Caspase 1/11 Deficiency or Pharmacological Inhibition Mitigates Psoriasis-Like Phenotype in Mice

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Inflammatory caspases, activated within the inflammasome, are responsible for the maturation and secretion of IL-1β/IL-18. Although their expression in psoriasis was shown several years ago, little is known about the role of inflammatory caspases in the context of psoriasis. Here, we confirmed that caspases 1, 4, and 5 are activated in lesional skin from psoriasis patients. We showed in three psoriasis-like models that inflammatory caspases are activated, and accordingly, caspase 1/11 invalidation or pharmacological inhibition by Ac-YVAD-CMK (i.e., Ac-Tyr-Val-Ala-Asp-chloromethylketone) injection induced a decrease in ear thickness, erythema, scaling, inflammatory cytokine expression, and immune cell infiltration in mice. We observed that keratinocytes were primed to secrete IL-1β when cultured in conditions mimicking psoriasis. Generation of chimeric mice by bone marrow transplantation was carried out to decipher the respective contribution of keratinocytes and/or immune cells in the activation of inflammatory caspases during psoriasis-like inflammatory response. Our data showed that the presence of caspase 1/11 in the immune system is sufficient for a fully inflammatory response, whereas the absence of caspase 1/11 in keratinocytes/fibroblasts had no impact. In summary, our study indicates that inflammatory caspases activated in immune cells are implicated in psoriasis pathogenesis.


INTRODUCTION
Caspases are a family of proteases (i.e., Cysteine-Aspartic proteases), highly conserved evolutionarily, with a key role in apoptosis and inflammatory signaling pathways (Lamkanfi et al., 2007). In relation to their biological function, caspases have been classified in apoptotic (caspases 2, 3, 6, 7, 8, 9, and 10) and proinflammatory (caspases 1, 4, 5, 11, and 12) (Li and Yuan, 2008), mainly involved in cell death signaling pathways and regulation of cytokine maturation during inflammation, respectively. Nevertheless, proinflammatory caspases have been also implicated in pyroptosis, another programmed cell death (Lamkanfi and Dixit, 2014; Man and Kanneganti, 2015). Although proinflammatory caspases 1 and 12 are functional orthologues between humans and mice, caspases 4 and 5, only present in humans, are the homologues of mouse caspase 11 (Liu and Lieberman, 2017).

Proinflammatory caspases, first produced as inactive zymogens, are activated through multiprotein complexes called inflammasomes after cellular stimulation via engagement of pattern recognition receptors (Martinon et al., 2002). Once activated, inflammatory caspases mediated immune response against infectious stress through the maturation and secretion of proinflammatory cytokines such as IL-1β and IL-18. Caspase 1 activation and subsequent IL-1β production have been associated with a large variety of inflammatory and autoimmune diseases (reviewed in Gabay et al., 2010; McIlwain et al., 2013; and Patel et al., 2017) such as rheumatoid arthritis, type 2 diabetes (Ruscitti et al., 2015), and inflammatory bowel diseases (Perera et al., 2017).

Psoriasis is a chronic autoinflammatory/autoimmune skin disease (Lowes et al., 2014) characterized by an intense dialog between keratinocytes and immune cells, that affects 2% of the worldwide population (Paris et al., 2013). Psoriasis has been classified according to several clinical manifestations, psoriasis vulgaris being the most common type, whose principal feature is the development of red patches with silver scales called psoriasis plaques (Deng et al., 2016). A large and complex network of several proinflammatory cytokines such as IL-1α, IL-1β, IL-17, IL-18, IL-22, IL-23, IFN-γ, and tumor necrosis factor (TNF)-α have been associated with the development and establishment of psoriasis plaques (Nickoloff et al., 2007), highlighting a key role of these cytokines in the pathogenesis of psoriasis.

