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► **To cite this version:**

L. E. Aira, D. Goncalves, J. P. Bossowski, C. Rubio-Patino, J. Chiche, et al.. Caspase 1/11 Deficiency or Pharmacological Inhibition Mitigates Psoriasis-Like Phenotype in Mice. *Journal of Investigative Dermatology*, 2019, 139 (6), pp.1306–1317. 10.1016/j.jid.2018.11.031 . hal-02195315

**HAL Id: hal-02195315**

**<https://hal.science/hal-02195315>**

Submitted on 25 Oct 2021

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## **Caspase 1/11 deficiency or pharmacological inhibition mitigates psoriasis-like phenotype in mice**

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### **Keywords**

Inflammatory response, pro-inflammatory caspases, pro-inflammatory cytokines, keratinocytes, human skin biopsies, psoriasis

**Running title:** Inflammatory caspases are involved in psoriasis

## **Abstract**

Inflammatory caspases, activated within inflammasome, are responsible for the maturation and secretion of IL-1 $\beta$ /IL-18. While their expression in psoriasis was demonstrated several years ago, little is known about the role of inflammatory caspases in the context of psoriasis. Here, we confirmed that caspase-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 injection induced a decrease in ear thickness, erythema, scaling, inflammatory cytokines expression and immune cells infiltration in mice. Interestingly, we observed that keratinocytes were primed to secrete IL-1 $\beta$  when cultured in condition mimicking psoriasis. Generation of chimeric mice by bone marrow transplantation was carried out in order 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 (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 (2, 3, 6, 7, 8, 9 and 10) and pro-inflammatory (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, pro-inflammatory 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 orthologous between human and mice; caspases 4 and 5, only present in human, are the homologous of mouse caspase 11 (Liu and Lieberman, 2017).

Pro-inflammatory caspases, firstly 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 pro-inflammatory cytokines such as interleukin (IL)-1 $\beta$  and IL-18. Caspase 1 activation and subsequent IL-1 $\beta$  production have been associated with a large variety of inflammatory and autoimmune diseases (reviewed in (Gabay et al., 2010, McIlwain et al., 2013, Patel et al., 2017)) such as rheumatoid arthritis, type 2 diabetes (Ruscitti et al., 2015) and inflammatory bowel diseases (IBD) (Perera et al., 2017).

Psoriasis is a chronic auto-inflammatory/autoimmune skin disease (Lowe et al., 2014) characterized by an intense dialogue between keratinocytes and immune cells, affecting 2% of the worldwide population (Parisi 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-1 $\alpha$ , IL-1 $\beta$ , IL-17, IL-18, IL-22, IL-23, interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$  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.

Albeit 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 pro-inflammatory caspases in this context. It has been showed that activated caspase 1 is increased in lesional

skin samples from psoriasis patients compared to non-lesional samples (Johansen et al., 2007, Marchetti et al., 2009). Moreover, an increase in the mRNA expression of caspase 5 was found in lesional biopsies (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 as Cho et al reported a decrease in the epidermal thickness associated with a significant reduction in IL-1 $\beta$  levels in caspase 1  $-/-$  mice treated with IMQ (Cho et al., 2012) whereas another recent study concluded that the invalidation of caspase 1 had no impact in reducing the psoriasis-like phenotype after IMQ treatment (Rabeony et al., 2015).

Therefore, we decided to address the implication of pro-inflammatory caspases in psoriasis pathogenesis. We first find in a large cohort of psoriasis patient biopsies that caspase 1 and 5 are activated in all lesional samples and that caspase 4 is activated in most lesional biopsies. Further, caspase 1/11 deficiency and pharmacological inhibition of pro-inflammatory caspases significantly reduce skin inflammatory disease in three mouse models of psoriasis like-phenotype. Moreover, stimulation of normal human keratinocytes (NHK) with TNF- $\alpha$  and IL-17A demonstrates that keratinocytes are primed to activate caspase 5 and secrete IL-1 $\beta$ . Finally, by adoptive transfer experiments we show that pro-inflammatory caspases activation in immune cells is sufficient to induce a complete inflammatory response in mice.

## Results

### **Pro-inflammatory caspases are activated in lesional skin biopsies from psoriasis patients**

Although increased mRNA expression of pro-inflammatory caspases 1, 4 and 5 in lesional biopsies 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 on a large cohort of psoriasis patients, the expression of caspases to confirm their activation status. To do that, western blot analysis of skin extracts was performed from non lesional and lesional biopsies. Pro-caspase 1, 4 and 5 were all expressed in non-lesional biopsies; however only a strong increase of pro-caspase 5 was observed in lesional skin as compared to non-lesional paired and healthy donor skin (Figure 1a,b,d; Figure S1a). Interestingly, high levels of cleaved caspase 1 and 5 were observed in lesional skin from all analyzed patients (Figure 1a-d; Figure S1a) and cleaved caspase 4 was seen in most analyzed lesional biopsies (Figure 1a,b,d; Figure S1a). Moreover, an increase in inflammasome component expression along with the mature forms of IL-1 $\beta$  and IL-18 was found (Figure S1b), as previously reported (Dombrowski et al., 2011, Johansen et al., 2007). Altogether, these data suggest that pro-inflammatory caspase activity is regulated in injured skin from psoriasis patients.

