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*J Immunol* 2009; 183:5593-5599; doi: 10.4049/jimmunol.0900476

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Thalidomide Inhibits Activation of Caspase-1

Martin Keller, Gabriel Sollberger, and Hans-Dietmar Beer2

Thalidomide is an efficient anti-inflammatory and anti-angiogenic drug, but its therapeutic use is problematic due to a strong teratogenic activity. Nevertheless, thalidomide was approved for the treatment of inflammatory skin diseases and certain types of cancer, and it is extensively tested for several other indications. Recently, we demonstrated that active caspase-1, whose activation is dependent on inflammasome complexes, is required for unconventional protein secretion of proinflammatory cytokines such as IL-1 and of the proangiogenic fibroblast growth factor 2. In this study, we show that pharmacological doses of thalidomide strongly reduced the secretion of both proteins. Thalidomide-treated cells also released less of other leaderless proteins, which require caspase-1 activity for their secretion. In line with these findings, the drug inhibited activation and activity of caspase-1 in cultured cells but not in vitro. The latter finding suggests that the pharmacological activity is exerted by a metabolite of the drug. The anti-inflammatory activity of thalidomide was also mediated via caspase-1 in mice. These findings represent a novel mechanism by which thalidomide exerts its pharmacological activity and suggest that inhibition of the activity of IL-1 might represent a novel strategy to substitute thalidomide. The Journal of Immunology, 2009, 183: 5593–5599.

More than 50 years ago thalidomide was introduced as a sedative, tranquilizer, and antiemetic for morning sickness (1, 2). In West Germany, it was also sold as an over-the-counter medication for pregnant women, but was withdrawn from the market in 1961, because more than 10,000 children were born with malformations related to the use of thalidomide by their mothers during pregnancy (1, 2). In the late 1960s, it turned out that thalidomide is an extremely efficient drug for the treatment of Erythema Nodosum Leprosum, a painful complication of leprosy, causing skin lesions, fever, and other symptoms, and the FDA approved thalidomide for this indication (3). In 1994, thalidomide was shown to inhibit angiogenesis, suggesting that this capability is responsible for the drug’s teratogenic activity (4, 5). In addition, it was speculated that inhibition of angiogenesis by thalidomide might also prevent the growth of tumors (4). For several years, thalidomide has been extensively tested as an anti-inflammatory, immunomodulatory, and anti-angiogenic drug for inflammatory skin diseases and cancer (3, 6). Recently, the FDA approved it in combination with dexamethasone for the treatment of multiple myeloma (7, 8).

Despite its pharmacological potency, the mechanisms by which thalidomide affects immune responses and angiogenesis are unclear (1, 2). Thalidomide is most likely not the biologically active compound. Rather, an as yet unidentified oxidized metabolite, which indirectly inhibits the transcription factor NF-κB, is suggested to be responsible for its pharmacological activity (1). Possible mediators are the proinflammatory cytokines IL-1 and TNF-α, which both can activate NF-κB (1, 2). Vice versa, the promoters of these genes were shown to be activated by NF-κB (1, 2). Furthermore, thalidomide reduces the mRNA stability of TNF-α, providing a possible explanation for the drug’s anti-inflammatory activity (9, 10).

We have recently demonstrated that active caspase-1 regulates secretion of several proteins, which lack a signal peptide and do not require the classical endoplasmic reticulum/Golgi-dependent pathway for release (11). These proteins are involved in inflammation, cytoprotection, apoptosis, and angiogenesis. Originally, caspase-1 was identified as an activator of the proinflammatory cytokines proIL-1β and proIL-18, which also lack a signal peptide (12, 13). Caspase-1 itself is activated by innate immune complexes, the inflammasomes (12, 13). Because caspase-1 activity links inflammation with angiogenesis and because both processes are inhibited in vivo by thalidomide, we analyzed whether thalidomide can inhibit caspase-1 activation.