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Abbreviations: Ac-YVAD-CMK, Ac-Tyr-Val-Ala-Asp-chloromethylketone; dKO, double knockout; IMQ, imiquimod; TNF, tumor necrosis factor; WT, wild type

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Although the role of IL-1 and IL-18 cytokines has been well described in psoriasis pathogenesis (Companjen et al., 2004; Rabeony et al., 2015; Shimoura et al., 2017; Singh et al., 2016; Uribe-Herranz et al., 2013), little is known about the contribution of proinflammatory caspases in this context. It has been showed that activated caspase 1 is increased in lesional skin samples from psoriasis patients compared with nonlesional samples (Johansen et al., 2007; Marchetti et al., 2009). Moreover, an increase in the mRNA expression of caspase 5 was found in lesional biopsy samples (Salskov-Iversen et al., 2011), and a constitutive expression of this caspase was observed in the epidermis of normal skin (Zwicker et al., 2017). However, the contribution of inflammatory caspases in psoriasis pathogenesis is controversial, because Cho et al. (2012) reported a decrease in the epidermal thickness associated with a significant reduction in IL-1β levels in caspase 1/−/− mice treated with imiquimod (IMQ) (Cho et al., 2012), whereas another recent study concluded that the invalidation of caspase 1 had no impact on reducing the psoriasis-like phenotype after IMQ treatment (Rabeony et al., 2015).

Therefore, we decided to address the implication of proinflammatory caspases in psoriasis pathogenesis. First, in biopsy samples from a large cohort of psoriasis patients, we found that caspases 1 and 5 were activated in all lesional samples and that caspase 4 was activated in most lesional samples. Furthermore, caspase 1/11 deficiency and pharmacological inhibition of proinflammatory caspases significantly reduced skin inflammatory disease in three mouse models of psoriasis-like phenotype. Moreover, stimulation of normal human keratinocytes with TNF-α and IL-17A showed that keratinocytes are primed to activate caspase 5 and secrete IL-1β. Finally, by adoptive transfer experiments, we showed that proinflammatory caspase activation in immune cells is sufficient to induce a complete inflammatory response in mice.

RESULTS
Proinflammatory caspases are activated in lesional skin biopsy samples from psoriasis patients
Although increased mRNA expression of proinflammatory caspases 1, 4, and 5 in lesional biopsy samples from psoriasis patients has been already observed (Salskov-Iversen et al., 2011; Zwicker et al., 2017) and expression of cleaved caspase 5 could be detected in one psoriasis patient (Zwicker et al., 2017), we decided to assess the expression of caspases to confirm their activation status in a large cohort of psoriasis patients. We performed Western blot analysis of skin extracts from nonlesional and lesional biopsy samples. Pro-caspase 1, 4, and 5 were all expressed in nonlesional samples; however, only a strong increase of pro-caspase 5 was observed in lesional skin compared with nonlesional paired and healthy donor skin (Figure 1a, b, and d, and see Supplementary Figure S1a online). High levels of cleaved caspase 1 and 5 were observed in lesional skin from all analyzed patients (Figure 1a–d, and see Supplementary Figure S1a), and cleaved caspase 4 was seen in most analyzed lesional biopsy samples (Figure 1a, b, and d, and see Supplementary Figure S1a). Moreover, an increase in inflammasome component expression, along with the mature forms of IL-1β and IL-18, was found (see Supplementary Figure S1b), as previously reported (Dombrowski et al., 2011; Johansen et al., 2007). Altogether, these data suggest that proinflammatory caspase activity is regulated in injured skin from psoriasis patients.

Figure 1. Proinflammatory caspases are activated in lesional skin biopsy samples from psoriasis patients. Protein extraction was performed from total skin of healthy donors (HD) and nonlesional (NL) and lesional (L) skin biopsy samples from psoriasis patients, and then Western blot analyses were carried out. (a–c) Caspases 1, 4, and 5 were blotted in HD and in NL and L biopsy samples from different psoriasis patients, showing caspase activation in lesional samples. (d) Total of analyzed samples, showing number of samples in which activated caspases were present. *Unspecific band.
Deficiency in proinflammatory caspases 1/11 impaired psoriasis-like disease development in different mouse models

Although the role of inflammasome-processed cytokines IL-1β (Feldmeyer et al., 2010) and IL-18 (Compagno et al., 2004) is well-known in psoriasis, the role of proinflammatory caspases in this scenario is controversial. Therefore, to clearly define the role of proinflammatory caspases in psoriasis-like disease, we decided to use three different mouse models: (i) LynΔN transgenic mice (Marchetti et al., 2009), which spontaneously develop psoriasis after birth; (ii) IMQ-induced skin dermatitis (van der Fits et al., 2009); and (iii) IL-23 intradermal ear injections (Hedrick et al., 2009).

