This information is current as of April 19, 2017.

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*J Immunol* published online 4 April 2014

http://www.jimmunol.org/content/early/2014/04/04/jimmunol.1203524

Supplementary Material

http://www.jimmunol.org/content/suppl/2014/04/04/jimmunol.1203524.DCSupplemental

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The Journal of Immunology is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Human Procaspe-1 Variants with Decreased Enzymatic Activity Are Associated with Febrile Episodes and May Contribute to Inflammation via RIP2 and NF-κB Signaling

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The proinflammatory enzyme caspase-1 plays an important role in the innate immune system and is involved in a variety of inflammatory conditions. Rare naturally occurring human variants of the caspase-1 gene (CASP1) lead to different protein expression and structure and to decreased or absent enzymatic activity. Paradoxically, a significant number of patients with such variants suffer from febrile episodes despite decreased IL-1β production and secretion. In this study, we investigate how variant (pro)caspase-1 can possibly contribute to inflammation. In a transfection model, such variant procaspase-1 binds receptor interacting protein kinase 2 (RIP2) via Caspase activation and recruitment domain (CARD)/CARD interaction and thereby activates NF-κB, whereas wild-type procaspase-1 reduces intracellular RIP2 levels by enzymatic cleavage and release into the supernatant. We approach the protein interactions by coimmunoprecipitation and confocal microscopy and show that NF-κB activation is inhibited by anti–RIP2-short hairpin RNA and by the expression of a RIP2 CARD-only protein. In conclusion, variant procaspase-1 binds RIP2 and thereby activates NF-κB. This pathway could possibly contribute to proinflammatory signaling. The Journal of Immunology, 2014, 192: 000–000.

The proinflammatory enzyme caspase-1 plays an important role in the innate immune system and is involved in a variety of inflammatory conditions such as periodic fevers or gout. Normally, inflammasome activation results in procaspase-1 auto-processing to generate the active form. This heterotetrameric enzyme containing two p10 and two p20 subunits is able to process the proforms of IL-1β and IL-18 into their biologically active mature forms (1–8). These cytokines subsequently participate in an appropriate response of the immune system to tissue damage or pathogen invasion (9) but also can play a role in autoinflammatory conditions (10). Recently, we reported seven rare naturally occurring genetic variants of human procaspase-1, which exhibited altered protein structure and showed decreased or absent autoprocessing and enzymatic activity (11). However, such procaspase-1 variants with decreased activity were detected in patients with autoinflammatory disease. Therefore, we discussed possible disease modifying effects of the (pro)caspase-1 variants because they may have attenuated an otherwise more severe disorder (11). In addition, Lankin et al. (12) showed that procaspase-1 with a defective active center can bind receptor interacting protein kinase 2 (RIP2) and thereby activate NF-κB. This NF is known as a key player in orchestrating inflammation (13). Therefore, we explored whether the naturally occurring procaspase-1 variants also can have a proinflammatory effect.

Materials and Methods

Abs

The following Abs were used: anti–procaspase-1 A-19 (sc-622), anti–caspase-1 p20 h297 (sc-22163), and anti–RIP2 N-19 (sc-8610) all from Santa Cruz Biotechnology (Santa Cruz, CA); anti–RIP2 (PX093) from Cell Sciences (Canton, MA); anti–RIP2 (SP6260P) from Acris (Hidenhausen, Germany); anti–RIP2 D10B11 from Cell Signaling Technology (Beverly, MA); anti–RIP2 (8427) from Abcam (Cambridge, U.K.); anti–GAPDH (H86045M) from Meridian Life Science (Saco, ME); anti–vesicular stomatitis virus (VSV) (V5507) from Sigma-Aldrich (St. Louis, MO); HRP-linked anti-rabbit (NAS540) from GE Healthcare (Freiburg, Germany); HRP-linked anti-mouse (P0260) from DakoCytomation (Glostrup, Denmark); and Alexa Fluor 488-linked anti-rabbit (A-11008) and Alexa Fluor 568-linked anti-rabbit (A-11011) both from Life Technologies (Darmstadt, Germany).

