Enhanced Inflammasome Activity in Patients with Psoriasis Promotes Systemic Inflammation

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Psoriasis is linked to systemic inflammation and cardiovascular comorbidities, but studies of the underlying cellular mechanisms are lacking. The NLRP3 inflammasome is genetically associated with psoriasis, and its activation is increasingly linked with cardiovascular disease. In this study, we show that patients with psoriasis exhibited higher plasma levels of inflammasome-generated IL-1β and IL-18, without any correlation to skin lesion severity. Increased constitutive expression of the inflammasome sensors NLRP3, NLRP1, and AIM2 was found in peripheral blood cells of the patients and also of those with mild disease, and this was accompanied by an increased caspase-1 reactivity in the myeloid blood subsets. TNF-α was found to activate selectively the NLRP3 inflammasome without the requirement for a priming signal. TNF-α was found to signal through the TNFR–caspase-8–caspase-1 alternative inflammasome pathway, which proceeds independently of pyroptosis. Patients who received anti-TNF therapy had normalized plasma IL-1β and IL-18 levels as well as normalized caspase-1 reactivity. This was in contrast to the patients treated with methotrexate who exhibited persistent, increased caspase-1 reactivity. Thus, we show that the TNF-α–mediated activation of NLRP3 inflammasomes in patients with psoriasis may contribute to systemic inflammation. Anti-TNF therapy normalized inflammasome function, suggesting a mechanism for the cardiovascular risk–reducing effect.


INTRODUCTION
Psoriasis is an immune-mediated systemic disorder that is characterized by inflammatory reactions in the skin and joints (Nestle et al., 2009; Takeshita et al., 2017). The systemic nature of the disease, which entails immune dysregulation beyond the skin, is increasingly being recognized (Boehncke, 2018; Nestle et al., 2009). Psoriasis is associated with increased risks for systemic comorbidities, including obesity, atherosclerosis, and cardiovascular disease (Armstrong et al., 2013; Boehncke, 2018; Gelfand et al., 2006; Mehta et al., 2011), risks that are shared with other chronic inflammatory diseases. A causal link between systemic inflammation and the comorbidities seen in psoriasis remains to be fully elucidated.

In psoriasis, there is a complex interplay between components of the innate and adaptive immune systems (Lowes et al., 2014; Schön, 2019). Innate immunity seems to be important in the active, severe form that involves systemic and comorbid manifestations (Christophers and van de Kerkhof, 2019; Fanoni et al., 2019). The IL-23/T helper type 17 immune axis plays a critical role in the chronic state, which is supported by the efficacy of immunotherapies directed against TNF-α, IL-17, and IL-23 in the treatment of psoriasis (Harden et al., 2015).

The inflammasomes form the central hub of innate immune regulation. Members of the NLR family of proteins, including NLRP1, NLRP3, NLRC4, and AIM2, assemble into multiprotein complexes (termed inflammasomes) upon sensing microbial or danger signals (Broz and Dixit, 2016). Among the NLRs, NLRP3 is the most extensively studied, and it has been implicated in several chronic inflammatory disorders (Liu et al., 2017; Martinon et al., 2006; Masters et al., 2010; Mathews et al., 2014). The activation of the NLRP3 inflammasome requires an initial priming signal, which triggers the transcriptional upregulation of the NLRP3 and pro–IL-1β genes (Bauerle and Eberl, 2009). This is followed by a second signal, which results in inflammasome assembly, cleavage of pro-caspase-1 to active caspase-1, and the production of IL-1β and IL-18 (Latz et al., 2013).

A role of inflammasomes in psoriasis pathogenesis is supported by genetic data showing associations with NLRP1, NLRP3, and AIM2 (Broz and Dixit, 2016; Carlström et al., 2012; Ekman et al., 2014; Zuo et al., 2015). Moreover, previous studies have reported increased caspase-1 activity (Johansen et al., 2007) and caspase-5 expression (Salskov-Iversen et al., 2011) in psoriatic skin as well as upregulated NLR-signaling genes in psoriatic dermis (Tervaniemi et al., 2016). Constitutive IL-1β activation or the lack of an IL-1 receptor antagonist results in a T helper type 17–mediated response and a psoriatic phenotype in mice (Guo et al., 2009; Shepherd et al., 2004).

