly skin was cut into little pieces and irradiated with UVB (as described above) in DMEM or mock treated. The skin was embedded 24 h after irradiation and cut into 7-μm cryo-sections. Sections were fixed in –20°C methanol for 10 min and washed three times in PBS-T for 10 min, respectively. After washing with PBS and blocking in 3% BSA and 0.1% Nonidet P-40 in PBS for 1 h at room temperature, the first Ab was incubated in the same buffer, and the sections were incubated overnight in a humid chamber at 4°C. After washing three times with PBS-T, secondary Abs (1:250) were added for 1 h at room temperature in the same buffer with Hoechst (1:1000). Slides were washed three times with PBS and embedded in Mowiol.

Statistical analysis and repetition of experiments

For statistical analysis, one-way ANOVA with a Dunnett post-test of the Prism Software (GraphPad Software) was used. All experiments were performed at least three times with technical replicates. The line represents the border between dermis and epidermis. Scale bars, 200 μm.

FIGURE 1. Keratinocytes express caspase-4 in vitro and in vivo. A. Human primary keratinocytes were irradiated with UVB (0.26 mW/cm², 6 min) or mock treated and harvested after 1.5, 3, and 4.5 h. Western blots of lysates and of supernatants; the latter were concentrated by acetone precipitation. Unspecific bands are marked by an asterisk. B. Representative pictures of indirect immunofluorescence with an Ab against caspase-4 of ex vivo UV-irradiated (24 h after irradiation) and control human skin or with the secondary Ab only (lower panel). The line represents the border between dermis and epidermis. Scale bars, 200 μm.
repeated at least one time. The result of a representative experiment is shown.

**Results**

**Caspase-4 is secreted by keratinocytes upon UVB irradiation**

Human caspase-4 and -5 represent potential functional orthologs of murine caspase-11, and the latter is required for activation of caspase-1 in macrophages upon certain stimuli (8, 27, 28). We have recently demonstrated that human primary keratinocytes express several inflammasome proteins (11). Using a siRNA approach, we identified the NLRP-3 inflammasome to be required for UVB-induced secretion of IL-1β (11, 36). Interestingly, a siRNA-mediated knockdown of caspase-5 expression did not influence UVB-induced secretion of IL-1β, although this experiment does not exclude the possibility that small amounts of the protease are sufficient for activation of caspase-1 (11, 19). In contrast to caspase-5, mRNA expression of caspase-4 is high in human epidermis (37). Thus, we wondered whether caspase-4 might be required for maturation of proIL-1β in keratinocytes. At first, we performed Western blot analysis of lysates and supernatants of UVB-irradiated human primary keratinocytes (Fig. 1A). We detected a band with the expected size of caspase-4. Upon transfection of keratinocytes with two different siRNAs specific for caspase-4 mRNA, the intensity of this band decreased, demonstrating that it most likely indeed represents caspase-4 (Fig. 2B).

Most importantly, we also detected large amounts of caspase-4 protein in the supernatant of UVB-irradiated keratinocytes in contrast to the supernatant of mock-treated cells (Fig. 1A). As caspase-4 lacks a signal peptide, this result suggests that the protease is secreted independently of the classical ER/Golgi-dependent pathway together with caspase-1 and IL-1β.

Expression of caspase-4 is not restricted to keratinocytes in vitro. Using indirect immunofluorescence, we detected the protease in keratinocytes in vivo in human epidermis throughout all cell layers (Fig. 1B). It has recently been reported that caspase-4 protein expression is strongly induced in the lesional stratum corneum of patients suffering from psoriasis, a common chronic inflammatory disease (37). However, we were not able to detect significantly higher amounts of caspase-4 or of caspase-1 under inflammatory conditions in keratinocytes in vivo, neither in lesional psoriasis nor during wound healing, nor in ex vivo UVB-irradiated human skin (results not shown, Fig. 1B).

**Keratinocytes require caspase-4 for UVB-induced unconventional protein secretion**

We have recently shown that human keratinocytes secrete not only IL-1β, but also inflammasome proteins upon UVB irradiation (11). The secretion of caspase-4 by keratinocytes upon UVB irradiation raises the possibility that the protease may be involved in activation of caspase-1 and in turn in maturation of proIL-1β.