Plasmids

Plasmids were encoding procaspase-1, VSV-tagged RIP2-kinase negative, and caspase activation and recruitment domain (CARD)<sup>Δ</sup> RIP2 and were gifts to the laboratory by Prof. Dr. Dirk Lindemann, Department of Virology, Medical Faculty of Technische Universität Dresden, Germany; and Prof. Dr. Thomas Tilmann, Institute for Virology, Medical Faculty of Technische Universität Dresden, Germany.
from Prof. J. Tschope (University of Lausanne, Lausanne, Switzerland). The luciferase reporter plasmid pBluIVuci was a gift from Prof. G. Nemerow (University of Michigan, Ann Arbor, MI). All plasmids for procaspase-1 variants have been described in Ref. 11. RIP2-wild-type (wt) was created by site-directed mutagenesis of the VSV-tagged RIP2-kisane-negative plasmid using the QuikChange II XL Site-Directed Mutagenesis Kit from Agilent Technologies (Santa Clara, CA), according to the manufacturer’s protocol. The CrmA plasmid was a gift from Dr. J. Bugert (Cardiff University, Cardiff, U.K.).

**Transfection protocol**

For transfection, HEK 293T cells were seeded in 6-well plates, transfected 24 h later with the respective plasmids with 1.75 μg/ml polyethyleneimine, and evaluated 24 h later.

**NF-κB luciferase reporter assay**

NF-κB activity was determined using the Luciferase Assay System from Promega (Manheim, Germany), according to the manufacturer’s protocol. The activity of the luciferase was measured sequentially on a Mithras LB940 (Berthold, Bad Wildbad, Germany).

**Knockdown of RIP2 in HEK 293T cells**

HEK 293T cells were transduced with anti-RIP2 short hairpin RNA (shRNA) lentiviral particles (sc-37389-V) or control shRNA lentiviral particles (sc-108080); both from Santa Cruz Biotechnology using 3 μg/ml Polybrene, according to the manufacturer’s protocol. Cells were cultured in IMDM 10% FCS and selected with 4 μg/ml puromycin. Protein knockdown was analyzed by Western blotting using anti-RIP2 PX093 from Cell Sciences. Loaded protein was normalized to total protein concentration determined with DC Protein Assay from Bio-Rad (Munich, Germany).

**Immunofluorescence staining**

Human peripheral blood monocytes were isolated from PBMCs using CD14-magnetic beads from Miltenyi Biotec (Bedford, MA) and differentiated to macrophages by incubation in M-CSF (20 ng/ml) for 7 d. The cells were then incubated with or without crude LPS (10 ng/ml) for 4 h, fixed in 4% paraformaldehyde for 10 min at 4˚C, permeabilized and permeabilized in PBS containing 0.04% saponin and 1% BSA for 1 h. The cells were then incubated with the appropriate primary Ab for 2 h at 37˚C and secondary Ab for 45 min. Cover slips were mounted on glass slides in Vectashield mounting medium from Vector Laboratories (Burlingame, CA). Cells were imaged using a Zeiss LSM 510 confocal microscope from Carl Zeiss (Jena, Germany) or a Leica TCS SP5 from Leica Microsystems (Wetzlar, Germany) with a ×40 or a ×63 1.4NA objective lens. An argon laser was used for excitation of Alexa488 at wavelengths of 488 nm, whereas a helium-neon laser (543 nm) was used for excitation of Alexa568.

Quantification of colocalization was performed with Fiji (14).

**Lentiviral transduction of THP-1 cells**

Expression of endogenous (pro)caspase-1 was knocked down using the shRNA expression vector pKO.1 (Addgene, Cambridge, MA). The following target sequences were used: CASP1-3'- untranslated region, “AGAGATCTCTCTTGAAAGGT;” and control, “AGAGCTCTTCTGTTAAGAGT.” The knockdown was confirmed by Western blotting. An internal ribosome entry site (IRES) cassette was annexed to the Flag-tagged cDNA of wt or rarest variants (R221C/wt, L265S/wt, T267I/wt, R240Q/R240Q, C285A with a defective active center was included as control. The expression of endogenous (pro)caspase-1 was knocked down using the pBluIVuci plasmid and evaluated 24 h later.