Although there is accumulating evidence for the involvement of inflammasomes in cardiovascular diseases (An et al., 2019), studies elucidating the systemic role of inflammasomes in patients with psoriasis is lacking. In this study, we...
demonstrate increased inflammasome priming and activity in patients with psoriasis and identify TNF-\(\alpha\) as the main regulator of inflammasome expression and function in the peripheral blood of patients with psoriasis.

**RESULTS**

Enhanced plasma levels of IL-1\(\beta\) and IL-18 in untreated patients with psoriasis compared with those in healthy controls

To determine whether there is an increased inflammasome activity in patients with psoriasis, we analyzed the free circulating levels of the inflammasome-generated cytokines IL-1\(\beta\) and IL-18 in the plasma samples of untreated patients with psoriasis (\(n = 18\)) and healthy controls (\(n = 19\)) using Luminex (R&D Systems, Minneapolis, MN). The PASI score of the included patients was in the range of 0–25, encompassing the entire spectrum of low to severe disease. We found significantly higher plasma levels of IL-1\(\beta\) and IL-18 (Figure 1a and b) in the patients than in the controls, suggesting increased constitutive inflammasome activity, which may have implications for systemic inflammation. The IL-1\(\beta\) levels in the patient group demonstrated a highly significant correlation with the IL-18 levels (\(r = 0.59, P = 0.009\), Pearson’s correlation coefficient) (Supplementary Figure S1). We found no correlations between the investigated cytokines and disease severity (PASI), suggesting sources of IL-1\(\beta\) and IL-18 other than the lesional skin.

Enhanced constitutive expression of inflammasome sensors in the peripheral blood subsets of untreated patients with psoriasis

We next investigated the baseline levels of inflammasome sensors in patients with psoriasis (mean age = 49.25 ± 14.2 years, mean PASI = 5.6 ± 6.9) compared with those in healthy controls (mean age = 51.5 ± 15 years). Using a multicolor flow cytometry panel, we determined the
expression levels of NLRP1, NLRP3, and AIM2 in the CD14+ CD16+, CD4+, and CD8+ subsets of red blood cell–lysed fresh whole blood. Significantly enhanced baseline expression levels of NLRP3 and NLRP1 were noted in the CD14+ monocyte, CD16+ neutrophil, and CD4+ lymphocyte subsets from patients with psoriasis compared with those from the healthy controls (Figure 1c) (Supplementary Figure S2). Similar results were observed with an increased AIM2 expression in the CD14+ and CD16+ subsets in the patients (Supplementary Figure S2). This suggests that the inflammasome sensors exist in a primed state in the immune cell subsets in the peripheral blood of the patients.

### Higher caspase-1 reactivity to lipopolysaccharide in the untreated patients with psoriasis than in healthy controls

Next, owing to the lack of correlation between inflammasome-generated cytokines and severity, we selected patients with mild PASI and investigated whether the constitutively upregulated peripheral-blood NLRP3 expression results in increased inflammasome function. Using fresh whole-blood samples from patients with mild psoriasis (mean PASI = 3.8 ± 2.6) and healthy controls, we found no detectable differences in spontaneous caspase-1 activity between the two groups (data not shown), suggesting that inflammasome priming in the peripheral blood does not result in its spontaneous activation. Interestingly, in a patient who was suffering an exacerbation of severe psoriasis (PASI = 25) after the sudden withdrawal of systemic treatment, we found increased basal caspase-1 activity in the CD4+ and CD8+ cell subsets. The same patient, who later exhibited chronic-phase psoriasis (PASI = 9), demonstrated normal basal caspase-1 activity in the CD4+ and CD8+ cell subsets (Supplementary Figure S3). These findings suggest that an acute inflammatory state can provoke the constitutive involvement of inflammasomes in the lymphoid subsets, and this is supported by recent data showing NLRP3-dependent IL-1β release in the CD4+ cells from patients with gain-of-function NLRP3 mutations (Arbore et al., 2016).