Materials and Methods

Material and Abs

The following reagents were used: (±)-Thalidomide, MIT, siRNA, sodium pyruvate, and primers from Sigma-Aldrich; caspase-1 inhibitor Ac-Tyr-Val-Ala-Asp-chloromethylketone (YVAD)3 and monosodium urate (MSU) from Alexis. For Western blotting, the following Abs were used: anti-caspase-1 (rabbit, sc-622; used for detection of procaspase-1 and the caspase recruitment domain (CARD)); anti-Bid (rabbit, sc-11423) (Santa Cruz Biotechnology); anti-thioredoxin-1 (rabbit, ab16835) (Abcam); anti-β-actin (mouse, A5441) and anti-gelsolin (mouse, G4896) (Sigma-Aldrich); anti- apoptosis-associated speck-like protein containing a CARD (ASC) (rabbit, ALX-210–905-R100) and anti-peroxiredoxin-1 (rabbit, ALX-210–524) (Alexis); anti-IL-1β (mouse; MAB201, R&D Systems); anti-fibroblast growth factor (FGF)-BP was described (14); and anti-Aip1 (rat) was provided by Dr. J. Li, Harvard Medical School, Boston, MA. Secondary Abs were alkaline phosphatase conjugated and were from Promega. For immunohistochemistry an anti-Ly-6-G Ab (rat, 553124, BD Pharmingen) and an anti-rat secondary Ab conjugated with biotin (donkey, 112–065–003, Jackson ImmunoResearch Laboratories) were used.

3 Abbreviations used in this paper: MSU, monosodium urate; CARD, caspase recruitment domain; FGF, fibroblast growth factor; WT, wild type; LDH, lactate dehydrogenase; Asc, apoptosis-associated speck-like protein containing a CARD; IL-1Ra, IL-1 receptor antagonist; NALP, NACHT, LRR, and Pyrin domain-containing protein; YVAD, Ac-Tyr-Val-Ala-Asp-chloromethylketone.
Cell isolation and culture

Human primary keratinocytes were isolated from foreskin and cultured up to passage 6 in Keratinocyte-SFM (Invitrogen) supplemented with epidermal growth factor and bovine pituitary extract. COS-1 cells were cultured in DMEM (Sigma-Aldrich) supplemented with 10% FCS (Abimed-BioConcept) and 1% penicillin/streptomycin.

Human primary fibroblasts were isolated from foreskin and cultured in DMEM supplemented with 20% FCS and 1% penicillin/streptomycin. Human primary mononuclear cells were purified from the buffy coat fraction of different donors via centrifugation through a Ficoll density gradient (Ficoll-Paque, Amersham Biosciences). Cells were cultured in RPMI 1640 medium supplemented with 10% FCS, 1% penicillin/streptomycin, and 1 mM sodium pyruvate.

Animals

Mice with targeted caspase-1 ablation (15) were provided by Dr. C. Grimm (University Hospital Zurich, Switzerland) and backcrossed into the C57BL/6 genetic background. Experiments involving mice were performed with permission from the local veterinary authorities of Zurich, Switzerland.

Cell treatment and stimulation of protein secretion

Cells were treated 2 h before stimulation with thalidomide, caspase-1 inhibitor or solvent only (DMSO) with a final DMSO content of 0.1%. Human keratinocytes were grown to 70% confluency and irradiated with UVB at a dose of 50 mJ/cm² as described (11). Cells and secreted proteins were collected 4 h later. Human mononuclear cells were seeded in 48-well plates at a density of 1.2 × 10⁶ cells per well, grown overnight and stimulated with 20 μg/ml MSU. Cells and secreted proteins were collected 4 h later. Human fibroblasts were grown to confluence, starved overnight in medium without FCS, and supplemented with 1 U/ml heparin. Cells were then irradiated with 5 J/cm² UVA in medium lacking phenol red. Cells and secreted proteins were collected 4 h later.