First, we used the LynΔN mice (Marchetti et al., 2009), which recapitulate the main features of human psoriasis, as illustrated by the increased skin expression of the principal cytokines implicated in psoriasis pathogenesis (see Supplementary Figure S2a online). An increased mRNA expression of some inflammasome components, such as caspase 11, AIM-2, and NLRP-3 (see Supplementary Figure S2b), associated with an increase in IL-1β expression at both the mRNA and protein levels was observed (see Supplementary Figure S2b and c)). Next, we crossed LynΔN mice with C1/C11-deficient mice (Kuida et al., 1995) to obtain LynΔN C1/C11−/− mice, referred to hereafter as LynΔN double knockout (dKO) mice. The absence of proinflammatory caspases hampered the skin inflammatory phenotype developed by LynΔN mice over time, based on the adapted Psoriasis Area and Severity Index score that we established (Figure 2a, and see Supplementary Figure S3a online), producing a significant delay in the initiation of the psoriasis-like phenotype in LynΔN dKO mice. Moreover, both the lack of weight gain linked to the onset of the disease and the reported death of LynΔN mice (Marchetti et al., 2009) were significantly reduced in LynΔN dKO mice (Figure 2b, and see Supplementary Figure S3b). The improved phenotype observed in LynΔN dKO mice was associated with a decrease in epidermal hyperplasia (Figure 2c and d), along with a decrease in the mRNA expression of inflammasome components such as AIM-2 and NLRP-3 and several proinflammatory cytokines (Figure 2e, and see Supplementary Figure S3c), with the exception of IL-23 and TNF-α, for which similar levels were obtained. Concomitantly, the absence of proinflammatory caspases significantly reduced the levels of IL-1β and IL-18 protein expression observed in LynΔN mice skin (Figure 2f). Therefore, activation of caspase 1/11 is necessary for the development of complete inflammatory skin disease in the LynΔN mouse model.

To confirm the contribution of proinflammatory caspases in psoriasis pathogenesis, we induced a psoriasis-like phenotype either by topical IMQ cream application onto the back skin (Figure 3) or by IL-23 intradermal injections into ears (see Supplementary Figure S4 online) of wild-type (WT) and C1/C11-deficient mice, hereafter referred to as dKO mice. In both models (IMQ and IL-23), the absence of proinflammatory caspases delayed the onset of the clinical score measured. Erythema, scaling, and cumulative scores quantified in the IMQ model (Figure 3a) and ear thickness measured in the IL-23 model (see Supplementary Figure S4a and b) were significantly reduced in dKO mice compared with WT mice. Furthermore, hematoxylin and eosin staining of skin sections showed a decrease in epidermal hyperplasia (Figure 3b and c, and see Supplementary Figure S4c and d), along with a reduction in the inflammatory cell infiltration (Figure 3d, and see Supplementary Figure S4e), with a reduced number of CD45.2+ immune cells in the dKO mice skin, evidenced more precisely by a significant decrease in the number of inflammatory monocytes and neutrophils. Additionally, inflammasome components, proinflammatory cytokine mRNA expression (Figure 3e, and see Supplementary Figures S4f and Figure S5 online) and IL-1β protein levels were significantly reduced in dKO mice in both models (Figure 3f, and see Supplementary Figure S4g online). Surprisingly, IL-18 protein level was differentially modulated only in the IMQ model (Figure 3f). Altogether, these data indicate that regulation of proinflammatory caspase activity is necessary to produce an inflammatory response in psoriasis.

Pharmacological inhibition of caspases 1/11 by Ac-YVAD-CMK compound reduces IMQ-induced psoriasis-like phenotype in mice

It has been previously shown in mice with acute gastric injury that treatment with the selective caspase 1 inhibitor Ac-Tyr-Val-Ala-Asp-chloromethylketone (Ac-YVAD-CMK) led to protection through the attenuation of NLRP-3 inflammasome activity (Zhang et al., 2016). Therefore, we next decided to analyze the effect of Ac-YVAD-CMK treatment in the IMQ model. We treated WT mice with IMQ cream, together with either DMSO or Ac-YVAD-CMK compound injected intraperitoneally.