To elucidate the extent of inflammasome reactivity toward stimuli in the immune cells, we made use of the toll-like receptor 4 agonist lipopolysaccharide (LPS). A total of 1 hour of LPS exposure of fresh whole-blood samples was followed by the determination of caspase-1 activity in the CD14+, CD16+, CD4+, and CD8+ cell subsets. Studies in mice have suggested NLRP3 as an important modulator of age-associated inflammatory changes (Stout-Delgado et al., 2012). Using 18 healthy individuals, we found a distinct negative correlation between LPS-induced caspase-1 reactivity in the CD14+ cells and the age of the individuals (r = −0.5, P = 0.02), suggesting an immune aging of the human monocytes (Supplementary Figure S4). No correlations of caspase-1 reactivity to body mass index and gender were found (data not shown). Using age-matched patients and healthy controls (Table 1), we observed significantly increased caspase-1 reactivity in the CD14+ and CD16+ cell subsets of the patients (Figure 2) compared with those of the controls, which suggests that the priming of NLRP3 in these cells promotes inflammasome reactivity. In contrast, no differences in caspase-1 reactivity were observed in the CD4+ and CD8+ cell subsets (data not shown). We did not detect caspase-3 and/or caspase-7 in the CD14+ and CD16+ blood cells, showing that the fluorescence inhibitor of caspase-1
activity we observed did not arise from the apoptotic caspases but from the inflammasome response (Supplementary Figure S5). However, the detection of other inflammatory caspases cannot be ruled out.

**Exposure to TNF-α selectively primes NLRP3 but not NLRP1 or AIM2 or NLRC4**

To investigate the mechanism of inflammasome priming in patients with psoriasis, we investigated the effects of exposing whole-blood samples for 1 hour to the key psoriasis-related cytokines on the gene expression of NLRP1, NLR31, NLRC4, and AIM2. The cytokines IL-17, TNF-α, IL-23, and IFN-γ have all been shown to be increased systemically in psoriasis (Enerbäck, 2011). Among the cytokines, exposure to TNF-α alone and in combination with IL-17 induced a substantial 10-fold increase in NLRP3 gene expression compared with unstimulated samples (Figure 3a). Except for the significant decrease in NLRC4 to the combination of IL-17 and TNF-α, no changes in the expression levels of NLRP1, NLRC4, and AIM2 to psoriasis cytokines were observed (Supplementary Figure S5).

These results suggest that among the cytokines that are strongly implicated in psoriasis, TNF-α induces substantial and specific priming of NLRP3 but not of any of the other inflammasome-forming NLRs.

**TNF-α treatment upregulates the expression of pro-IL-1β and pro-IL-18**

We next examined the role of TNF-α in the transcriptional regulation of pro-IL-1β and pro-IL-18 in whole blood. We found that the 1-hour exposure of whole blood to TNF-α resulted in a remarkable (>160-fold) increase in pro-IL-1β expression (Figure 3b). A comparatively smaller but significant increase in pro-IL-18 was observed (Figure 3c). These findings indicate that TNF-α induces the transcriptional upregulation of both pro-IL-1β and pro-IL-18. The combination of IL-17 with TNF-α resulted in a small but significant increment in pro-IL-1β gene expression, which was not observed when IL-17 was used alone. Thus, TNF-α primes not only NLRP3 but also IL-1β and IL-18.

**TNF-α activates caspase-1 without the requirement for a second stimulus**

To determine whether any of the psoriasis-associated cytokines induce a fully activated inflammasome, thereby activating caspase-1, we exposed fresh whole blood for 1 hour to IL-17, TNF-α, IL-23, or IFN-γ, followed by flow cytometry. TNF-α exposure resulted in a significant increase in caspase-1 activity, which was present in the innate immune CD14+ and CD16+ populations but was absent in the lymphoid CD4+ and CD8+ cell subsets (Figure 4a and b). A significant increase in caspase-1 activity was also observed when using the combination of IL-17 and TNF-α but not when IL-17 alone was used, primarily indicating a TNF-α effect. IL-23 addition gave rise to a very small but significant increase in caspase-1 activity. Our data suggest that TNF-α both primes and activates the NLRP3 inflammasome, thereby bypassing the requirement for a second signal.

**TNF-α–induced inflammasome activity is dependent upon NLRP3 and ROS**

To elucidate whether TNF-α–induced inflammasome activity is dependent upon NLRP3, we added the specific NLRP3 inhibitor MCC950 at 1 hour before exposing the whole blood to the combination of TNF-α and IL-17. This resulted in a substantially decreased pro-IL-1β expression (from ~160-fold to ~30-fold) (Figure 5a). In contrast, there was no decrease in pro-IL-18 on preincubation with MCC950 (data not shown). ROS generation is a well-known trigger for inflammasome activation (Tschopp and Schroder, 2010). The addition of the ROS scavenger N-acetyl-L-cysteine resulted in a significant decrease in the TNF-α + IL-17–induced pro-IL-1β gene expression, suggesting that ROS are involved in TNF-α + IL-17–induced pro-IL-1β activation. (Figure 5a).