### **Deficiency in pro-inflammatory caspases 1/11 impaired the psoriasis-like disease development in different mouse models**

While the role of inflammasome-processed cytokines IL-1 $\beta$  (Feldmeyer et al., 2010) and IL-18 (Companjen 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 pro-inflammatory caspases in psoriasis-like disease, we decided to use three different mouse models: i) the Lyn $\Delta$ N transgenic mice (Marchetti et al., 2009), which develop spontaneously psoriasis after birth; ii) the IMQ-induced skin dermatitis (van der Fits et al., 2009), and iii) the IL-23 intradermal ear injections (Hedrick et al., 2009).

First, we used the Lyn $\Delta$ N 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 (Figure S2a). Interestingly, an increased mRNA expression of some inflammasome components, such as caspase-11, Absent In Melanoma (AIM)-2 and NLRP-3 (Figure S2b), associated to an increase in IL-1 $\beta$  expression both at the mRNA and protein level was observed (Figure S2b,c). Next, we crossed Lyn $\Delta$ N mice with

C1/C11 deficient mice (Kuida et al., 1995) to obtain Lyn $\Delta$ N C1/C11  $-/-$  mice, named hereafter Lyn $\Delta$ N dKO mice. Strikingly, the absence of pro-inflammatory caspases hampered the skin inflammatory phenotype developed by Lyn $\Delta$ N mice over time, based on the adapted PASI score that we established (Figure 2a; Figure S3a), producing a significant delay in the initiation of the psoriasis-like phenotype in Lyn $\Delta$ N dKO mice. Moreover, both the no weight gain linked to the onset of the disease and the reported death of Lyn $\Delta$ N mice (Marchetti et al., 2009) were significantly reduced in Lyn $\Delta$ N dKO mice (Figure S3b; Figure 2b). The improved phenotype observed in Lyn $\Delta$ N dKO mice was associated to a decrease in epidermal hyperplasia (Figure 2c,d), along with a reduction in the mRNA expression of inflammasome components such as AIM-2 and NLRP-3 and several pro-inflammatory cytokines (Figure 2e; Figure S3c), with an exception for IL-23 and TNF- $\alpha$ , for which similar levels were obtained. Concomitantly, the absence of pro-inflammatory caspases significantly reduced the levels of both IL-1 $\beta$  and IL-18 protein expression observed in Lyn $\Delta$ N mice skin (Figure 2f). Therefore, activation of caspase 1/11 is necessary for the development of a complete inflammatory skin disease in the Lyn $\Delta$ N mice model.

To confirm the contribution of pro-inflammatory 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 (Figure S4) of WT and C1/C11 deficient mice, hereafter named dKO mice. In both models (IMQ and IL-23), the absence of pro-inflammatory caspases delayed the onset of the clinical score measured. Indeed, erythema, scaling and cumulative scores quantified in the IMQ model (Figure 3a) and ear thickness measured in the IL-23 model (Figure S4a,b) were significantly reduced in dKO mice compared to WT mice. Further, H&E staining of skin sections revealed a decrease in epidermal hyperplasia (Figure 3b,c; Figure S4c,d), along with a reduction in the inflammatory cell infiltration (Figure 3d; Figure S4e), with a reduced number of CD45.2<sup>+</sup> immune cells into the dKO mice skin, evidenced more precisely by a significant decrease number of inflammatory monocytes and neutrophils. Additionally, inflammasome components, pro-inflammatory cytokine mRNA expression (Figure 3e; Figure S4f; Figure S5) and IL1 $\beta$  protein level were significantly reduced in dKO mice in both models (Figure 3f; Figure S4g). Surprisingly, IL-18 protein level was only differentially modulated in the IMQ model (Figure 3f). Altogether, these data indicate that regulation of pro-inflammatory 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 demonstrated in mice suffering from acute gastric injury that treatment with the selective caspase 1 inhibitor AC-YVAD-CMK led to a 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. To do that, WT mice were treated with IMQ cream together with either DMSO or AC-YVAD-CMK compound injected intra-peritoneally.

While the co-treatment had no additive effect on both weight loss (Figure S6a) and splenomegaly induced by IMQ treatment (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). It is worth noting that the delay observed upon pharmacological inhibition of caspase 1 started later compared to deficient mice for C1/11 (dKO mice) (Figure 3a). However, the skin phenotype attenuation was associated with a significant decrease in epidermal hyperplasia (Figure 4b,c) and mRNA expression of different inflammasome components and pro-inflammatory cytokines (Figure 4d; Figure S6c). Additionally, the pharmacological inhibition of caspase 1 was linked to a significant decrease in IL-1 $\beta$  and IL-18 protein levels (Figure 4e,f).

Next, we assessed the AC-YVAD-CMK as a potential therapeutic treatment. To do that, WT mice were first treated with IMQ and then daily treated with four intra-peritoneal injections of AC-YVAD-CMK after two IMQ applications (Figure S6d) and a significant reduction in the induced psoriasis-like disease was observed. Moreover, a trend toward to a significant decrease was found in the pro-inflammatory cytokines and NLRP-3 inflammasome mRNA expression (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 pro-inflammatory caspase activation is required for the complete achievement of the inflammatory process implicated in psoriasis pathogenesis.