Although the co-treatment had no additive effect on either weight loss (see Supplementary Figure S6a online) or splenomegaly induced by IMQ treatment (see Supplementary Figure S6b) as reported (van der Fits et al., 2009), a significant reduction in erythema, scaling, and cumulative score was achieved in mice treated with the selective caspase 1 inhibitor (Figure 4a). The delay observed upon pharmacological inhibition of caspase 1 started later compared with mice deficient for C1/11 (dKO mice) (Figure 3a). However, the skin phenotype attenuation was associated with a significant decrease in epidermal hyperplasia (Figure 4b and c) and mRNA expression of different inflammasome components and proinflammatory cytokines (Figure 4d and see Supplementary Figure S6c). Additionally, the pharmacological inhibition of caspase 1 was linked to a significant decrease in IL-1β and IL-18 protein levels (Figure 4e and f).

Next, we assessed Ac-YVAD-CMK as a potential therapeutic treatment. First, we treated WT mice with IMQ and then treated them daily with four intraperitoneal injections of Ac-YVAD-CMK after two IMQ applications (see Supplementary Figure S6d), and a significant reduction in the induced psoriasis-like disease was observed. Moreover, a trend toward a significant decrease was found in the proinflammatory cytokines and NLRP-3 inflammasome mRNA expression (see Supplementary Figure S6e), which could corroborate the attenuation obtained in the observed phenotype, although no differences in IL-17 mRNA expression were seen. Altogether, these data confirm that proinflammatory caspase activation is required for complete...
Figure 2. Crossing LynΔN mice with C1/C11-deficient mice leads to a reduction in psoriasis-like phenotype. LynΔN mice were crossed with C1/C11-deficient mice (dKO mice). LynΔN C1/C11-deficient mice (LynΔN dKO mice) were killed, together with WT and LynΔN mice, 11 days after birth, and abdomen skin was harvested. (a) Adapted PASI score was assessed at three time points (see Materials and Methods), and number of analyzed mice is showed below the graph. (b) Survival of WT, LynΔN, and LynΔN dKO mice through the 11 days of the study. (c) Representative histological sections stained with hematoxylin and eosin. Original magnification ×10 (top) and ×20 (bottom). Scale bars = 50 μm. (d) Epidermal hyperplasia quantification through hematoxylin and eosin staining (WT, n = 5; LynΔN, n = 7; LynΔN dKO, n = 10). (e) Skin was processed for total RNA isolation, and gene expression was determined by quantitative PCR (WT, n = 4; LynΔN, n = 4; LynΔN dKO, n = 8). (f) Total protein was prepared, and IL-1β (WT, n = 5; LynΔN, n = 8; LynΔN dKO, n = 14) and IL-18 (WT, n = 3; LynΔN, n = 5; LynΔN dKO, n = 7) levels were quantified by ELISA. Data presented were obtained from pooled mice of several experiments. Error bars represent mean ± standard deviation. One-way analysis of variance with the uncorrected Fisher least significant difference multiple comparison test was applied to determine statistical significance in the PASI score. Long-rank test (Mantel-Cox test) was applied for the survival experiment, and Student t test was applied for the rest of the panels. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001. A.U., arbitrary unit; dKO, double knockout; ns, not significant; PASI, Psoriasis Area and Severity Index; WT, wild type.
Figure 3. Deficiency in C1/C11 genes in mice decreases IMQ-induced psoriasis-like phenotype. WT and C1/C11-deficient mice (dKO mice) were treated daily for 6 days with vehicle (Vaseline; Unilever, London, UK) or IMQ cream, and after they were killed, back skin was harvested. (a) Severity of IMQ-induced psoriasis-like phenotype represented by erythema, scaling, and cumulative score measurements (Vasel, n = 2; IMQ-WT, n = 4; IMQ-dKO, n = 8).
achievement of the inflammatory process implicated in psoriasis pathogenesis.