Similarly, 1 hour of preincubation with MCC950 before the exposure of the whole blood to TNF-α resulted in significantly decreased caspase-1 activity, demonstrating that TNF-α–induced inflammasome activity is largely dependent on NLRP3 (Figure 5b).

**TNF-α–induced inflammasome activity involves caspase-8**

Caspase-8 signaling has been suggested to act upstream of NLRP3 in the LPS-mediated alternative inflammasome

Figure 3. Effect of psoriasis-related cytokines on mRNA levels of NLRP3, pro-IL-1β, and pro-IL-18. (a) The effect of 1 hour of exposure of whole blood at 37 °C to IL-17, TNF-α, IL-23, and IFN-γ on the expression of NLRP3 was determined. The effects of 1 hour of exposure of whole blood to psoriasis-related cytokines on the expression levels of (b) pro-IL-1β and (c) pro-IL-18 were determined. The samples were analyzed using qRT-PCR and the results are shown as fold-changes relative to the unstimulated sample (n = 3–5). A paired t-test was used for the comparisons. Error bars represent mean ± SEM. *P < 0.05, **P < 0.001. ns, not significant.
signaling in human monocytes (Gaidt et al., 2016). To determine whether the TNF-α–induced inflammasome activity in blood cells involves caspase-8, we added the caspase-8 inhibitor (Z-IETD-FMK) at 1 hour before stimulation with TNF-α, followed by the determination of caspase-1 activity. We found a significant decrease in caspase-1 activity on the inhibition of caspase-8 (Figure 5c), thereby confirming that TNF-α signaling in monocytes proceeds through the TNF receptor–caspase-8–caspase-1 pathway.

**TNF-α activates the alternative inflammasome pathway in monocytes**

The recently described alternative inflammasome pathway in human monocytes has been shown to bypass the need for a second stimulus and proceed independently of pyroptosis (Gaidt et al., 2016). On the basis of our observation that TNF-α did not require a second stimulus for inflammasome activation, we investigated whether the TNF-α exposure resulted in pyroptosis by determining the release of lactate dehydrogenase (LDH). Because potassium ion efflux is sufficient to activate NLRP3, we made use of nigericin as a positive control (Muñoz-Planillo et al., 2013). We found that just as in the case with LPS, TNF-α did not result in LDH release, whereas robust LDH release was observed when nigericin was used. Our data suggest that TNF-α activates the alternative inflammasome pathway (Figure 5d).

**Patients with psoriasis treated with TNF-α inhibitors display normalized inflammasome function**

Our data demonstrated an important role for TNF-α in inflammasome priming and activation. As anticipated, we found that the TNF-α levels in the plasma were elevated in the patients with psoriasis (n = 18) compared with those in the healthy individuals (n = 23) (Supplementary Figure S5c), thereby confirming the finding that TNF-α is present in the circulation and may act as a trigger for inflammasome activation (Bai et al., 2017).

TNF-α inhibition using neutralizing antibodies is highly effective in treating psoriasis (Harden et al., 2015). In patients with psoriasis who were treated with anti–TNF-α antibodies for at least 8 months, we found an attenuated LPS-induced upregulation of pro–IL-1β gene expression in the whole blood compared with that in the whole blood of the untreated patients, suggesting that the anti–TNF-α treatment reduces the priming of the inflammasome (Figure 6a). These patients also exhibited lower IL-1β and IL-18 plasma levels than the untreated patients (although not reaching statistical significance) but without a difference in plasma levels compared with those in the healthy controls (Supplementary Figure S7).

Interestingly, compared with the untreated patients (mean PASI = 3.8 ± 2.6), the patients treated with TNF-α inhibitors (mean PASI = 2.1 ± 1.98), despite having comparable PASI levels with the untreated groups, displayed significantly reduced caspase-1 reactivity in the CD14+ and CD16+ cells (Figure 6b). In fact, the caspase-1 reactivity displayed by these patients was similar to the levels displayed by healthy controls.

Methotrexate is a folate antagonist that at a standard dose of 15–20 mg per week is a well-established therapy for inflammatory conditions such as psoriasis. Intriguingly, an increased caspase-1 reactivity in the CD14+ and CD16+ subsets persisted in the patients with psoriasis who were treated with methotrexate (mean PASI = 2.4 ± 1.53) compared with the reactivity in the patients treated with anti–TNF-α (Figure 6c).