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** Keratinocytes require expression of caspase-4 for unconventional protein secretion. A and B, Human primary keratinocytes were transfected with scrambled siRNA (ctr.) or with siRNA against vascular endothelial growth factor, caspase-5, caspase-1, and caspase-4. Forty-eight hours after transfection, keratinocytes were harvested and analyzed for the expression of caspase-1, caspase-4, and β-actin (A, lower panel) or irradiated with UVB (0.26 mW/cm², 6 min) (A, upper panel, B). A, Four hours after UV irradiation, the relative amount of IL-1β was determined by ELISA measurements of the cytokine concentrations in the supernatant and lysate (IL-1β supernatant/IL-1β [supernatant + cells] × 100). LDH activity served as a control for cell lysis. Bars represent mean ± SD of three experiments. ***p < 0.001. B, Four hours after UV irradiation, lysates and supernatants were harvested, the supernatants were concentrated by acetone precipitation, and Western blots were performed for the analysis of the expression and secretion of the indicated proteins. The unspecific bands are marked by an asterisk.
Therefore, we transfected human keratinocytes with two different siRNAs that target caspase-4 mRNA. As controls, we knocked down expression of caspase-1, caspase-5, and the unrelated vascular endothelial growth factor-A, or we used scrambled siRNA. Western blotting demonstrated that expression of caspase-1 and -4 was efficiently downregulated (Fig. 2A), and we recently demonstrated the functionality of the other siRNAs (11, 19). Then we irradiated the cells with a physiological dose of UVB. Four hours later, we measured the secretion of IL-1β by ELISA and the release of the cytoplasmic enzyme LDH; the latter reflects the amount of cell lysis. As expected, a knockdown of caspase-1 expression almost completely abolished secretion of IL-1β as well as secretion of the CARD of caspase-1, which reflects its processing and activation (Fig. 2A). Surprisingly, a knockdown of caspase-4 expression had a comparable effect on the secretion of these processed polypeptides. This experiment suggests that caspase-4 expression is required for activation of caspase-1 by the NLRP-3 inflammasome and in turn for maturation of proIL-1β.

We have recently demonstrated that caspase-1 is a general regulator of unconventional protein secretion (19). To examine whether caspase-4 expression is also required in this context, we performed Western blots of lysates and supernatants of UVB-irradiated keratinocytes, which we transfected with siRNAs, as described above (Fig. 2B). As expected, a knockdown of caspase-1 expression strongly reduced secretion of caspase-4, which demonstrates that caspase-4 is unconventionally secreted dependent on caspase-1 expression. Interestingly, a reduction of caspase-4 expression resulted in reduced secretion of several leaderless proteins (also described in 19, 34). Most importantly, the reduction of these proteins in the supernatant correlated with the knockdown efficiency of the two siRNAs and therefore with expression of caspase-4. In contrast, the amount of the cytoplasmic or conventionally secreted proteins β-actin, gelsolin, and fibroblast growth factor-binding protein in the supernatant did not depend on caspase-1 or -4 expression (Fig. 2B). This experiment demonstrates that caspase-4 expression is required for UVB-induced unconventional protein secretion, most likely through activation of caspase-1.

**Overexpression of active caspase-4 rescues UVB-induced secretion of IL-1β by caspase-4 knockdown keratinocytes**

Then we wondered whether caspase-4 promotes UVB-induced secretion of IL-1β in keratinocytes through its enzymatic activity. Although the tetrapeptide Z-LEVD-FMK is supposed to inhibit only caspase-4 and -5 (38, 39), it also blocked activity of overexpressed caspase-4 (results not shown) and, therefore, cannot be used for the specific inhibition of caspase-4 activity.

Thus, we knocked down expression of caspase-4 with siRNA in keratinocytes for 4 d and transfected an expression plasmid encoding either caspase-4 or enzymatically inactive caspase-4 with an alanine residue instead of the active site cysteine, both with a HA tag. After a medium change, the cells were irradiated with UVB, and lysate and supernatant were analyzed by Western blots (Fig. 3A). Most importantly, UVB-induced IL-1β and IL-18 secretion was rescued much more efficiently by overexpression of active caspase-4 in knockdown cells than by the dead mutant. This effect is most likely mediated by caspase-1 because more processed caspase-1 was detected in the supernatant of keratinocytes, which overexpressed active caspase-4. Interestingly, we detected a higher expression and secretion of the full-length inactive version of caspase-4 compared with the active protein. This effect was not seen in COS-1 cells (Supplemental Fig. 1, Fig. 4A, 4C).

Overexpression of active and inactive caspase-4 in mock-treated keratinocytes without a knockdown also revealed significantly higher amounts of the full-length form of the dead mutant in cell lysate and supernatant (Fig. 3B). Interestingly, overexpression of the active enzyme resulted in activation of caspase-1 and secretion of mature IL-1β and IL-18. Secretion of IL-1β and of the CARD...
of caspase-1 was also detected in keratinocytes overexpressing enzymatically inactive caspase-4 or in cells transfected with an empty vector, however, at a very low level, suggesting that transfection of DNA weakly activates caspase-1 in keratinocytes (Fig. 3B, results not shown).