**Communoprecipitation using HEK 293T cells**

Cells were transfected in 6-well plates with RIP2-V5 (800 ng) and/or variants of procaspase-1-Flag (200 ng) plasmids. After 24 h, cells were lysed with lysis buffer containing 20 mM Tris/HCl (pH 7.4), 150 mM NaCl, 10 mM EDTA, 0.2% Nonidet P-40, 1 mM PMSE, and complete EDTA-free protease inhibitor (Roche Applied Science, Mannheim, Germany). Lysates were clarified and analyzed by SDS-PAGE and Western blotting using anti-V5 or anti–procaspase-1 A-19. For immunoprecipitation, lysates were incubated with anti-V5 for RIP2 precipitation or anti–procaspase-1 A-19 for 2 h at 4˚C with rotation. Precipitates were analyzed by SDS-PAGE and Western blotting. All experiments were repeated at least three times.

**RIP2 cleavage experiments**

For RIP2 cleavage experiments, zVAD-FMK (50 μM) from Calbiochem (catalog number 219007; Merck Millipore, Billerica, MA) was added to the cell media 3 h prior harvesting. CrmA was supplied by cotransfection of a CrmA plasmid. RIP2 protein was detected by Western blot analysis using the anti-RIP2 Ab SPP266P.

In addition, the tandem fusion construct mCherry-RIP2-EGFP was introduced into the vector p6NST51. p6NST51 is a variant of p6NST50 (16) having the SFFV U3-driven IRES EGFP-Zeo expression cassette replaced by a IRES-Zeo resistance cassette. mCherry and EGFP genes have been purchased from BD Clontech (Mountain View, CA). RIP2 protein levels were determined by Western blot using anti-RIP2 Ab FX093 (Cell Sciences) for N-terminal RIP2 and anti-RIP2 Ab D10811 (Cell Signaling Technology) for C-terminal RIP2.

**RIP2 release experiment**

To study the release of RIP2, HEK 293T cells were transfected with plasmids coding for RIP2 and either wt procaspase-1 or the enzymatically inactive variant C285A. Proteins in the supernatant were precipitated with methanol/chloroform. The cells had been cultured in media including insulin-transferrin-selenium-ethanolamine (Life Technologies) instead of PBS for the last 24 h.

**Statistics**

Significance of the NF-κB assay results and of Table I was determined by the paired Wilcoxon rank-test (Holm adjusted) and Fisher’s exact test, respectively.

**Results**

**Procaspase-1 variants are associated with febrile episodes**

Recently, we characterized structure and function of different genetic variants of procaspase-1 (p.R221C, p.R240Q, p.N263S, p.L265S, p.T267I, p.K319R, and p.A329T) found in a healthy control population and in patients with recurrent febrile episodes (11). These procaspase-1 variants did not segregate with disease within families. Almost all relatives of patients with and without the variants were immunologically healthy. This excludes the variants from causing a confounding disorder. However, we observed a statistically significant difference for the procaspase-1 variants to occur more often in patients than in healthy donors (Table I). Ethnicity is probably not a confounding factor because results are still significant within each group of Arabs, Turks, and whites. The rarest variants (R221C/wt, L265S/wt, T267I/wt, R240Q/R240Q, and R240Q/K319R) occur in patients only.

Taken together, these results support the assumption that at least some of the variants are disease promoting cofactors.