Primary human keratinocytes are primed to secrete IL-1β in mimicking psoriasis conditions in an inflammasome-dependent manner

Psoriasis is characterized by a dialog between keratinocytes in the epidermis and the immune cell infiltration, mainly in the dermis, through the release of cytokines. Thus, to decipher in which skin compartments inflammatory caspases and IL-1β were induced upon IMQ treatment in mice, an epidermis/dermis dissociation experiment was performed (see Supplementary Figure S7 online). A significant increase in IL-1β mRNA expression was found both in the epidermis and dermis compartments, whereas caspase 1 and 11 expression levels were increased only in the dermis. We next wanted to know whether proinflammatory caspases could be activated in normal human keratinocytes in vitro upon stimulation with two cytokines implicated in psoriasis pathogenesis: IL-17A and TNF-α (Johansen et al., 2016). As expected, a significant increase in the mRNA expression levels of IL-6 and IL-8 (Bertelsen et al., 2017; Fujishima et al., 2010) were observed, validating our in vitro model (see Supplementary Figure S8a and b online). Although no differences in caspase 1 and 4 (Figure 5a and e) expression levels were observed, a significant increase in caspase 5, IL-1β, NLRP-1, and NLRP-3 expression levels (Figure 5a–d) both at the mRNA and protein levels, were observed. Moreover, there was not only an increase of pro-caspase 5 but also of its activated form at long-term stimulation time points (Figure 5d). Furthermore, to determine if those primed keratinocytes could be responsible for IL-1β release upon inflammasome activation (Stout-Delgado et al., 2012), we added nigericin to normal human keratinocytes pretreated for 24 hours with TNF-α plus IL-17A. Significant increases in the release of mature IL-1β and activated caspase 1 were observed (Figure 5g and h), confirming that keratinocytes in a psoriasis environment can be implicated in IL-1β secretion in an inflammatory caspase-dependent manner.

Caspase 1/11 activation in immune cells is sufficient for the development of psoriasis-like disease in mice

Because we had shown that (i) the activation of inflammatory caspases is necessary to obtain a complete psoriasis-like disease and (ii) inflammatory caspase activation can take place both in immune cells and keratinocytes, we decided to perform bone marrow transplantation experiments to elucidate in which cell type their activation is responsible for the psoriasis pathogenesis. As expected, dKO mice receiving dKO bone marrow showed a significant decrease in the adapted Psoriasis Area and Severity Index score (Figure 6a), epidermal hyperplasia (Figure 6b and c), mRNA expression of inflammasome components and proinflammatory cytokines (Figure 6d), and IL-1β protein levels (Figure 6e) compared with WT mice transplanted with WT bone marrow. After IMQ treatment, WT mice transplanted with dKO bone marrow showed a significant reduction in erythema and scaling scores (Figure 6a), epidermal hyperplasia (Figure 6b and c), mRNA expression of different proinflammatory cytokines (Figure 6d), and IL-1β protein levels (Figure 6e) compared with WT mice, which received WT bone marrow. The reduction in the phenotype observed in this chimeric mouse is comparable to what was observed in dKO mice reconstituted with dKO bone marrow. Conversely, dKO mice transplanted with WT bone marrow developed a phenotype comparable to WT mice reconstituted with WT bone marrow. However, although an identical increase in epidermal hyperplasia was obtained (Figure 6b and c), the mRNA expression levels of cytokine and inflammasome components (Figures 6d, and see Supplementary Figure S9a online) were less pronounced. This discrepancy could be explained by either the absence of radiosensitive γδ T cells, which require thymocyte transfer to be repopulated (in our study, only bone marrow cells were transferred), or the fact that lethal irradiation of mice could modify their response to proinflammatory challenge.

Nevertheless, altogether these results provided evidence of a major role of inflammatory caspase activation in immune cells rather than keratinocytes to trigger a complete psoriasis-like disease. Furthermore, similar results were obtained with the IL-23 mouse model (see Supplementary Figure S9b and c), reinforcing the notion that activation of proinflammatory caspases in immune cells is sufficient to induce a complete proinflammatory response leading to a psoriasis-like phenotype in treated mice. FACS-sorting of immune cells showed that caspase 1 and caspase 11 expression levels were highly increased in macrophages upon IMQ treatment compared with granulocytes and CD11c+/CD11b− cells (see Supplementary Figure S10 online), suggesting that it is their expression/activation within macrophages that should be implicated in psoriasis pathogenesis. However, further experiments are needed to clearly identify in which immune cells inflammatory caspases are activated and required for psoriasis development.