These experiments demonstrate that, in keratinocytes, enzymatically active caspase-4 is required for efficient UVB-induced activation of caspase-1 and in turn for IL-1β and IL-18 secretion, and that overexpression of active caspase-4 is sufficient for inflammasome activation.

Caspase-4 processes caspase-1 and enhances secretion of IL-1β in transfected COS-1 cells

We have recently shown that transfected COS-1 cells are a useful tool for the examination of inflammasomes (35). Thus, we overexpressed different combinations of active and inactive caspase-1 and caspase-4 in these cells and analyzed the processing of both proteases in the lysate and supernatant (Fig. 4A). Overexpression of active, but not of inactive caspase-4 resulted in processing to two variants of ∼30 kDa in the lysate and in the supernatant, which was not influenced by coexpression of caspase-1. Overexpression of active, but not of inactive caspase-1 alone induces its self-processing, which is known (35). Most importantly, coexpression of active caspase-4 together with inactive caspase-1 restores processing of caspase-1. In addition, inactive caspase-4 prevented activation of active caspase-1. This experiment demonstrates that caspase-4 directly or indirectly supports activation of caspase-1, but not vice versa, suggesting that caspase-4 may act upstream of caspase-1.

Then we coexpressed proIL-1β with caspase-1 and varying amounts of caspase-4 (Fig. 4B, Supplemental Fig. 2). Expression of wild-type (wt) caspase-4 increased secretion of the cytokine (Fig. 4B, data not shown), depending on the amount of the transfected plasmid. In addition, overexpression of wt caspase-4 resulted in cytotoxicity, reflected by slightly enhanced amounts of β-actin in the supernatant, and has been demonstrated earlier (40, 41). In contrast, overexpression of an enzymatically inactive version of caspase-4 had a dominant-negative effect and inhibited caspase-1–dependent secretion of IL-1β in a dose-dependent manner. Surprisingly, overexpression of caspase-4 with caspase-1 resulted also in high amounts of the mature cytokine in the supernatant (Fig. 4B). Processing of proIL-1β by caspase-4 resulted in cytokotoxicity, reflected by slightly enhanced amounts of β-actin in the supernatant, and has been demonstrated earlier (40, 41). In contrast, overexpression of an enzymatically inactive version of caspase-4 had a dominant-negative effect and inhibited caspase-1–dependent secretion of IL-1β in a dose-dependent manner. Surprisingly, overexpression of caspase-4 without caspase-1 resulted also in high amounts of the mature cytokine in the supernatant (Fig. 4B). Processing of proIL-1β by caspase-4 yielded three different bands for IL-1β on Western blots, and two of them migrated faster than mature IL-1β generated by caspase-1 overexpression alone. This suggests that processing of proIL-1β by caspase-4 is less accurate than by caspase-1.

For a better comparison, we overexpressed proIL-1β and caspase-1 with low, but similar amounts of caspase-4 versions. Then we analyzed processing of proIL-1β by Western blotting (Fig. 4C). Cotransfection of plasmids coding for caspase-4 strongly enhanced processing of proIL-1β and secretion of IL-
In contrast, maturation was inhibited by expression of enzymatically inactive caspase-4. Expression of proIL-1β with caspase-4, but without caspase-1, resulted in only slightly reduced amounts of mature IL-1β compared with coexpression of caspase-1, confirming that overexpressed caspase-4 can directly or indirectly process proIL-1β. Overexpression of enzymatically active caspase-4 induced partial cell lysis, which is reflected by enhanced amounts of β-actin in the supernatant (Fig. 4C). Thus, IL-1β in the supernatant results not only from secretion, but also from passive release.

These overexpression experiments in COS-1 cells demonstrate that caspase-4 supports caspase-1 processing as well as caspase-1-dependent activation of proIL-1β. They also suggest that overexpressed caspase-4 alone may be sufficient for processing and secretion of the cytokine.