**Procaspase-1 variants activate NF-κB**

Next, we analyzed a possible mechanism that could explain how the procaspase-1 variants contributed to proinflammatory signaling. For this purpose, we transfected HEK 293T cells with plasmids coding for wt and variant procaspase-1. The artificial variant C285A with a defective active center was included as control. The release of active NF-κB from the intrinsic NF-κB/IκB complex was measured by a luciferase reporter assay. The variant R221C was only weakly expressed at the protein level (11) and therefore...
not analyzed in this study. The other variants (N263S, K319R, R240Q, L265S, and C285A) and wt procaspase-1 were equally expressed after transfection (Fig. 1A). Furthermore, protein levels of endogenous RIP2 were also similar (Fig. 1A). The caspase-1 variants induced an increase in NF-κB activity that was significant in R240Q, L265S, and C285A (Fig. 1B). This increase roughly correlated inversely with enzymatic activity.

Enhanced NF-κB activity was not accompanied by a increase or decrease of cell death rates of the transfected HEK 293T cells as measured by caspase-3 activation or ethidiumbromide staining of the transfected cells (Supplemental Fig. 1A, 1B). Hence, altered cell death rates do not explain differences in NF-κB activity.

The increase in NF-κB activity induced by variant procaspase-1 depends on RIP2

To investigate the participation of RIP2 in the observed enhanced NF-κB activation, we performed a cotransfection experiment with a plasmid coding for the CARD domain of RIP2 in HEK 293T cells. The coexpression of this plasmid alone abolished the procaspase-1–mediated NF-κB activation probably by blocking the

Table I. Procaspase-1 variants are associated with febrile episodes within different ethnic groups

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Patients&lt;sup&gt;a&lt;/sup&gt;: Variant Alleles/Total Alleles</th>
<th>Control&lt;sup&gt;b&lt;/sup&gt;: Variant Alleles/Total Alleles</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whites</td>
<td>22/800</td>
<td>1/214</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>Turks</td>
<td>7/102</td>
<td>2/200</td>
<td>p = 0.008</td>
</tr>
<tr>
<td>Arabs</td>
<td>3/12</td>
<td>4/200</td>
<td>p = 0.004</td>
</tr>
</tbody>
</table>


<sup>a</sup>Patients suffer from recurrent febrile episodes.

<sup>b</sup>Healthy blood donors.

FIGURE 1. NF-κB activation by procaspase-1 variants is mediated by RIP2. (A) Similarity of the expression levels of endogenous RIP2 as well as the different overexpressed procaspase-1 variants in HEK 293T was illustrated by Western blot using anti-RIP2 Ab D10B11 (C-terminal; Cell Signaling Technology) and anti-caspase-1 Ab A19 (Card-Domain; Santa Cruz Biotechnology). (B) Fold increase of intrinsic NF-κB activation in HEK 293T cells as measured by a luciferase reporter after transfection with plasmids coding for procaspase-1 variants as compared with transfection with empty plasmid (–, set to 1), n = 9. (C) Similar to (B), but cotransfected with a plasmid coding for the CARD domain of RIP2 where indicated; ordinate: arbitrary fluorescence units of luciferase substrate, n = 3. (D) Similar to (B), but transfection with plasmids coding for procaspase-1 variants after transduction of cells with anti-RIP2 shRNA (a-RIP2) or with irrelevant control shRNA (ctrl), n = 10. Inset confirms knockdown of RIP2 by a Western blot analysis with anti-RIP2 Ab PX093 (N-terminal; Cell Sciences).
interaction of the CARD domain of procaspase-1 with the CARD domain of complete RIP2 (Fig. 1C).

Furthermore, we knocked down RIP2 in HEK 293T cells with anti–RIP2-shRNA. Strongly decreased protein levels of RIP2 were confirmed (inset in Fig. 1D). Knockdown of RIP2 led to a significant reduction of NF-κB activation by the variants L265S and C285A compared with control cells (Fig. 1D). Possible shRNA off-target effects were excluded using anti–RIP2-shRNA constructs targeting two additional coding sequences of RIP2 (Supplemental Fig. 2).

Taken together, these data show that the procaspase-1 variants activate NF-κB via RIP2 in a transfection model.