DISCUSSION

Psoriasis is a chronic inflammatory skin disease with an unpredictable course (Sabat et al., 2007). Although many efforts have been made to identify molecular factors implicated in the initiation and maintenance of the disease, which allowed the identification of new biological drugs targeting TNF-α, IL-17, and IL-23 (Lowes et al., 2014), the etiology of psoriasis is still a matter of concern. In this context, controversial results are found in the literature regarding the impact of the inflammasome pathway leading to inflammatory caspase...
Figure 4. Inhibition of caspase 1 by Ac-YVAD-CMK compound reduces IMQ-induced psoriasis-like phenotype in mice. WT mice were treated daily for 6 days with vehicle (Vaseline; Unilever, London, UK) or IMQ cream in combination with DMSO or the caspase 1 inhibitor Ac-YVAD-CMK (8 mg/kg). (a) Severity of IMQ-induced psoriasis-like phenotype represented by erythema, scaling and cumulative score measurements. (b) Representative histological sections stained with hematoxylin and eosin. Original magnification ×10. Scale bars = 50 μm. (c) Epidermal hyperplasia quantification through hematoxylin and eosin staining. (d) Skin was processed for total RNA isolation, and gene expression was determined by quantitative PCR. (e) Skin was processed for total protein extraction, and IL-1β and IL-18 levels were quantified by ELISA. Error bars represent mean ± standard error of the mean. Student t test, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001. In a and d: Vasel/DMSO, n = 6; Vasel/AC-YVAD-CMK, n = 2; IMQ/DMSO, n = 7; IMQ/AC-YVAD-CMK, n = 11 pooled mice from two independent experiments. In c and e: data represent one experiment representative of two independent experiments. Ac-YVAD-CMK, Ac-Tyr-Val-Ala-Asp-chloromethylketone; A.U., arbitrary unit; dKO, double knockout; IMQ, imiquimod; WT, wild type.
Figure 5. Primary human keratinocytes are primed to secrete IL-1β and activate caspase 5 in mimicking psoriasis conditions. Primary human keratinocytes were cultured in keratinocyte growth medium and then stimulated with the proinflammatory cytokines TNF-α (10 ng/ml) and IL-17A (200 ng/ml) at different time points. After total RNA isolation, (a) caspase 1, caspase 4, and caspase 5; (b) IL-1β; and (c) NLRP-1 and NLRP-3 gene expression levels were determined by quantitative PCR (n = 3 independent experiments). (d–f) Total lysates were prepared from stimulated keratinocytes, and Western blot analyses were performed (n = 3 independent experiments). (g) Primary human keratinocytes were stimulated with TNF-α (10 ng/ml) and IL-17A (200 ng/ml) in the presence or not of nigericin to activate the inflammasome; then IL-1β and caspase 1 expression levels were determined intracellularly and extracellularly (n = 3 independent experiments). (h) After keratinocyte stimulation with TNF-α (10 ng/ml) and IL-17A (200 ng/ml) in the presence of nigericin at two time points, supernatant was harvested, and IL-1β secretion was quantified by ELISA (n = 3 independent experiments). Error bars represent mean ± standard error of the mean. Student t test, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001. A.U., arbitrary unit; Casp, caspase; Cleav, cleaved; Ctrl, control; h, hour; T, tumor necrosis factor-α (10 ng/ml); TNF, tumor necrosis factor.
Figure 6. Caspase 1/11 deficiency in immune cells is enough to reduce IMQ-induced psoriasis-like phenotype. WT and C1/C11-deficient (dKO) mice were irradiated and then reconstituted with bone marrow from WT (WT→WT and WT→dKO) and dKO (dKO→dKO and dKO→WT) mice, which were left to recuperate for 8 weeks. Then, the four groups of mice were treated daily for 6 days with vehicle (Vaseline; Unilever, London, UK) or IMQ cream. After treatment,
activation and mature IL-1β/IL-18 secretion in psoriasis. Indeed, besides strong demonstration of the implication of IL-1 family members in psoriasis (Rabeony et al., 2015), the role of inflammatory caspases in this respect is still a matter of debate, because Cho et al. (2012) and Raboey et al. (2015) reported opposite results using the IMQ psoriasis-like model. However, our results argue in favor of a major role of inflammatory caspases in cytokine maturation in psoriasis, because the invalidation or pharmacological inhibition of proinflammatory caspases leads to a significant reduction in the induced inflammatory phenotype in several psoriasis mouse models. Moreover, even if other proteases such as caspase 8 and neutrophil-derived proteases have been reported to participate in the maturation of IL-1β and IL-18 (Clancy et al., 2017; Pierini et al., 2012), our study pointed out inflammatory caspases as major contributors of IL-1β/IL-18 maturation in skin disease. In fact, it has been shown that IL-1β can potentiate the production of IL-17A by γδ T cells, reinforcing the establishment of the disease (Cai et al., 2011; Kessel et al., 2017). Thus, we can speculate that the significant decrease in IL-1β expression obtained in our study, through the invalidation or inhibition of inflammatory caspases, contributes to attenuating the induced psoriasis-like phenotype by decreasing the secretion of IL-17A by γδ T cells. This finding is supported by the study of Douglas et al. (2015), which showed that atopic dermatitis/psoriasis-like disease developed by Sharpin (cpldm) mice is significantly reduced when caspase 1 and 11 are deficient (Douglas et al., 2015). Indeed, neither lesions nor epidermal hyperplasia were observed, showing the main role of proinflammatory caspases in the induction of dermatitis and the function of inflammasome activation as an initiating signal in Sharpin mice. The delay in the dermatitis onset in Sharpin-C1/C11 mice is consistent with the delay observed in LynΔN dKO mice used in this study, showing the improvement in psoriasis-like phenotype through the invalidation of proinflammatory caspases. Although genetic ablation of TNFR1 in Sharpin (Rickard et al., 2014) or LynΔN (Marchetti et al., 2009) mice produced a complete rescue of the phenotype, the invalidation of inflammatory caspases was associated with a delay in the inflammation symptoms, showing that TNF signaling is one of the principal pathways to induce skin inflammation, whereas inflammatory caspase pathways are more implicated in the maintenance and exacerbation of the disease. Additionally, the level of psoriasis-like phenotype inhibition in the absence of proinflammatory caspases is consistent with other studies in which other important components for psoriasis disease development, like T cells, are deleted (van der Fits et al., 2009).