Caspase-1 activity assay

A colorimetric assay (R&D Systems) with a chromogenic substrate (WEDH) was used to measure caspase-1 activity according to the manufacturer’s instructions. For the experiment described in Fig. 3B, human keratinocytes were treated and irradiated as described above. Four hours after irradiation, cells were lysed and 1/30 of a 14-cm dish was incubated with the chromogenic substrate. For the experiment described in Fig. 3C, 1/8 of a 10-cm dish lysate of COS-1 cells overexpressing caspase-1/Asc (see below) was incubated with thalidomide, caspase-1 inhibitor, or solvent only (DMSO), and with the substrate. For the experiment described in Fig. 3D, human keratinocytes with an siRNA-mediated caspase-1 knock-down (see below) were treated with thalidomide or solvent only (DMSO) for 5 h. Cells were then lysed and optionally treated with 100 μg/ml proteinase K (Roche) for 30 min at 55°C. Proteinase K was heat inactivated for 10 min at 95°C. An amount of this lysate corresponding to 1/12 of a 14-cm dish was then incubated with lysate corresponding to 1/16 of a 10-cm dish of COS-1 cells overexpressing caspase-1/Asc (see above) and with the substrate. After 90 min incubation, the OD was measured at 405 nm. Each experiment was measured in triplicates.

Quantification of neutrophil infiltration after UVB irradiation

Eight-week-old caspase-1 knockout and wild-type (WT) mice were i.p. injected with 200 μg per gram body weight thalidomide suspended in 0.5% carboxymethylcellulose or with carboxymethylcellulose only. The injections were performed during 3 days with one injection each day. On the third day, mice were anesthetized and the back skin of the mice was shaved with an electric animal shaver. The mice were irradiated with a dose of 75 mJ/cm² UVB by placing them under a UV light source (Medison HP-54, Schmid, and Bohm). Twenty-four hours later mice were sacrificed and four skin biopsies of ~1 cm in length were sampled. Biopsies were fixed in acidic ethanol, and paraffin embedded sections were immunohistochemically stained with an Ab against the neutrophil marker Ly-6G and counterstained with H&E. The number of Ly-6G positive cells per mm² dermis was determined using the open laboratory software (Improvision). The percent release was calculated for each well individually according to the formula % release = SN/S(N+S-Lys), where SN is the amount in the supernatant and Lys the amount in the lysate. Each experiment was performed with triplicate dishes. ELISA DuoSet development kits for IL-1β and FGF2 were from R&D Systems and LDH activity assay was from Promega. ELISA and LDH measurements were performed according to the supplier’s instructions.

MTT assay

Medium was supplemented with 1/5 volume of MTT solution (0.5% in PBS). After 60 min incubation, the supernatant was aspirated and the cells were lysed in 40 mM HCl/isopropanol for 10 min. The reaction was stopped by addition of an equal volume of water. OD was measured at 590 nm. Each experiment was performed with triplicate dishes.

Real-time RT-PCR

RNA was isolated from cells grown on a 12-well plate using the High Pure RNA isolation kit and cDNA was synthesized using the Transcriptor First Strand CDNA kit, respectively (Roche). Relative quantification of gene expression was performed using the SYBR Green kit (PE Applied Biosystems) on a LightCycler 480 Real-Time PCR System (Roche). All primer pairs amplified cDNA fragments of ~100 bp in length and flanked one intron. Expression levels of genes were normalized to expression levels of the unrelated gene β-actin. All measurements were performed in triplicates.

Statistical analysis

Statistical analysis was performed using the Prism Software (GraphPad Software). Cell culture experiments were analyzed by one-tailed Student’s t test, and the animal experiment by a one-tailed Mann-Whitney U test as described in the figure legends.