The above data strongly support the assumption that TNF-α plays a key role in the increased inflammasome reactivity in patients with psoriasis. The marked difference in caspase-1 reactivity between the patients treated with methotrexate and those treated with anti–TNF-α antibodies suggests that targeting TNF-α is superior in treating the inflammasome-induced systemic inflammation in psoriasis.

**DISCUSSION**

Psoriasis entails a low-grade systemic inflammation, as evidenced by the increased C-reactive protein levels in patients compared with those in healthy subjects (Dowlatshahi et al., 2013). The systemic manifestations of psoriasis, including obesity and atherosclerosis, lead to cardiovascular disease and an increased risk of major adverse cardiovascular events.
Insulin resistance and endothelial dysfunction, both of which are associated with atherosclerosis, have been suggested as the pathogenic link between psoriasis and cardiovascular comorbidity (Boehncke, 2018). In line with this concept, TNF-α, which is a major insulin antagonist and a known inducer of endothelial dysfunction, has been found to be systemically enhanced in cases of psoriasis (Hotamisligil et al., 1993; Picchi et al., 2006). Although this provides a solid conceptual basis for the linkage between the systemic inflammation in psoriasis and cardiovascular comorbidity, there remains a lack of mechanistic evidence (Boehncke et al., 2011; Yamanaka and Mizutani, 2015).

In this study, we have investigated the inflammasomes and provide mechanistic data about their role in triggering the systemic inflammation in psoriasis. We demonstrate that patients with psoriasis have increased systemic levels of IL-1β and IL-18 and possess primed inflammasome components. No correlations of the above cytokines with PASI were observed in the patients with mild-to-severe PASI, suggesting that the inflammasome-dependent systemic inflammation in these patients is independent of their cutaneous manifestations. Using the blood from patients with mild-to-moderate psoriasis (PASI < 7.5) in all the caspase-1 reactivity experiments, we could show that the myeloid cells from untreated patients with psoriasis exhibit enhanced caspase-1 production in response to LPS. Importantly, we show that TNF-α selectively activates the NLRP3 inflammasome without the requirement for a priming signal, resulting in the activation of caspase-1. In accordance with this, we show that patients with psoriasis who are treated with anti-TNF-α antibodies do

![Figure 5. TNF-α-primes and activates the inflammasome.](image)

(Armstrong et al., 2013; Boehncke, 2018; Gelfand et al., 2006; Mehta et al., 2011).
not have increased plasma levels of IL-1β and IL-18 and do not exhibit increased caspase-1 reactivity compared with untreated patients.

IL-1β signaling plays a well-documented role in the pathogenesis of psoriasis (Lowes et al., 2014). IL-1β has been shown to be critical for the differentiation and activation of IL-17-producing T cells (Sutton et al., 2009), and IL-1β protein levels in the psoriatic skin have been shown to correlate with disease severity and treatment response (Cai et al., 2019). It is increasingly recognized that activation of the NLRP3 inflammasome contributes to the pathogenesis of cardiovascular diseases (An et al., 2019). NLRP3 expression in adipose tissue has been shown to be associated with coronary atherosclerosis (Bando et al., 2015). The NLRP3-specific inhibitor MCC950 prevents diet-induced cardiovascular disease in mice through autophagy induction (Pavillard et al., 2017). Data from the Canakinumab Anti-Inflammatory Thrombosis Outcome Study trial (Ridker et al., 2017) reveal a statistically significant lower rate of recurrent cardiovascular events in patients treated with the anti-IL-1β antibody canakinumab, suggesting a mechanistic role for the inflammasome in linking systemic inflammation with cardiovascular diseases (Ridker et al., 2017). Moreover, the antithrombogenic effect of statins has been attributed to reduced NLRP3 and IL-1β expression (Satoh et al., 2014).