### **Primary human keratinocytes are primed to secrete IL-1 $\beta$ in mimicking psoriasis condition in an inflammasome dependent manner**

Psoriasis is characterized by a dialogue 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-1 $\beta$  were induced upon IMQ treatment in mice, epidermis/dermis dissociation experiment was performed (Figure S7). A significant increase in IL-1 $\beta$  mRNA expression was found both in epidermis and dermis



compartment, while caspase 1 and 11 expression were only increased in the dermis. We next wanted to know whether pro-inflammatory caspases could be activated in normal human keratinocytes (NHK) in vitro upon stimulation with two cytokines implicated in psoriasis pathogenesis: IL-17A and TNF- $\alpha$  (Johansen et al., 2016). As expected, a significant increase in the mRNA expression of IL-6 and IL-8 (Bertelsen et al., 2017, Fujishima et al., 2010) was observed, validating our in vitro model (Figure S8a,b). While no difference in caspase 1 and 4 (Figure 5a,e) expression levels was observed, a significant increase of caspase 5, IL-1 $\beta$ , NLRP-1 and NLRP-3 expression (Figure 5a-d,f), both at the mRNA and protein level, was observed. Moreover, it is interesting to note that there was not only an increase of pro-caspase 5 but also of its activated form at long-term stimulation time points (Figure 5d). Moreover, to know if those primed keratinocytes could be responsible for IL-1 $\beta$  release upon inflammasome activation (Stout-Delgado et al., 2012), nigericin was added to NHK pre-treated for 24h with TNF- $\alpha$  + IL-17A. Interestingly, a significant increase in the release of mature IL-1 $\beta$  and activated caspase 1 was observed (Figure 5g,h), confirming that keratinocytes in a psoriasis environment can be implicated in IL-1 $\beta$  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**

As we showed that 1) the activation of inflammatory caspases is necessary to obtain a complete psoriasis-like disease, and 2) 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 PASI score (Figure 6a), epidermal hyperplasia (Figure 6b,c), mRNA expression of inflammasome components and pro-inflammatory cytokines (Figure 6d) and IL-1 $\beta$  protein levels (Figure 6e) compared to 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,c), mRNA expression of different pro-inflammatory cytokines (Figure 6d) and IL-1 $\beta$  protein levels (Figure 6e) compared to 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. On the opposite, 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,c), the mRNA expression levels of cytokine and inflammasome components (Figures 6d and S9a) were less pronounced. This discrepancy could be explained by either the absence of radio-sensitive  $\gamma\delta$  T cells, that require thymocyte transfer to be repopulated (in our study only bone marrow cells were transferred) or the fact that mice lethal irradiation could modify their response to pro-inflammatory challenge.

Nevertheless, altogether these results provided evidence of a major role of inflammatory caspase activation into immune cells rather than keratinocytes to trigger a complete psoriasis-like disease. Furthermore, similar results were obtained with the IL-23 mice model (figure S9 b,c), reinforcing the notion that activation of pro-inflammatory caspases in immune cells is sufficient to induce a complete pro-inflammatory response leading to a psoriasis-like phenotype in treated mice. Interestingly, FACS-sorting of immune cells showed that caspase-1 and caspase-11 expression was highly increased in macrophages upon IMQ treatment as compared to granulocytes and CD11c<sup>+</sup>/CD11b<sup>-</sup> cells (figure S10), 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). While many efforts have been done to identify molecular factors implicated in the initiation and maintenance of the disease, which allowed the identification of new biological drugs targeting TNF- $\alpha$ , IL17 and IL-23 (Lowe 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 inflammasome pathway leading to inflammatory caspase activation and mature IL-1 $\beta$ /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 as Cho et al (Cho et al., 2012) and Rabeony et al (Rabeony et al., 2015) reported opposite results using IMQ psoriasis-like model. However, our results argue in favor of a major role of inflammatory caspases in cytokine maturation in psoriasis as the invalidation or the pharmacological inhibition of pro-inflammatory 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-1 $\beta$  and IL-18 (Clancy et al., 2017, Pierini et al., 2012), our study pointed out inflammatory caspases as major contributors of IL-1 $\beta$ /IL-18 maturation in skin disease. In fact, it has been demonstrated that IL-1 $\beta$  can potentiate the production of IL-17A by  $\gamma\delta$ -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-1 $\beta$  expression obtained in our study, through the invalidation or inhibition of inflammatory caspases, contributes to attenuate the induced psoriasis-like phenotype by decreasing the secretion of IL-17A by  $\gamma\delta$ -T cells. This finding is supported by the study of Douglas et al, showing that atopic dermatitis/psoriasis-like disease developed by Sharpin (cpdm) mice, is significantly reduced when caspase 1/11 are deficient (Douglas et al., 2015). Indeed, neither lesions nor epidermal hyperplasia were observed, demonstrating the main role of pro-inflammatory caspases in the induction of dermatitis and the function of inflammasome activation as an initiating signal in Sharpin mice. Interestingly, the delay in the dermatitis onset in Sharpin-C1/C11  $-/-$  mice is consistent with the delay observed in Lyn $\Delta$ N dKO mice used in the present study, showing the improvement in psoriasis-like phenotype through the invalidation of pro-inflammatory caspases. It is worth noting that while genetic ablation of TNFR1 in Sharpin (Rickard et al., 2014) or Lyn $\Delta$ N (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, demonstrating that TNF signaling is one of the principal pathway to induce skin inflammation, while inflammatory caspases pathway is more implicated in the maintenance and exacerbation of the disease. Additionally, the level of psoriasis-like phenotype inhibition in the absence of pro-inflammatory caspases is consistent with other studies where other important components for psoriasis disease development, like T cells, are deleted (van der Fits et al., 2009).