**Interaction and colocalization of procaspase-1 and RIP2 in THP-1 cells and human macrophages**

In a next step, we examined whether endogenous procaspase-1 interacts with endogenous RIP2 in cells that belong to the immune system. Therefore, native THP-1 cells, differentiated with PMA and stimulated with crude LPS (cLPS) for different time intervals, were lysed, and endogenous RIP2 was immunoprecipitated. We found that endogenous procaspase-1 coprecipitated in a time-dependent manner (Fig. 2A). The strongest RIP2–procaspase-1 interaction occurred after 60–80 min and decreased after ∼3 h.

Next, we localized RIP2 and procaspase-1 in human monocyte–derived macrophages using Ab staining and confocal microscopy. After stimulation with cLPS, RIP2 and procaspase-1 colocalized in a cytoplasmic compartment adjacent to the nucleus of the macrophages. This happened in a time dependent manner and in accordance with the communoprecipitation (co-IP) experiment (Fig. 2A). The highest rate of cells showing this colocalization was found 60–80 min after the beginning of cLPS stimulation (Fig. 2B, 2C). Medium exchange alone did not induce colocalization of RIP2 and procaspase-1 (Supplemental Fig. 3). The colocalization diminished over ∼3 h and then reached the level of nonstimulated cells. The localization of procaspase-1 and RIP2 in the same area was further ensured by determining Pearson’s correlation coefficient [which will be close to 1 if green and red channel distributions are linked (17)] and Manders’ overlap coefficient (Fig. 3A). In accordance with the cell staining, fluorescence intensity profiles of both colors correlated in a corresponding histogram (Fig. 3B).

**Analysis of interaction of procaspase-1 variants with RIP2 by Co-IP**

To investigate the interaction of RIP2 with an inactive procaspase-1 variant, we established THP-1 cells that had been transduced with shRNA targeting a sequence of the 3′-untranslated region of wt procaspase-1 mRNA to knock down endogenous wt procaspase-1. The reconstitution with either wt procaspase-1 or the enzymatically inactive variant L265S was realized by a second transduction. Western blot analysis showed similar amounts of introduced procaspase-1 and procaspase-1 (Supplemental Fig. 2). However, interaction of the enzymatically inactive variant L265S with RIP2 was increased compared with wt procaspase-1 (Fig. 4A, IP: a-RIP2, bottom row). Although the enzymatically inactive variant was expressed 1.3-fold stronger than reconstituted wt procaspase-1 the lysate, the precipitation of the L265S variant with RIP2 was 2.3-fold higher as determined densitometrically.

In parallel, we cotransfected HEK 293T cells with plasmids coding for VSV-tagged RIP2 and either wt procaspase-1 or one of the enzymatically inactive variants L265S and C285A to gain higher levels of these proteins within cells. Again, IP of VSV-RIP2 led to coprecipitation of procaspase-1 and vice versa (Fig. 4B).
However, we noticed that cotransfection with a plasmid coding for wt procaspase-1 yielded much fainter VSV-RIP2 bands compared with cells cotransfected with plasmids coding for L265S or C285A variants (Fig. 4B, lysate, upper row). The differences of the VSV-RIP2 protein levels became even stronger in the co-IP (Fig. 4B, a-procaspase-1 IP). Similar to the anti-RIP2 IP with the transduced THP-1 cells, anti–VSV-RIP2 IP in transfected HEK 293T cells yielded much stronger bands of enzymatically inactive procaspase-1 variants compared with wt procaspase-1 (Fig. 4B, a-VSV IP).

These data indicate that interaction of enzymatically inactive procaspase-1 variants with RIP2 was enhanced.

wt but not variant procaspase-1 decreased intracellular RIP2 levels by enzymatic cleavage and by release of RIP2 into the supernatant

The previous experiments suggested decreased VSV-RIP2 protein levels in cells cotransfected with a plasmid coding for wt procaspase-1. Next, we investigated this phenomenon. First, plasmids coding for different procaspase-1 variants were transfected together with a plasmid coding for VSV-RIP2 into HEK 293T cells. In Western blot analysis, wt and enzymatically residual active procaspase-1 variants (e.g., N263S) yielded indeed fainter bands for VSV-RIP2 than inactive procaspase-1 forms (Fig. 5A). Moreover, an 8-kDa C-terminal cleavage product of RIP2 could be found when active procaspase-1 had been cotransfected (Fig. 5A).