Although it was previously shown that the cleaved form of caspase 5 is present in lesional skin biopsy samples from psoriasis patients (Zwicker et al., 2017), we confirmed in a large number of lesional and nonlesional skin biopsy samples that cleaved forms of caspases 1, 4, and 5 are found in all lesional samples of tested psoriasis patients, whereas no cleaved forms are found in healthy donor samples. Moreover, cleaved forms of proinflammatory caspases were associated with an increase in the NLRP-3 and AIM-2 inflammasomes. Caspase inhibitors have been evaluated in clinical trials, specifically the small molecule inhibitors VX-740 and VX-765, also known as pralicasan and belnacasan, respectively; which are potent and selective inhibitors of caspase 1 and caspase 4 (Vertex Pharmaceuticals, Cambridge, MA). In our study, we co-treated mice with IMQ and the selective caspase 1 inhibitor Ac-YVAD-CMK and found a significant decrease in erythema, scaling, and proinflammatory cytokine production, reinforcing the notion that targeting proinflammatory caspases could be of interest to treat psoriasis disease. Indeed, in a model of acute gastric injury, mice were pretreated with Ac-YVAD-CMK, and an inhibition of IL-1β production was observed, leading to the protection of these mice, an effect correlated with the impairment of NLRP-3 inflammasome activity (Zhang et al., 2016). Moreover, like in Ac-YVAD-CMK–treated mice, Zhang et al. found a strong diminution in other proinflammatory cytokines including IL-6, IL-8, and TNF-α. Actually, not only are caspase inhibitors evaluated as potential drugs to treat psoriasis disease, but IL-1, IL-18, and inflammasomes inhibition could be possible targets to treat inflammatory skin diseases (Fenini et al., 2017). Along these lines, psoriasis patients were treated in an open-label, phase I/II clinical trial with the human monoclonal antibody against IL-1α, MABp1, and an encouraging clinical response was achieved that might be improved with an increase in dose/frequency (Coleman et al., 2015).