Results

Thalidomide inhibits secretion of IL-1β and FGF2

UVB irradiation of skin-derived human primary keratinocytes activates caspase-1 in an inflammasome-dependent manner, which in turn results in activation of proIL-1β and secretion of the mature cytokine (16). The activation of caspase-1 requires expression of the inflammasome proteins Nalp, Lrr, and Pyrin domain containing protein (NALP3); Asc, and to a lesser extent NALP1 (16). Pharmacological doses of thalidomide (1, 17) added to keratinocytes before UVB irradiation inhibited secretion of IL-1β in a dose-dependent manner (Fig. 1, A and B). Because UV-induced secretion of IL-1β by keratinocytes requires proliferation of the cells, substances or conditions, which inhibit cell growth or induce differentiation, negatively affect IL-1β secretion (16). Although thalidomide has been shown to inhibit cell proliferation at high concentrations (3), a growth inhibitory effect for keratinocytes was
Thalidomide inhibits IL-1β and FGF2 secretion. Different types of primary human cells were treated with the indicated concentrations of thalidomide (Thld) or with caspase-1 inhibitor (Ci; ac-YVAD-cmk) 2 h before stimulation of IL-1β or FGF2 secretion. Secretion of these proteins was stimulated in keratinocytes by irradiation with 50 mJ/cm² UVB (A and B), in blood-derived mononuclear cells by treatment with 20 µg/ml MSU (C and D) and in skin-derived fibroblasts by irradiation with 5 J/cm² UVA (E and F). Four hours after stimulation, the percentage of cytokine release was determined by ELISA measurements of levels in the supernatant and the cell lysate. Accordingly, the percentage of LDH release was determined and served as a control for cell lysis (left axis). Cell viability was determined by measuring MTT activity (right axis). A, C, and E. Values from cells treated with the most effective dose of thalidomide or with caspase-1 inhibitor are shown. B, D, and F. Dose-response curves for secretion of IL-1β or FGF2 and LDH and MTT activity upon treatment with thalidomide are shown. Values obtained with untreated cells were arbitrarily set as 1. All values are expressed as mean ± s.d. of triplicate measurements. *** p < 0.001 of a one-tailed Student’s t test.

not observed at the pharmacological concentrations that we used, and the viability of UV-irradiated keratinocytes was even slightly enhanced (Fig. 1, A and B). Thus, the drug reduced the extent of cell lysis, which is reflected by the decrease in the activity of the cytoplasmic enzyme LDH in the supernatant of cells. In addition, thalidomide enhanced the MTT activity of UV-irradiated cells, which mirrors mitochondrial activity and thereby viability. Interestingly, addition of the caspase-1 inhibitor YVAD to keratinocytes before irradiation had the same effect (Fig. 1A). As it is known that caspase-1 activity is cytotoxic for keratinocytes and other cell types (11, 16), these experiments suggest that thalidomide inhibits activation of caspase-1. The extent of reduction of secretion of IL-1β through thalidomide was comparable to the effect of the caspase-1 inhibitor YVAD (Fig. 1A). To analyze whether inhibition of IL-1β secretion by thalidomide is a more general effect we used an in vitro model for gout (18): When human primary monocytes are treated with MSU crystals, they activate caspase-1, which requires the NALP3 inflammasome (18). Active caspase-1 then processes proIL-1β and the mature cytokine is secreted. Indeed, thalidomide also inhibited secretion of IL-1β in MSU-treated monocytes in a dose-dependent manner (Fig. 1, C and D).

FGF2 is a potent proangiogenic growth factor, which is also secreted by an unconventional pathway (5, 19). We have recently shown that UVA-induced secretion of FGF2 by human primary skin-derived fibroblasts requires caspase-1 activity (11). Strikingly, thalidomide inhibited this secretion to a similar extent as the caspase-1 inhibitor YVAD (Fig. 1, E and F). These experiments demonstrate that thalidomide inhibits secretion of IL-1β and FGF2 from different types of primary cell. The inhibition of secretion was not due to a cytotoxic effect of thalidomide. Rather, under the described conditions, the drug slightly enhanced cell viability to a similar extent as a caspase-1 inhibitor. In addition, thalidomide also significantly reduced absent in melanoma 2-dependent poly(dA:dT)-induced secretion of IL-1β by THP-1 cells (20), although in these experiments thalidomide was less efficient than YVAD (results not shown).

Thalidomide inhibits unconventional protein secretion without affecting expression of inflammasome proteins