We performed our experiments using unmanipulated whole blood in order to reflect accurately the in vivo conditions (Mallone et al., 2011). The NLRP3 inflammasome is mainly described in myeloid cells, with CD14+ monocytes being the main source of IL-1β. Neutrophils have recently been shown to possess functional inflammasome components (Bakele et al., 2014), and the involvement of CD4+ cells in inflammasome hyperactivity has recently been demonstrated in patients with gain-of-function mutations in NLRP3 (Arbore et al., 2016). Consistent with the above studies, we find increased expression of NLRP3 not only in the CD14+ subset but also in the CD16+ and CD4+ subsets of patients with psoriasis. Increased LPS-induced caspase-1 reactivity in the CD14+ and CD16+ cells of the untreated patients was observed, which is in line with our data showing NLRP3 priming in the patients’ cells. The observed lack of correlations between IL-1β and IL-18 and the severity of psoriasis as well as with the increased caspase-1 reactivity in patients with mild PASI suggests an ongoing, low-grade inflammatory process in the blood that is acting independently of the skin manifestations. Moreover, our data support that psoriasis may be a part of the autoinflammatory keratinization diseases (Akiyama, 2020; Akiyama et al., 2017). The CD4+ T cells, despite the primed NLRP3, did not display enhanced caspase-1 reactivity in response to short-term exposure to LPS, which may reflect a requirement for additional T-cell activation, as shown by Arbore et al (2016).

TNF-α expression is increased in adipose tissues, including adipocytes and macrophages in obese patients, and this potentially contributes to its circulating levels while also leading to the amplification of pre-existing inflammatory processes (Yost and Gudjonsson, 2009). Inhibitors of TNF-α have previously been demonstrated to reduce cardiovascular risk in patients with psoriasis as well as reducing the risk for diabetes, which is in line with the insulin-antagonist effect of TNF-α (Hotamisligil et al., 1993). Interestingly, the risk-reducing effect of TNF-α was demonstrated to be superior to that of methotrexate (Wu et al., 2017). Furthermore, data
from a recent Cardiovascular Inflammation Reduction Trial show that among patients with stable atherosclerosis, treatment with methotrexate does not reduce the levels of IL-1β, IL-6, and C-reactive protein and does not result in fewer cardiovascular events compared with placebo (Ridker et al., 2019). Intriguingly, we show that the patients treated with methotrexate, like the untreated patients, have significantly increased caspase-1 reactivity, which is not seen in the patients treated with inhibitors of TNF-α.

TNF-α has been shown to be a key transcriptional regulator of NLRP3 inflammasome components in murine models (McGeough et al., 2017). Previous studies using isolated human monocytes and polymorphonuclear neutrophils have shown that exposure to TNF-α results in significantly increased expression of NLRP3 (O’Connor et al., 2003) and pro–IL-1β (Marucha et al., 1990), respectively. Surprisingly, we found that exposing whole blood to TNF-α resulted in the production of NLRP3-dependent active caspase-1, without the need for a second signal. The recently described alternative inflammasome signaling utilized by toll-like receptor 4-LPS in human monocytes has been shown to bypass the requirement for a second signal, leading to caspase-1 activation through the receptor-interacting serine/threonine-protein kinase 1 and caspase-8 pathways (Gaidt et al., 2016) and is independent of pyroptosis. Accordingly, we show that TNF-α–induced caspase-1 activation in monocytes is dependent on caspase-8 and proceeds without pyroptotic cell death. TNF-α signaling takes place through the TNF receptor 1 and engages the receptor-interacting serine/threonine-protein kinase 1-TRADD-caspase-8 pathway, leading to regulated necroptosis (Vanden Berghe et al., 2014). Pyroptotic cell death characterizes the classical but not the alternative inflammasome pathway, thereby allowing the cells in the latter case to respond with IL-1β production without committing to cell death, which may be instrumental in mediating a low-grade, chronic inflammation. Our study demonstrates that TNF-α in the blood of patients with psoriasis stimulates the NLRP3 inflammasome. However, additional data are required to validate the contribution of TNF-α–mediated NLRP3 activation in the systemic inflammation and comorbidities of psoriasis.

In summary, we show that there is an enhanced NLRP3 inflammasome function in blood samples from patients with psoriasis. Our data support a role for innate immune system and inflammasomes in systemic inflammation in psoriasis, with TNF-α being the main mediator of inflammasome activation. The cardiovascular risk–reducing effect of TNF-α inhibitors may be mechanistically explained by attenuated inflammasome activity.

**MATERIALS AND METHODS**

The detailed protocols are described in the Supplementary Materials.