Caspase-4 physically interacts with caspase-1

Then we examined a possible direct physical interaction between caspase-1 and -4 with CoIP experiments using lysates of transfected COS-1 cells. Because overexpression of caspase-4 in COS-1 cells resulted in self-processing of the protease and in lysis (Supplemental Fig. 2, Fig. 4B, 4C), we used an enzymatically inactive mutant of the protease. Indeed, overexpressed, Myc-tagged, and inactive caspase-4 clearly interacted with caspase-1 (Fig. 5A), but neither with caspase-3, -5, and -9 nor with NLRP-1, -3, or Aim2 (data not shown). Then we wondered which domain of caspase-1 is bound by caspase-4. As both proteases carry a CARD at their N termini, binding can be due to homotypic interaction or caspase-4 might bind to the p10 or p20 subunit of caspase-1. The latter is the interaction domain for binding of unconventionally secreted proteins such as proIL-1α, fibroblast growth factor 2, or Aip-1 (19) (our unpublished results). Caspase-4 clearly bound to the p20 subunit of caspase-1, but not to its CARD (Fig. 5B, 5C, data not shown). As an additional control for the functionality of the CARD construct of caspase-1, we performed a CoIP with Myc- and HA-tagged caspase-1 CARDs, which demonstrated a strong CARD–CARD interaction (data not shown). The binding domain of caspase-4 is its p20 subunit and not its CARD (Fig. 5C, data not shown). However, also the p10 subunits of caspase-1 and -4 are involved in the interaction of the proteases (Supplemental Fig. 3).

Overexpression of caspase-4 with proIL-1β resulted in large amounts of mature IL-1β, which suggests a processing of the cytokine by the protease due to a direct physical interaction (Fig. 4B, 4C). As expected, caspase-4 interacted with HA-tagged proIL-1β in transfected COS-1 cells (Fig. 5D).

**FIGURE 5.** Caspase-4 physically interacts with caspase-1 and with proIL-1β. CoIP of caspase-4 (or its p20 subunit) and caspase-1 (or its p20 subunit), proIL-1β, or Asc from lysate of transfected COS-1 cells (A–E) or from lysate of UVB-irradiated human primary keratinocytes (F). Abs used for immunoprecipitation or CoIP and Western blots are indicated. When tagged proteins were expressed, Abs against the epitopes were used. A–E, COS-1 cells were transfected with plasmids coding for the indicated proteins. To avoid toxic effects, the inactive mutants caspase-1 (C285A) and caspase-4 (C258A) were overexpressed. Incubation with protein A-Sepharose served as a control (ctr.). F, Endogenous caspase-1 or proIL-1β was precipitated with a rabbit (caspase-1) or mouse (proIL-1β) Ab (20 μg) and protein A-Sepharose from lysate of human primary keratinocytes, which had been irradiated with UVB 3 h before lysis. The same amount of a rabbit Ab against HA (caspase-1) or a mouse Ab against Flag (proIL-1β) served as a negative (isotype) control.
Recently, it has been demonstrated that caspase-1 can bind to Asc due to a CARD–CARD interaction (33). However, we did not detect an interaction of caspase-4 with Asc (Fig. 5E), not even upon coexpression of caspase-1 (data not shown). Caspase-4 bound to itself (data not shown), raising the possibility that activation of overexpressed caspase-4 is a direct intermolecular process (Fig. 4A).

Then we examined a possible interaction of caspase-4 with caspase-1 and proIL-1β at the endogenous level in keratinocytes. We immunoprecipitated caspase-1 and proIL-1β; HA and Flag Abs served as a negative (isotype) control. Caspase-4 was detected in the caspase-1, but not in the proIL-1β precipitate (Fig. 5F). In contrast, caspase-1 clearly interacted with proIL-1β, as expected (data not shown).

These CoIP experiments demonstrate that caspase-4 can directly interact with caspase-1 when overexpressed and at the endogenous level. In contrast, detectable amounts of caspase-4 bind to proIL-1β only upon overexpression.

Caspase-4 is required for inflammasome activation in activated THP-1 cells

Showing that caspase-4 expression is needed for activation of the NLRP-3 inflammasome in UVB-irradiated keratinocytes, we wondered whether caspase-4 plays a more general role in inflammasome activation. Therefore, we generated stable THP-1 short hairpin RNA (shRNA) cell lines by lentiviral transduction. We used the siRNA sequences directed against caspase-4 (Fig. 2) and a sequence against lamin as a control. The knockdown efficiency was determined by quantitative RT-PCR as well as by Western blot, showing a very efficient downregulation of caspase-4 mRNA and protein expression by sequence 1 and a less efficient knockdown by sequence 2 (Fig. 6A, Supplemental Fig. 4A, data not shown). Interestingly, stimulation of the NLRP-3 inflammasome with MSU or ATP as well as the Aim2 inflammasome with poly(dA:dT). Interestingly, a knockdown of caspase-4 resulted in a reduction of the activation of caspase-1 and in less secretion of IL-1β for all applied stimuli as well as in reduced secretion of IL-18 from MSU- or poly(dA:dT)-stimulated cells (Fig. 6B–E, Supplemental Fig. 4D).