What is the cause for the reduced secretion of IL-1β and FGF2 upon treatment of cells with thalidomide? The drug does not negatively influence expression of both proteins (Fig. 2A and results not shown). The secretion of IL-1β and FGF2 requires caspase-1 activity (Fig. 1, A, C, and E) and we recently identified several other leaderless proteins, whose secretion also depends on activity of this protease. These include caspase-1 itself, Asc, Bid, Aip1, peroxiredoxin-1, and thioredoxin-1 (11). Interestingly, thalidomide also inhibited UVB-induced secretion of these proteins by primary keratinocytes to a similar extent as the caspase-1 inhibitor YVAD (Fig. 2A). By contrast, secretion of proteins, which use the classical secretory pathway (gelsolin and FGF-binding protein) was not affected. In addition, the amount of the cytoplasmic protein β-actin, which is not secreted at all but only passively released upon cell lysis, remained constant in the supernatant (Fig. 2A).
Caspase-1 is activated in recently identified innate immune complexes called inflammasomes (12, 13). A reduction of expression of these inflammasome proteins (caspase-1, ASC, NALP-1/3, tripartite motif-16) inhibits activation of caspase-1 and in turn inhibits activation of caspase-1 and in turn inhibits activation of post-ribosomal secretion (11, 16, 19). But the inhibitory effect of thalidomide was not due to suppression of expression of inflammasome proteins (Fig. 2, A and B). Interestingly, mRNA expression of NALP-3 was strongly down-regulated upon UVB irradiation (Fig. 2B and results not shown). This down-regulation was mediated by an unknown factor, which is secreted dependent on caspase-1 activity (results not shown).

**Thalidomide inhibits activation and activity of caspase-1 in cultured cells but not in vitro**

Then, we analyzed caspase-1 in thalidomide-treated cells in more detail (Fig. 3A). Keratinocytes quickly secrete active caspase-1 and other inflammasome proteins after UV irradiation (16). Interestingly, the secretion of the CARD of caspase-1, which results from processing and activation of procaspase-1 (11), was strongly reduced in thalidomide-treated and irradiated keratinocytes compared with mock-treated cells (Fig. 3A). In addition, we directly measured caspase-1 activity in irradiated keratinocytes and found a strong reduction upon thalidomide treatment (Fig. 3B). To test the possibility that thalidomide directly inhibits caspase-1 activity and thereby its activation, we incubated lysate of COS-1 cells overexpressing caspase-1 and Asc, and caspase-1 activity was measured as in B. D, Human keratinocytes with an siRNA-mediated knock-down of caspase-1 activity (Fig. 3D, ctr). Values in B, C, and D represent mean ± s.d. of triplicate measurements. ***, p < 0.005; ***, p < 0.001; ns p > 0.01 of a one-tailed Student’s t test.
Intriguingly, lysate from thalidomide-treated keratinocytes significantly reduced caspase-1 activity in comparison to mock-treated keratinocytes (Fig. 3D). Digestion of the keratinocyte lysate with proteinase K and inactivation of the enzyme through incubation for 10 min at 95°C did not affect the inhibition of caspase-1, suggesting that the inhibition is not caused by a protein or peptide. In conclusion, thalidomide indirectly inhibits caspase-1 activity, most likely via an as yet unidentified metabolite. As caspase-1 contributes to its own processing, further activation of caspase-1 is also inhibited as well as unconventional protein secretion, which is a downstream event of caspase-1 activation.

Caspase-1 is required for the anti-inflammatory activity of thalidomide in mice

Does thalidomide also inhibit caspase-1 activation in vivo? The drug does not affect murine development (1, 2), but its anti-inflammatory and anti-angiogenic activities are well documented in these animals, although higher doses of the drug are necessary than in humans (21, 22). Therefore, mice can be used as a model organism to determine the activity of thalidomide in vivo. We recently showed that neutrophil immigration into the dermis of mice upon UVB irradiation is partly dependent on caspase-1 expression, suggesting that IL-1 secreted by keratinocytes plays an important role in UV-induced skin inflammation (16, 23). Therefore, we treated caspase-1 knockout mice and WT littermates with thalidomide at a pharmacologically active concentration (21, 22) or with vehicle only, irradiated these mice with UVB, and determined the number of neutrophils in the dermis below the irradiated epidermis (Fig. 4). UV irradiation strongly increased the number of these inflammatory cells in WT mice but to a significantly lesser extent in animals lacking caspase-1, confirming our recent data (16). Most importantly, we found similar numbers of neutrophils in thalidomide-treated mice irrespective of caspase-1 expression. If the inhibitory effect of thalidomide were largely independent of caspase-1, one would expect a stronger reduction in neutrophil recruitment in thalidomide- vs mock-treated caspase-1 knockout mice. Thus, these results demonstrate that thalidomide and caspase-1 act in the same pathway in vivo in mice.