**Study subjects and samples**

All the patients included in the study were examined, and the diagnosis of psoriasis was verified by a dermatologist at the Department of Dermatology at Linköping University Hospital, Linköping, Sweden. PASI was used to appraise disease severity. The recruited patients had PASI between 0 and 25. The study was approved by the Local Ethics Committee and conducted according to the Declaration of Helsinki principles. Written informed consent was obtained from all patients and healthy controls. Peripheral blood samples from the study subjects were collected in and immediately processed for flow cytometry or RNA isolation. Plasma was collected using standard procedures.

**In vitro stimulations of blood cells**

Peripheral whole-blood samples were left untreated or were pre-incubated with MCC950 (1 μM; Invivogen, Toulouse, France) and Z-IEFD-FMK (5 μM; BD Biosciences, Franklin Lakes, NJ) for 1 hour at 37 °C, followed by 1 hour at 37 °C with the indicated stimuli before further processing using either qPCR or flow cytometry. The following stimuli and concentrations were used: IL-17 (10 ng/ml); TNF-α (10 ng/ml); IFN-γ (20 ng/ml); IL-23 (10 ng/ml); IL-4 (20 ng/ml); N-acetyl-L-cysteine (5 mM; Sigma-Aldrich, St. Louis, MO); nigericin (6.5 μM; Invivogen), and LPS (100 ng/ml, Sigma-Aldrich). All cytokines were purchased from R&D Systems.

**RNA extraction and quantitative real-time PCR**

RNA was extracted from whole blood using the QiAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Quantitative real-time PCR was performed to detect the genes of interest using SYBR green (Applied Biosystems, Foster City, CA) or predesigned TaqMan assays in the 7500HT PCR apparatus (Applied Biosystems). GAPDH or RPLPO were used as reference genes.

**Flow cytometric analysis**

**NLRP1, NLRP3, and AIM2 detection in white blood cell subsets.** After red blood cell lysis, the following surface staining antibodies were used: CD14-Pacific Blue, CD16-APC H7, CD4-PE Cy7, CD8-V500, and CD209-PCP Cy5.5 (all from BD Biosciences). Intracellular staining was performed for 40 minutes at 4 °C using the antibodies NLRP1-AF700, NLRP3-phycoerythrin, and AIM2-AF647 (all from Novus Biologicals, Centennial, CO).

**Active caspase 1 and caspase-3 and/or caspase-7 detection using fluorescence inhibitor of caspase-1 flow cytometry.** Peripheral blood samples were left untreated or treated with LPS for 1 hour at 37 °C. The red blood cells were lysed and stained with CD4-pacific blue, CD14-APC-Cy7, CD8-phycoerythrin, and CD16-PE-Cy7 (all from BD Biosciences). Caspase-1 activity was determined using a caspase-1 fluorescence inhibitor of caspase-1 kit and caspase-3 and/or caspase-7 activity was detected using FLICA 660 (ImmunoChemistry Technologies, Bloomington, MN) according to the manufacturer’s instructions. All flow cytometric analyses were performed on the Gallios flow cytometer (Beckman Coulter LS, Brea, CA) and analyzed using Kaluza Analysis, version 1.3 (Beckman Coulter). The gating strategy is shown in Supplementary Figure S8.

**Cytokine determinations in plasma samples using Luminex**

The plasma levels of IL-1β, IL-18, and TNF-α were determined using Luminex kits (R&D Systems) following the manufacturer’s instructions.

**LDH assay**

Monocytes were isolated from PBMCs using EasySep Human Monocyte Enrichment Kit without CD16 Depletion (STEMCELL Technologies, Vancouver, Canada). The Pierce LDH cytotoxicity kit (Life Technologies, Carlsbad, CA) was used to determine pyroptotic cell death according to the manufacturer’s instructions.
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Statistical analysis
Data analysis was performed in GraphPad Prism, version 8.3 (GraphPad Software, San Diego, CA). Differences between the patients and the healthy individuals were determined using either the Mann–Whitney U test or the unpaired t-test with Welch’s correction. The pairwise comparisons in the in vitro experiments were determined using a paired t-test. A P-value ≤ 0.05 was considered statistically significant. Data are represented as mean ± SEM. Correlations were determined using Pearson’s correlation coefficients.

Data availability statement
No datasets were generated or analyzed during this study.

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CONFLICT OF INTEREST
The authors state no conflicts of interest.

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AUTHOR CONTRIBUTIONS
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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.2020.07.012.