These experiments demonstrate that caspase-4 expression is required for activation of the NLRP-3 and Aim2 inflammasomes in THP-1 cells, pointing to a general role of caspase-4 in inflammasome activation.

Discussion

Although the gene coding for caspase-4 is located on the same locus as the caspase-1 gene, experimental data have suggested to date that caspase-4 is implicated in ER stress-induced apoptosis (30). In this study, we describe an essential function of caspase-4 for the activation of the inflammatory caspase-1 in different inflammasome complexes and in different cell types.

In mice, caspase-11 is required for activation of caspase-1 (27); however, humans express caspase-4 and -5 from the caspase-11 locus, and it is not clear whether they represent functional orthologs of murine caspase-11 (8). The hypothesis that caspase-4 is involved in inflammasome activation is supported by the ob-
servation that caspase-4 is highly expressed in the epidermis (37) (Fig. 1B) and by keratinocytes in vitro (Fig. 1A). UV irradiation of keratinocytes induced release of caspase-4 (Fig. 1A), which was dependent on caspase-1 expression (Fig. 2B). Because caspase-4 lacks a signal peptide, this release most likely represents ER/Golgi-independent unconventional protein secretion (20). As expected, caspase-4 could also bind to caspase-1 when overexpressed as well as at the endogenous level in keratinocytes (Fig. 5A, 5B, 5F), and this interaction is most likely mediated by the mature part of caspase-1 and -4 (Fig. 5C, Supplemental Fig. 3). Processing of endogenous caspase-4 could not be detected, most likely due to the quality of our Abs. However, a band of ∼30 kDa appeared in the supernatant of keratinocytes and in the lysate and supernatant of COS-1 cells, when the active version of caspase-4 was overexpressed (Figs. 3B, 4A). Interestingly, in contrast to COS-1 cells, we found significantly lower levels of active caspase-4 compared with an inactive mutant upon transfection in keratinocytes, suggesting that active caspase-4 undergoes self-processing in keratinocytes following by degradation, which has also been shown for caspase-1 (42) (Fig. 3, Supplemental Fig. 1). Overexpressed caspase-4 was able to support processing of caspase-1 in keratinocytes and COS-1 cells, but not vice versa (Figs. 3, 4A), suggesting that caspase-4 activates caspase-1 and acts upstream of the inflammasome. Surprisingly, caspase-4 interacted with proIL-1β, when both proteins were overexpressed (Fig. 5D). In line with this finding, proIL-1β was processed directly or indirectly by caspase-4 in overexpression experiments (Fig. 4B, 4C, Supplemental Fig. 2). Caspase-1, -4, and -5 share a very similar substrate specificity in vitro (38, 39), which represents an explanation for this unexpected finding. The detection of smaller IL-1β fragments upon overexpression of proIL-1β and of caspase-4 may represent inaccurate (42) or indirect processing of the cytokine precursor by the protease, and supports this hypothesis. Most importantly, at the endogenous level in UV-irradiated keratinocytes, expression of both caspase-1 and -4 was required for efficient IL-1β secretion and for unconventional secretion of other proteins (Fig. 2). These experiments suggest that caspase-1 is the true IL-1β–converting enzyme in UV-irradiated keratinocytes, whereas expression of caspase-4 is required for activation of caspase-1.

To address the question whether caspase-4 expression is also required in other cell types for NLRP-3 inflammasome activation, we generated stable caspase-4 shRNA knockdown THP-1 cells required in other cell types for NLRP-3 inflammasome activation, UV-irradiated keratinocytes, whereas expression of caspase-4 is only required for noncanonical inflammasome activation (28), caspase-4 in human cells seems to have a much broader role. Therefore, caspase-4 plays an important role in innate immunity, which is in line with the fact that its gene is located on the caspase-1 locus.

Acknowledgments

We thank M. Spalinger for excellent technical assistance and S. Werner for helpful support and for providing the psoriatic biopsies, human skin wounds, and belly skin. The actin-interacting protein 1 Ab was a generous gift of J. Yuan (Harvard Medical School, Boston, MA), and the annexin A2 Ab was from V. Gerke (University of Münster, Germany). We thank Richard D. Iigo (Bordeaux, France) for the vector pAB286.1 and Jürg Tschopp (Lausanne, Switzerland) for pMD2-VSVG, pCMV-βg.R8.91, and the expression constructs for NLRP-1 and -3.

Disclosures

The authors have no financial conflicts of interest.

References