Thalidomide suppresses TNF-α production predominantly by modulation of IL-1 secretion

It has previously been shown that thalidomide reduces the stability of TNF-α mRNA, which may explain the drug’s anti-inflammatory activity (9, 10). In this study, however, we found that caspase-1 is inhibited by thalidomide, resulting in decreased secretion of IL-1 and several other unconventionally released proteins. IL-1 often acts downstream of TNF-α in local and systemic inflammatory diseases, because TNF-α can induce proIL-1β expression (24). However, in contrast to macrophages, expression of proIL-1α and -β is constitutive in human keratinocytes and does not require TNF-α (16). Therefore, we analyzed whether TNF-α expression might be downstream of IL-1 in this cell type. Indeed, knock-down of caspase-1 expression or inhibition of its enzymatic activity strongly reduced TNF-α mRNA expression upon UVB irradiation, possibly via regulation of secretion of IL-1 (Fig. 5, A and B). Most likely, siRNA-mediated reduction of caspase-1 expression was more efficient because the protease activates NF-κB also independently of its enzymatic activity (25). In addition, administration of mature IL-1β strongly induced TNF-α expression in cultured keratinocytes (Fig. 5C). UVB irradiation also increased the amount of TNF-α mRNA, but this induction was mainly IL-1-mediated, because it could be blocked by addition of IL-1 receptor antagonist (IL-1Ra) (Fig. 5C). As expected, thalidomide also reduced TNF-α expression (Fig. 5D). However, this reduction was only moderate, compared with TNF-α expression of keratinocytes, which had been treated with IL-1Ra before irradiation (Fig. 5D). In contrast to mock-treated control and UVB-irradiated cells (Fig. 5D, left and middle) thalidomide did not significantly reduce TNF-α expression of UV-irradiated keratinocytes treated with IL-1Ra (Fig. 5D, right). This may be due to the low expression level of TNF-α mRNA in the absence of IL-1-induced transcription. In addition, the extent of TNF-α induction varied between different experiments (compare Fig. 5, C and D and results not shown). Most likely, differences in cell lysis resulted in different amounts of active proIL-1α in the medium (16) and therefore in different levels of TNF-α mRNA expression. To address the question whether thalidomide influences TNF-α mRNA expression independently

FIGURE 4. UVB-induced skin inflammation in mice is reduced by thalidomide in a caspase-1-dependent manner. Caspase-1 knockout mice and WT mice were injected i.p. with 200 μg thalidomide (Thld) per gram body weight three times on consecutive days. After the last injection, mice were shaved and irradiated with 75 mJ/cm2 UVB. One day after irradiation, sections from back skin biopsies were stained with an Ab against the neutrophil marker Ly-6G. A, Micrographs are shown for representative areas of each group. The dotted lines indicate the border between the dermis and epidermis. Arrowheads indicate Ly-6G-positive cells. Bars represent 200 μm. B, Ly-6G-positive cells/mm² dermis were counted. Box plots display median, second and third quartile (box), and first and fourth quartile (whiskers) of mean values from two to four biopsies per mouse. N: number of animals, n: number of biopsies. *, p < 0.01; **, p < 0.005; ns p ≥ 0.01 of a one-tailed Mann-Whitney U test.
Cells were treated with 20 mJ/cm² UVB. Western blots show expression of infected with siRNA as indicated and two days later irradiated with 50 pg/ml IL-1 with the presence or absence of 4.5 pg/ml IL-1 Ra on the expression of TNF-α. Values were normalized to 1 (noninduced). Bars represent mean ± s.d. of triplicate measurements.

**Discussion**

This study demonstrates that thalidomide inhibits activation of caspase-1. The protease’s activity is required for activation of proinflammatory cytokines such as proIL-1β and for unconventional secretion of several other proteins, e.g., of the proangiogenic growth factor FGF2. Therefore, this finding represents a novel explanation for thalidomide’s strong anti-inflammatory and anti-angiogenic activities in vivo.