Although it was previously demonstrated that cleaved-form of caspase-5 is present in lesional skin biopsies from psoriasis patients (Zwicker et al., 2017), we confirmed in a large cohort of lesional and non-lesional skin biopsies that cleaved forms of caspase-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 Pralnacasan and Belnacasan, respectively; which are potent and selective inhibitors of caspase 1 and caspase 4 (Vertex Pharmaceuticals). In our study, we co-treated mice with IMQ and the selective caspase-1 inhibitor AC-YVAD-CMK and a significant decrease in the erythema, scaling and pro-inflammatory cytokine production was found, reinforcing the notion that targeting pro-inflammatory caspases could be of interest to treat psoriasis disease. Indeed, in a model of acute gastric injury, mice were pre-treated with AC-YVAD-CMK and an inhibition in the production of IL-1 $\beta$  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 pro-inflammatory cytokines including IL-6, IL-8 and TNF- $\alpha$ . Actually, not only caspase inhibitors are evaluated as potential drugs to treat psoriasis disease, but IL-1, IL-18 and inflammasome inhibition could be possible targets to treat inflammatory skin diseases (Fenini et al., 2017). In this line, psoriasis patients were treated in an open-label clinical trial I/II with the human monoclonal antibody against IL-1 $\alpha$ , MABp1, and an encouraging clinical response was achieved that must 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- $\alpha$  + IL-17A) are primed to secrete IL-1 $\beta$  in an inflammasome-dependent manner. Recently, it has been demonstrated that the stimulation of keratinocytes with TNF- $\alpha$  or with IL-17A only did not trigger caspase activation (Zwicker et al., 2017), but that the combination of at least

two pro-inflammatory cytokines is necessary (Cho et al., 2012), suggesting that in the presence of several pro-inflammatory cytokines (IFN- $\gamma$ , TNF- $\alpha$ , IL-17 and IL-22) a strong increase in pro-inflammatory caspases could be obtained. This hypothesis is in agreement with what happens in a psoriatic skin, where keratinocytes are in direct contact with a huge amount of pro-inflammatory cytokines secreted from the recruited immune cells and from themselves.

Our data showing that keratinocytes are primed to secrete IL-1 $\beta$  through the activation of inflammasome reinforces the idea that keratinocytes are immunological active cells (Feldmeyer et al., 2010). In fact, pro-IL-1 $\beta$  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 experiment indicated that the activation of pro-inflammatory caspases in immune cells is sufficient to induce a fully inflammatory response in mice, suggesting that caspase-mediated IL-1 $\beta$  production and secretion by the recruited macrophages, neutrophils and other immune cells could be sufficient to develop the psoriasis-like phenotype. It is noteworthy that inflammasome components are not so well expressed in murine keratinocytes and accordingly, murine keratinocytes are not able to activate inflammasome in response to stimuli (Sand et al., 2018). Therefore, although in our study we postulated that activation of pro-inflammatory caspases in immune cells, and more likely in macrophages, is sufficient to develop a psoriasis-like phenotype in mice, it appears that the case of humans the activation of inflammatory caspases in keratinocytes could be determinant in the development of the disease.

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

## **Materials and methods**

### **Study approval.**

This study was conducted on biopsies from 25 patients enrolled in a study registered in [clinicaltrials.gov](https://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, n° 2011-A00786-35.065), 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 from the French Minister for the High Education and Research approved all animal studies together with the Institutional Animal Care and Use Committee of the Centre Méditerranéen de Médecine Moléculaire (C3M), 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 Lyn $\Delta$ N) 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 six weeks. Both males and females were used in this study. WT mice (C57Bl6/J) were purchased from Envigo and C1/C11 deficient mice were a gift from Dr Richard A Flavell (Yale University, New Haven, CT, USA).

### **Conflict of interest**

There is no conflict of interest declared by the authors.