REFERENCES


SUPPLEMENTARY MATERIALS AND METHODS

Luminex
IL-1β and TNF-α levels were determined using the high-sensitivity Luminex kits (R&D Systems, Minneapolis, MN). Samples were acquired using Flex MAP 3D (Austin, TX). Five-parameter log-logistic was used to generate the standard curve. The data were analyzed using the MasterplexQT 2010 version 5.0 software (Hitachi MiraiBio Group, San Francisco, CA).

Lactate dehydrogenase assay
Cells were seeded at 80,000 cells per well in a 96-well plate. The next day, lipopolysaccharide or TNF-α was added 30 minutes before the addition of nigericin for 1.5 hours. Relative lactate dehydrogenase release was calculated as a percentage of the maximum lactate dehydrogenase lysis control.

Supplementary Figure S1. Correlation between plasma IL-1β and IL-18 in psoriasis patients (n = 18) was determined using Pearson’s correlation coefficient.

Supplementary Figure S2. Expression levels of NLRP1 and AIM2 are increased in psoriasis patients. Constitutive expression of inflammasome sensors was determined in the CD14+, CD16+, CD4+, and CD8+ subsets of red blood cell-lysed peripheral blood samples of patients with psoriasis (n = 12) and healthy controls (n = 5) using multicolor flow cytometry. Data are represented percentage gated cells. An unpaired t-test with Welch’s correction was performed. *P < 0.05, **P < 0.001, ***P < 0.0001. Error bars represent mean ± SEM. ns, not significant.
Supplementary Figure S3. Enhanced basal caspase-1 activity in CD4 and CD8 cell subsets during severe psoriasis, which normalizes during the chronic phase.

Basal FLICA activity was determined in the peripheral CD4+ and CD8+ cells from a patient who was suffering an exacerbation of severe psoriasis and later (from the same patient) during the chronic phase of the disease. CD4+ and CD8+ cells from two healthy controls were used for comparison. Data are represented as MFI. FLICA, fluorescence inhibitor of caspase-1 activity; MFI, mean fluorescence intensity.
Supplementary Figure S4. (a) Using peripheral blood samples from healthy controls (n = 18), caspase-1 reactivity in response to 1 hour of stimulation at 37 °C with LPS (100 ng/ml) was determined in the CD14+ cells using flow cytometry. Correlation between age and caspase-1 reactivity (using fold-changes stimulated/unstimulated sample) was determined using Pearson’s correlation coefficient. (b) No caspase-3 activity was detected in the CD14+ and CD16+ cells in unstimulated or LPS stimulated blood samples. FLICA, fluorescence inhibitor of caspase-1 activity; LPS, lipopolysaccharide; SS, side scatter.
Supplementary Figure S5. Effects of psoriasis-related cytokines on the expression levels of AIM2, NLRC4, and NLRP1. The effects of 1 hour of exposure at 37 °C of whole blood to psoriasis-related cytokines on the expression levels of AIM2, NLRC4, and NLRP1 were determined. The samples were analyzed using qRT-PCR and the results are shown as fold-changes relative to the unstimulated sample (n = 3–5). A paired t-test was performed to compare the cytokine-stimulated and unstimulated samples. Error bars represent mean ± SEM. *P < 0.05, **P < 0.001. LPS, lipopolysaccharide.

Supplementary Figure S6. Systemic levels of TNF-α are increased in the untreated patients. The plasma levels of TNF-α were determined in healthy controls (n = 23) and untreated patients (n = 18) using Luminex. The Mann-Whitney U test was used for the comparisons. Error bars represent mean ± SEM. *P < 0.05, **P < 0.001, ***P < 0.0001.
Supplementary Figure S7. IL-1β and IL-18 levels are normalized in anti–TNF-α-treated patients with psoriasis. The plasma levels of IL-1β and IL-18 were determined in healthy controls (n = 19), untreated patients (n = 18), and anti–TNF-α-treated patients (n = 9) using Luminex. Error bars represent mean ± SEM. *P < 0.05, **P < 0.001, ***P < 0.0001. ns, not significant.

Supplementary Figure S8. Gating strategy for the detection of caspase-1 using FLICA. The CD14+, CD16+, CD4+, and CD8+ cell populations were identified using side scatter from the singlets, followed by the identification of FLICA positive population by plotting FLICA against each cell type. MFI was used. FLICA, fluorescence inhibitor of caspase-1 activity; FS, forward scatter; MFI, mean fluorescence intensity; SS, side scatter.