Thalidomide inhibited secretion of the proinflammatory cytokine IL-1β from human primary keratinocytes and monocytes in a dose-dependent manner (Fig. 1A–D). In addition, the drug also reduced the release of the proangiogenic growth factor FGF2 and of several other leaderless proteins (Fig. 1E and F, and 2A). The secretion of all these proteins requires caspase-1 activity (11). The protease itself is activated by multimeric innate immune complexes, the inflammasomes (12, 13). Although thalidomide did not influence the expression of inflammasome proteins (Fig. 2A and B), it inhibited activation of caspase-1 to a similar extent as the caspase-1 inhibitor YVAD (Fig. 3A and B). However, in vitro thalidomide did not inhibit caspase-1 activity in contrast to YVAD (Fig. 3C). Interestingly, lysate of thalidomide-treated cells reduced the activity of the protease, even if this lysate was digested with proteinase K and heated to 95°C. An explanation for these results represents the fact that thalidomide itself has no biological activity (1). It is most likely an as yet unidentified oxidized metabolite of the drug, which exerts the anti-inflammatory and anti-angiogenic activity, and our results suggest that this metabolite inhibits activity and activation of caspase-1. Most importantly, we demonstrate that thalidomide exerts its anti-inflammatory effect in mice in a caspase-1-dependent manner. We have recently shown that UVB-induced skin inflammation in mice requires caspase-1 (16, 23). Interestingly, thalidomide also reduced UVB-induced recruitment of neutrophils by ~50%, to about the same number found in mice lacking caspase-1 (Fig. 4B). Because thalidomide has no significant effect in caspase-1 knockout mice, this experiment demonstrates that the drug mainly exerts its anti-inflammatory activity in mice through caspase-1. As it has been shown that thalidomide inhibits expression of the proinflammatory cytokine TNF-α (9, 10) we asked how this fits with our finding that the drug blocks activation of IL-1β via caspase-1. We recently showed that activation of caspase-1 in keratinocytes does not depend on a secreted factor (11). In addition, IL-1 is constitutively expressed in this cell type (11, 16). In contrast, expression of TNF-α was strongly induced by IL-1 in keratinocytes (Fig. 5A–E). In line with this finding, it has been shown that ablation of caspase-1 also reduces expression of TNF-α in macrophages, which demonstrates that caspase-1 is also an indirect regulator of TNF-α expression in these cells, most likely via IL-1 (15, 26). In addition, TNF-α expression levels in keratinocytes depended on the conditions of the cells and varied between different experiments (Fig. 5). These variations were most likely due to low but different amounts of biologically active proIL-1α in the medium of keratinocytes, as a result of secretion but also of cell lysis. The IL-1 in the medium then induces expression of TNF-α (16). Nevertheless, preincubation of keratinocytes with IL-1β demonstrated that TNF-α expression is also partly regulated by thalidomide independently of IL-1.
(Fig. 5E). This may also be the reason why thalidomide treatment of mice reduced the UV-induced infiltration of neutrophils to a slightly larger extent than ablation of caspase-1, although this difference was not statistically significant (Fig. 4B).

Intriguingly, it has been shown that thalidomide significantly inhibits UV-induced inflammation in human skin (27, 28). This may also provide an explanation for the drug’s therapeutic effect in photodermatological conditions, in autoinflammatory and autoimmune diseases as well as in certain types of cancer (1–3). It may well be that IL-1 plays a prominent role in these diseases. Therefore, the reduction of its activity via IL-1Ra may be a successful strategy for their treatment.

Acknowledgments
We thank S. Werner (ETH Zurich) for very helpful suggestions, discussions, and comments on the manuscript, D. Hohl (University of Lausanne) for stimulating discussions, J. Li (Harvard Medical School, Boston) for the recombinant version of IL-1Ra (1, 24). Thalidomide is effective in different dermatological conditions, in autoinflammatory and autoimmune diseases as well as in certain types of cancer (1–3). It may well be that IL-1 plays a prominent role in these diseases. Therefore, the reduction of its activity via IL-1Ra may be a successful strategy for their treatment.

Disclosures
The authors have no financial conflict of interest.

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