### **Acknowledgments**

This work was supported by grants from the Fondation pour la Recherche Médicale (DMP20101120387), from the Fondation ARC pour la Recherche sur le Cancer (PGA1RF201702053889), the INSERM and the University of Nice-Sophia-Antipolis. This work was supported by a grant from the Clinical Research Hospital Program (PHRC 2011) from the French Ministry of Health. This work and LEA were funded by the French Government (National Research Agency, ANR) through the 'Investments for the Future' LABEX SIGNALIFE: program reference #ANR-11-LABX-0028-01. We thank Dr Abdallah Khemis (Dermatology department, CHU Nice), Pr Marie-Aleth Richard (Dermatology department, CHU Marseille) and Pr Didier Bessis (Dermatology department, CHU Montpellier) for Patient recruitment and skin biopsies. We thank Pr Richard A. Flavell for providing C1/C11 deficient mice. We thank Dr Karine Bille and Dr Corine Bertolotto for provide us the Normal Human Keratinocytes. We thank the Animal facility and the Imaging Facility of C3M. We thank la Délégation à la Recherche Clinique et à l'Innovation for their help in the PHRC. We thank le Conseil Général des AM et de la region PACA et Corse for their help.

### **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 edit the manuscript. JER and PA provided intellectual input and edit the paper. SM conceived the project, supervised the study and obtained funding. All authors reviewed and approved the final version of the manuscript prior to its submission for publication.

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## Figure legends

### **Figure 1: Pro-inflammatory caspases are activated in lesional skin biopsies from psoriasis patients.**

Protein extraction was performed from total skin of healthy donors (HD) and non-lesional (NL) and lesional (L) skin biopsies from psoriasis patients and then, western blot analysis were carried out. **a**, **b** and **c**: Caspase 1, 4 and 5 were blotted in HD and in NL and L biopsies from different psoriasis patients showing caspase activation in lesional samples. **d**: Total of analyzed patients showing in how many patients activated caspases are present.

\* Unspecific band.

### **Figure 2: Crossing of 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). Newborn mice, LynΔN C1/C11 deficient mice (LynΔN dKO mice), were sacrificed, 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), number of analyzed mice is showed below the graph. **b**: Survival of WT, LynΔN and LynΔN dKO through the eleven days of the study. **c**: Representative histological sections stained with haematoxylin and eosin (10X and 20X), scale bars = 50 μm. **d**: Epidermal hyperplasia quantification through haematoxylin 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 qPCR (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.

A.U: arbitrary units. Errors bars represent mean ± SD. One-way ANOVA with the uncorrected Fisher's LSD multiple comparison test was applied to determine statistical significance in the PASI score. Long-rank test (Mantel-Cox test) was applied for survival experiment and Student t test was applied for the rest of panels, \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001, \*\*\*\* p ≤ 0.0001, ns: non-significant.

**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 six days with vehicle (Vaseline (Vasel)) or imiquimod (IMQ) cream and after sacrificed them back skin was harvested. **a:** Severity of IMQ-induced psoriasis like phenotype represented by Erythema, Scaling and Cumulative score measurement, (Vasel, n = 2; IMQ-WT, n = 4; IMQ-dKO, n = 8). **b:** Representative histological sections stained with haematoxylin and eosin (10X and 20X), scale bars = 50  $\mu$ m. **c:** Epidermal hyperplasia quantification through haematoxylin and eosin staining, (Vasel, n = 3; IMQ-WT, n = 4; IMQ-dKO, n = 8). **d:** The number of immune cell populations was quantified by FACS, (Vasel, n = 3; IMQ-WT, n = 4; IMQ-dKO, n = 8). **e:** Skin was processed for total RNA isolation and gene expression was determined by qPCR (Vasel, n = 3; IMQ-WT, n = 4; IMQ-dKO, n = 8). **f:** Skin was processed for total protein extraction and IL-1 $\beta$  and IL-18 levels were quantified by ELISA, (Vasel, n = 3; IMQ-WT, n = 4; IMQ-dKO, n = 8). Data represent one experiment representative of two independent experiments.

A.U: arbitrary units. Errors bars represent mean  $\pm$  SEM. Student t test, \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , ns: non-significant.

**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 six days with vehicle (Vaseline (Vasel)) or imiquimod (IMQ) cream in combination with dimethylsulfoxide (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 measurement. **b:** Representative histological sections stained with haematoxylin and eosin (10X), scale bars = 50  $\mu$ m. **c:** Epidermal hyperplasia quantification through haematoxylin and eosin staining. **d:** Skin was processed for total RNA isolation and gene expression was determined by qPCR. **e:** Skin was processed for total protein extraction and IL-1 $\beta$  and IL-18 levels were quantified by ELISA.

A.U: arbitrary units. Errors bars represent mean  $\pm$  SEM. Student t test, \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$ , ns: non-significant. (a,d : Vasel/DMSO, n = 6; Vasel/YVAD, n=2; IMQ/DMSO, n = 7; IMQ/YVAD, n=11 pooled mice from two independent experiments; c,e : data represent one experiment representative of two independent experiments).

**Figure 5: Primary human keratinocytes are primed to secrete IL-1 $\beta$  and activate caspase 5 in mimicking psoriasis conditions.**

Primary human keratinocytes were cultured in KGM and then stimulated with the pro-inflammatory cytokines TNF- $\alpha$  (10 ng/ml) and IL-17A (200 ng/ml) at different time points and after total RNA isolation, caspase 1, caspase 4, caspase 5 (**a**); IL-1 $\beta$  (**b**); and NLRP-1 and NLRP-3 (**c**) gene expression was determined by qPCR, (n = 3 independent experiments). **d**, **e**, and **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- $\alpha$  (10 ng/ml) and IL-17A (200 ng/ml) in the presence or not of nigericin to activate inflammasome, then IL-1 $\beta$  and caspase 1 expression was determined intracellular and extracellularly, (n = 3 independent experiments). **h**: After keratinocytes stimulation with TNF- $\alpha$  (10 ng/ml) and IL-17A (200 ng/ml) in the presence of nigericin at two time points, supernatant was harvested and IL-1 $\beta$  secretion was quantified by ELISA, (n = 3 independent experiments).