In our study, we showed that keratinocytes cultured in conditions mimicking psoriasis (TNF-α + IL-17A) are primed to secrete IL-1β in an inflammasome-dependent manner. Recently, it has been shown that the stimulation of keratinocytes with TNF-α alone or with IL-17A alone did not trigger caspase activation (Zwicker et al., 2017) but that the combination of at least two proinflammatory cytokines is necessary (Cho et al., 2012), suggesting that in the presence of several proinflammatory cytokines (IFN-γ, TNF-α, IL-17, and IL-22), a strong increase in proinflammatory caspases could be obtained. This hypothesis is in agreement with what happens in psoriatic skin, where keratinocytes are in direct contact with a huge amount of proinflammatory cytokines secreted from the recruited immune cells and from themselves.

Our data showing that keratinocytes are primed to secrete IL-1β through the activation of the inflammasome reinforces...
the idea that keratinocytes are immunologically active cells (Feldmeyer et al., 2010). In fact, pro-IL-1β is constitutively synthesized in keratinocytes, making these cells the principal source of IL-1 in the skin (Lee et al., 2009) released under stress conditions. However, bone marrow transplantation experiments indicated that the activation of proinflammatory caspases in immune cells is sufficient to induce a full inflammatory response in mice, suggesting that caspase-mediated IL-1β production and secretion by the recruited macrophages, neutrophils, and other immune cells could be sufficient to develop the psoriasis-like phenotype. Inflammasome components are not so well expressed in murine keratinocytes, and accordingly, murine keratinocytes are not able to activate the inflammasome in response to stimuli (Sand et al., 2018). Therefore, although in our study we postulated that activation of proinflammatory caspases in immune cells, and more likely in macrophages, is sufficient to develop a psoriasis-like phenotype in mice, it appears that in the case of humans, the activation of inflammatory caspases in keratinocytes could be a determinant in the development of the disease.

In conclusion, the findings presented herein confirm that proinflammatory caspases are involved in psoriasis pathogenesis, highlighting the notion that control of proinflammatory caspase activation and IL-1β maturation/secretion is essential for the improvement of chronic inflammatory disease.

MATERIALS AND METHODS

Study approval

This study was conducted on biopsy samples from 25 patients enrolled in a study registered at clinicaltrials.gov under the identifier NCT01538342: Role of Tyrosine Kinase Lyn and Cleaved Form by Caspases in Psoriasis. This study was approved by the local ethics committee (Comité de Protection des Personnes Sud-Méditerranée V, no. 2011-A00786-35.063) and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practices guidelines. All patients and healthy donors included signed informed consent before their inclusion in the study. The ethics review board of the French Minister for High Education and Research approved all animal studies, together with the Institutional Animal Care and Use Committee of the Centre Méditerranéen de Médecine Moléculaire, Nice, France (INSERM U1065).

Mice

Mice were housed under specific pathogen-free conditions and fed with standard laboratory food. All mice (WT, C1/C11 deficient, and LynΔN) were on a C57BL/6j genetic background and were used at 8–10 weeks of age, except for adoptive transfer, where mice were irradiated and reconstituted at 6 weeks. Both males and females were used in this study. WT mice (C57Bl6j) were purchased from Envigo, and C1/C11-deficient mice were a gift from Richard A. Flavell (Yale University, New Haven, CT).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

LEA and DG obtained the majority of data presented herein and were assisted by JPB, CRB, JC, LM, MG, PLO, and PC. JPO, JPL, and TP included patients and performed clinical examination. SM and LEA wrote and edited the manuscript. JER and PA provided intellectual input and edited the article. SM conceived the project, supervised the study, and obtained funding. All authors reviewed and approved the final version of the manuscript before its submission for publication.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.11.031.

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