Second, cleavage experiments with the tandem fusion protein mCherry-RIP2-EGFP (cartoon in Fig. 5B) revealed another RIP2 cleavage product at the N terminus fused to mCherry (80 kDa; Fig. 5B, first row). The C-terminal cleavage product fused to GFP (30 kDa) could be confirmed (Fig. 5B, second row).

Third, two different kinds of caspase-inhibitors, zV AD-CMK and CrmA, prevented the wt procaspase-1–mediated reduction of VSV-RIP2 protein level in the lysate (Fig. 5C). Finally, we tested the hypothesis that coexpression of wt procaspase-1 with VSV-RIP2 induces the release of VSV-RIP2 into the supernatant. As shown in Fig. 5D, VSV-RIP2 could be detected in the supernatant of cells, which had been cotransfected with a plasmid coding for wt procaspase-1 in contrast to cells with the inactive variant C285A or without procaspase-1 (Fig. 5D, supernatant). This release of VSV-RIP2 correlated with a reduction of intracellular VSV-RIP2 protein level (Fig. 5D, lysate). Activation...
of wt procaspase-1 was confirmed by the detection of p20 in the lysate, independent of VSV-RIP2 cotransfection. The p20 subunit was not detected in cells expressing the inactive variant C285A (Fig. 5D, lysate). However, p20 was only released into the supernatant of cells, which had been cotransfected with a plasmid coding for VSV-RIP2 and wt procaspase-1 (Fig. 5D, supernatant).

These data indicate that wt procaspase-1 but not enzymatically inactive procaspase-1 variants contributed to the reduction of intracellular VSV-RIP2 levels by enzymatic cleavage and by its release into the supernatant of transfected HEK 293T cells.

**Discussion**

Recently, we have described human hypomorphic genetic variants of procaspase-1 (11). Phagocytes and especially monocytes from individuals with such variants show a trend to produce decreased amounts of active IL-1β (11). Therefore, such individuals should be less prone to develop autoinflammation than others. Unexpectedly, we find a significant correlation between enzymatic less or inactive procaspase-1 variants and the development of autoinflammatory disorders (Table I). In an attempt to solve this paradox, we refer to the suggestion of Lamkanfi et al. (12) and investigate the ability of the naturally occurring human procaspase-1 variants to activate NF-κB via RIP2 interaction.

In a transfection model with endogenous RIP2, we find indeed a robust activation of intrinsic NF-κB by naturally occurring hypomorphic procaspase-1 variants (Fig. 1B). Interestingly, this activation is roughly inversely proportional to the residual enzymatic activity of the variants. It depends on RIP2 and is mediated by CARD/CARD interaction between procaspase-1 and RIP2 (Fig. 1C, 1D). In accordance with this interaction, endogenous RIP2 and procaspase-1 coimmunoprecipitate (Fig. 2A). Moreover, the endogenous proteins colocalize after activation of monocyte-derived macrophages (Figs. 2C, 3) and therefore putatively interact and take part in the same molecular pathways. Using transduction of THP-1 cells with procaspase-1 variants by lentiviral vectors with limited copy numbers per cell, we avoid strong protein overexpression and approximate a physiologic situation (Fig. 4A). As indicated by coimmunoprecipitation, interaction of the enzymatically inactive procaspase-1 variant L265S with endogenous RIP2 is stronger than the respective interaction with wt procaspase-1. Co-IP of procaspase-1 and VSV-RIP2 coexpressed in transfected HEK 293T cells strongly supports the data obtained in THP-1 cells. Enzymatically inactive procaspase-1 variants interact much stronger with RIP2 than wt procaspase-1 (Fig. 4B).