A.U: arbitrary units. Errors bars represent mean  $\pm$  SEM. Student t test, \* p  $\leq$  0.05, \*\* p  $\leq$  0.01, \*\*\*, p  $\leq$  0.001, \*\*\*\* p  $\leq$  0.0001, ns: non-significant. T: TNF- $\alpha$  (10 ng/ml), I: IL-17A (200 ng/ml).

**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 $\rightarrow$ WT and WT $\rightarrow$ dKO) and dKO (dKO $\rightarrow$ dKO and dKO $\rightarrow$ WT) mice, which were left to recuperate for 8 weeks. Then, the four groups of mice were treated daily for six days with vehicle (Vaseline (Vasel)) or imiquimod (IMQ) cream. After treatment back skin was harvested. **a**: Severity of IMQ-induced psoriasis like phenotype represented by Erythema, Scaling and Cumulative score measurement. **b**: Representative histological sections stained with haematoxylin and eosin (10X), scale bars = 50  $\mu$ m. **c**: Epidermal hyperplasia quantification through haematoxylin and eosin staining, (for each group of mice, Vasel, n = 3; IMQ, n = 6). **d**: Skin was processed for total RNA isolation and gene expression was determined by qPCR. **e**: Skin was processed for total protein extraction and IL-1 $\beta$  levels were quantified by ELISA, (for each group of mice, Vasel, n = 3; IMQ, n = 6).

A.U: arbitrary units. Errors bars represent mean  $\pm$  SEM. One-way ANOVA with the uncorrected Fisher's LSD multiple comparison test, \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$ , ns: non-significant, ND: non-detected. (a,d : BM WT-WT and BM dKO-dKO, n = 10; BM WT-dKO and BM dKO-WT, n = 14 pooled mice from two independent experiments; b,c,e : data represent one experiment representative of two independent experiments).

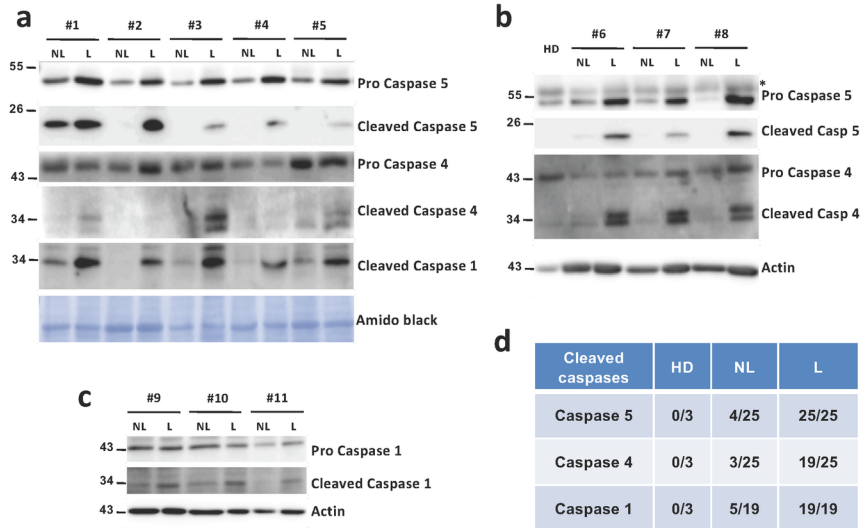


Figure 1, Aira-Caspases

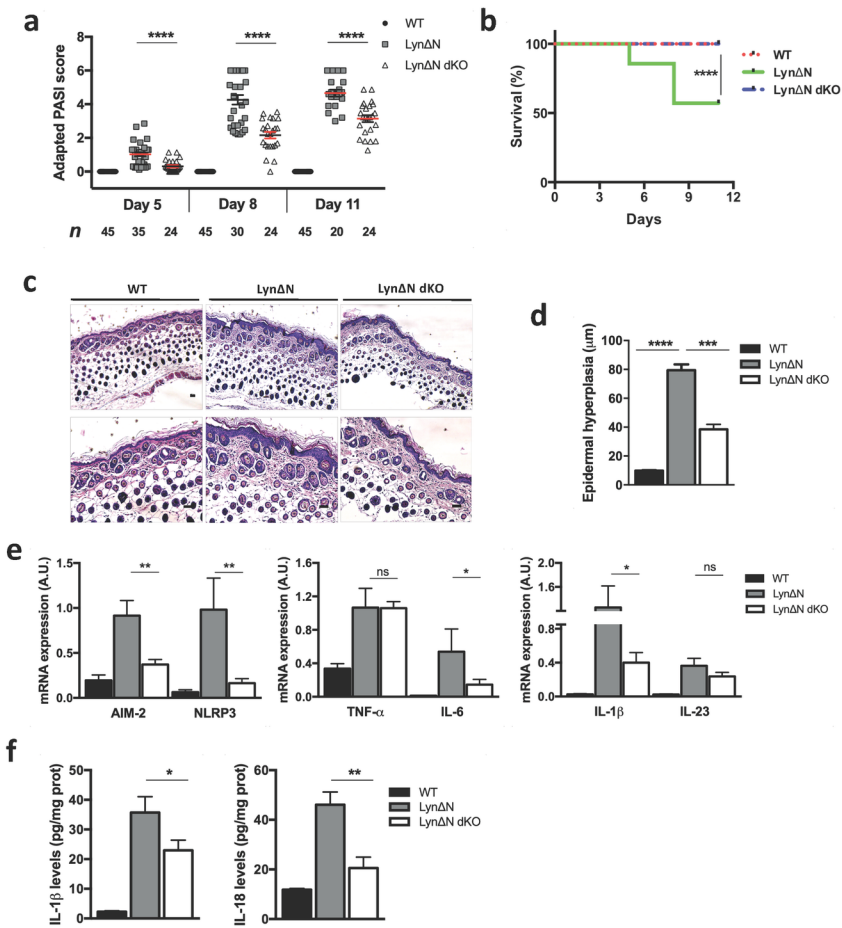


Figure 2, Aira-Caspases



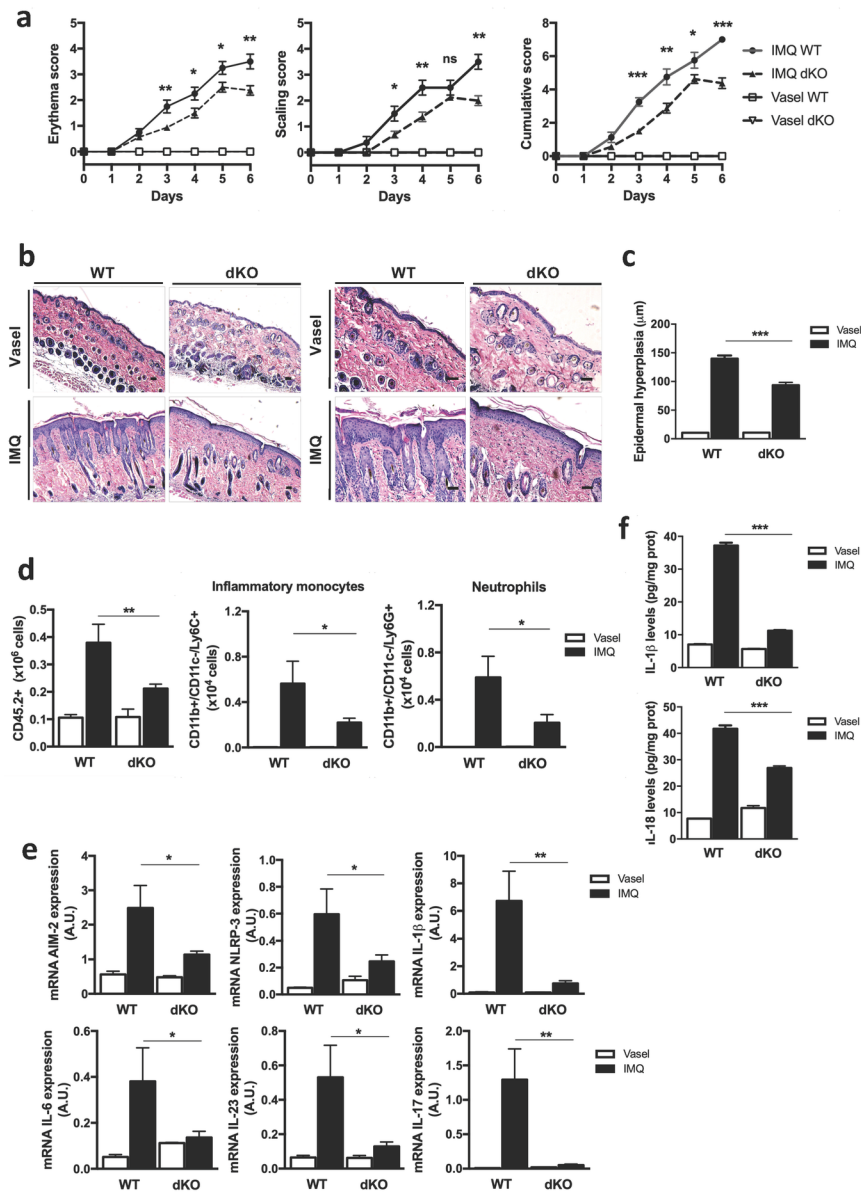