The activation of NF-κB by enzymatic inactive procaspase-1 described in our study is in accordance with the data from Lamkanfi et al. (12). However, in contrast to their study, we avoid wt procaspase-1–mediated cell death (Supplemental Fig. 1A) by reducing caspase-1 expression levels. Moreover, we show equal protein...
levels of wt procaspase-1 and all variants analyzed for NF-κB activation (Fig. 1A). Thereby, we are able to compare the NF-κB activating potential of different procaspase-1 variants with wt procaspase-1. We show an increased NF-κB activation mediated by variant procaspase-1. The underlying mechanism may be similar to NF-κB activation after binding of RIP2 to nucleotide-binding oligomerization domain-containing protein 2 (18). Hypothetically, the procaspase-1 variants with residual or no enzymatic activity induce a longer and/or stronger interaction with RIP2. NF-κB can subsequently bind to regulatory sequences of genes that encode proinflammatory proteins and thereby upregulate their expression (13). In contrast to enzymatically inactive procaspase-1, wt procaspase-1, and procaspase-1 variants with residual activity (e.g., N263S) reduce intracellular levels of overexpressed RIP2 (Fig. 5A). This effect as well as the faint VSV-RIP2 bands in the immunoprecipitation experiments (Fig. 4B) can be explained by RIP2 cleavage and release from cells induced by wt procaspase-1 (Fig. 5). The activation of caspase-1 and the simultaneous release of the p20 subunit by cotransfection of plasmids coding for wt procaspase-1 with RIP2 indeed has already been described (19). In this study, we additionally show the release of the overexpressed RIP2 from the cells. To our knowledge, cleavage of RIP2 by endogenous proteases has not been published to date.

So far, reduced enzymatic activity of caspase-1 realized by inhibition or knockout in mice is mainly thought to decrease inflammation (20–23). Considering the data of Kayagaki et al. (24), most studies on Casp1 knockout mice have been performed with a Casp1/ Casp1l double knockout strain. Therefore, the described reduction of inflammatory effects cannot be linked to Casp1 deletion with certainty. Furthermore, Oasa et al. (25) published data indicating a proinflammatory cytokine response and increased mortality in burn-injured mice treated with AC-YVAD-CMK. In addition, Menzel et al. (26) described a proinflammatory cytokine response and increased organ damage for Casp1 knockout mice in a model for hemorrhagic shock. These data may support our hypothesis as well as the model postulated by Lamkanfi et al. (12) in vivo.

Our patients are either heterozygous or exhibit at least residual enzymatic (pro)caspase-1 activity (11). If the transfection model really applies to these patients, the remaining enzymatic activity may promote inflammatory diseases by increased binding of RIP2 and thereby upregulate their expression (13). Furthermore, Osuka et al. (25) published data indicating a proinflammatory cytokine response and increased mortality in burn-injured mice treated with AC-YVAD-CMK. In addition, Menzel et al. (26) described a proinflammatory cytokine response and increased organ damage for Casp1 knockout mice in a model for hemorrhagic shock. These data may support our hypothesis as well as the model postulated by Lamkanfi et al. (12) in vivo.

Together, our study supports the following hypothesis (Fig. 6): Our patients are prone to autoinflammation. The procaspase-1 variants are disease modifying factors. As depicted in Fig. 6, they may promote inflammatory diseases by increased binding of RIP2 via CARD/CARD interaction and thereby induce an enhanced proinflammatory NF-κB signal. This signal may aggravate a manifest autoinflammatory disorder. It also may increase inflammation in predisposed patients above a certain threshold and thereby unmask such a disease.

Acknowledgments

We thank Prof. Jürg Tsopp (University of Lausanne) for psmiids for procaspase-1, VSV-tagged RIP2-kinase negative, CARDIRIP2, and procaspase-1 and Prof. Gabriel Nuñez (University of Michigan) for the luciferase reporter plasmid pBxIVluci. We also thank Diana Paul and Kathrin Höhne for excellent technical assistance.

Disclosures

The authors have no financial interests of conflict.

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