Figure 3, Aira-Caspases

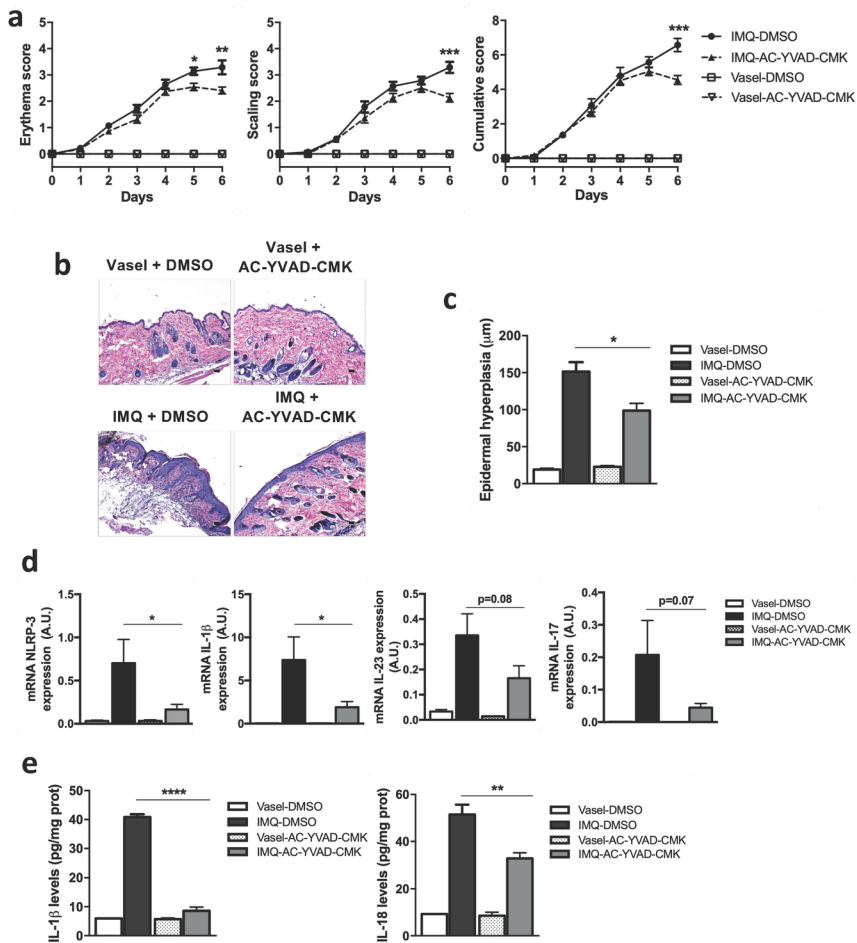


Figure 4, Aira-Caspases

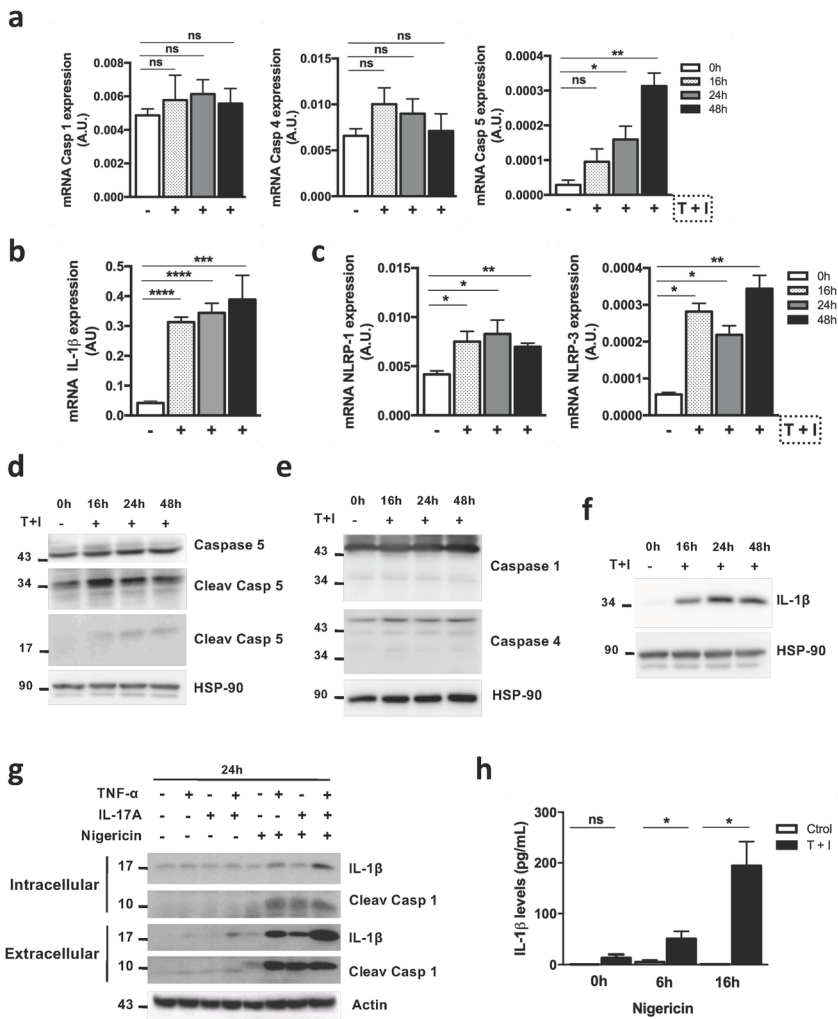


Figure 5, Aira-Caspases

