The IL-1 Pathway Is Hyperactive in Hidradenitis Suppurativa and Contributes to Skin Infiltration and Destruction

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Hidradenitis suppurativa (HS) (also designated *acne inversa*) is a chronic inflammatory disease characterized by painful purulent skin lesions and progressive destruction of skin architecture. Despite the high burden for the patients, pathogenetic pathways underlying HS alterations remain obscure. When we examined the HS cytokine pattern, IL-1β turned out to be a highly prominent cytokine, overexpressed even compared with psoriatic lesions. Analyses of IL-1β–induced transcriptome in various cell types showed overlapping profiles, with upregulations of molecules causing immune cell infiltration and extracellular matrix degradation, and of specific cytokines including IL-6, IL-32, and IL-36. Matching cellular IL-1 receptor levels, dermal fibroblasts showed both the strongest and broadest IL-1β response, which was not clearly shared or strengthened by other cytokines. The IL-1β signature was specifically present in HS lesions and could be reversed by application of IL-1 receptor antagonist. Search for blood parameters associated with IL-1β pathway activity in HS identified serum amyloid A, which was synergistically induced by IL-1β and IL-6 in hepatocytes. Consequently, strongly elevated blood serum amyloid A levels in HS correlated positively with the extent of inflammatory skin alterations. In summary, the IL-1β pathway represents a pathogenetic cascade, whose activity may be therapeutically targeted and monitored by blood SAA levels.


INTRODUCTION

Hidradenitis suppurativa (HS) (also referred to as *acne inversa*) is a chronic inflammatory disease affecting the intertriginous skin of especially axillary, inguinal, and peri-anal sites (Jemec, 2012). HS skin lesions contain painful inflamed nodules, abscesses, and fistulae that discharge malodorous purulent secretion. The advanced stage of HS is commonly associated with destruction of skin architecture.

HS is a common disease affecting approximately 1% of the population (Revuz et al., 2008). First manifestations usually appear during adolescence or young adulthood, and both sexes are affected. HS leads to a high extent of emotional and physical distress with social embarrassment, isolation, and depression (Kurek et al., 2013; Onderdijk et al., 2013). Furthermore, it profoundly disturbs the sexual health of affected patients (Janse et al., 2017; Kurek et al., 2012). Consequently, the impairment of the quality of life for HS is even far more severe than for various other dermatoses such as psoriasis, atopic eczema, or skin cancer (Matusiak et al., 2010; von der Werth et al., 2001). Moreover, many HS patients have metabolic and endocrinological alterations, including hypertriglyceridaemia, hypo-high density lipoprotein (HDL)-cholesterolemia, hyperglycemia, and central obesity, that increase the risk of cardiovascular disorders and reduce life expectancy (Egeberg et al., 2016; Sabat et al., 2012). As recently shown, HS patients also frequently suffer from spondyloarthritis (Richette et al., 2014; Schneider-Burrus et al., 2016).

Because of its high prevalence and severe physical and mental burden, HS has gained increased attention during the last years. Nevertheless, current health care of HS patients is still inadequate, and treatment options remain very limited (Kimball et al., 2016; Kohorst et al., 2016). This is mainly due to the lack of knowledge of the molecular pathways that lead
to the inflammatory skin destruction. Current understanding emphasizes infundibular hyperkeratosis with subsequent follicular plugging and stasis as initial events (Prens and Deckers, 2015). This allows commensal bacteria to propagate, and support immune cell infiltration. Subsequently, dilatation and rupture of the apocrine hair follicle unit facilitates expansion and chronification of inflammation (Wolk et al., 2011), leading to production of great amounts of pus that often contains bacteria and biofilms (Hessam et al., 2016; King et al., 2017). Emerging deep inflammatory nodules, abscesses, and fistulae represent the clinically relevant consequences. Although immune cells and their mediators are widely accepted as key players in HS pathogenesis (Hessam et al., 2018; Kelly et al., 2015; Lima et al., 2016; Thomi et al., 2017; Tsaousi et al., 2016; van der Zee et al., 2011; Wolk et al., 2011, 2017), the exact role of individual cytokines is currently unclear. This prompted us to investigate the cytokine milieu in HS skin lesions.

RESULTS

Cytokine expression pattern of HS lesional skin is marked by dominant IL-1β presence

Knowledge about the presence of immune mediators in inflamed tissue sheds light on the involved pathogenetic pathways and is a prerequisite for the development of any targeted anti-cytokine therapy. Although HS lesions are very disabling (Figure 1a), the local cytokine network in HS is still enigmatic. Therefore, we individually quantified the expression levels of about 30 mediators in lesional skin of HS patients and compared them with the expression patterns in healthy donor skin and in skin lesions of psoriasis, a model disease for chronic inflammatory skin conditions. For this, whole-thickness skin biopsy samples were analyzed by quantitative real-time PCR on reverse transcribed RNA (RT-qPCR). Expression of the majority of investigated mediators was significantly higher in HS lesions than in healthy control skin (Figure 1b and data not shown). Several of them, such as IL-17A and tumor necrosis factor (TNF)-α, reached even levels seen in psoriasis lesions. The cytokine, whose expression in HS lesions most exceeded that in psoriasis lesions, was IL-1β. In fact, IL-1β levels surpassed those in lesional psoriatic and healthy control skin by approximately 8-fold and approximately 130-fold, respectively (Figure 1b). Its sibling, IL-1α, which signals via an identical receptor complex, was only minimally increased in HS lesions compared with healthy and lesional psoriatic skin. Up-regulation of IL-1α transcripts in HS lesions was not paralleled by an increased expression of the antagonistic IL-1 family member IL-1RA, therefore differing from the situation in psoriasis. Further analyses showed that elevated IL-1β levels were already present, although to a lower extent, in perilesional skin of HS patients (Figure 1c). Finally, the lesional IL-1β expression in HS also exceeded the levels in other skin disorders such as atopic dermatitis and cutaneous T-cell lymphoma (Figure 1c and data not shown).

The IL-1β production is regulated not only transcriptionally but also posttranscriptionally by the inflammasome. The quantification of important inflammasome components in HS lesions by RT-qPCR showed a slight and moderate up-regulation of caspase-1 and NLRP3, respectively (Figure 1d), a finding in line with results from Kelly et al. (2015). This suggests an elevated production of active IL-1β protein in lesional HS skin. To substantiate this assumption, we put the skin biopsy samples in culture medium to allow the contained soluble mediators to diffuse into the medium, which was then assessed by ELISA. As shown in Figure 1e, active IL-1β protein was present in much larger quantities in active HS lesions than perilesional HS (∼35-fold) and healthy skin (∼55-fold). In contrast, the amount of IL-1x protein was not increased in perilesional or lesional HS skin cultures (see Supplementary Figure S1a online). The analyses of individual cell types separated from HS lesions by flow-cytometric cell sorting indicated macrophages/monocytes as major IL-1β producers (Figure 1f, and see Supplementary Figure S1b).

IL-1β induces a common transcriptional program in skin-relevant cell types

IL-1β emerged from our analysis as a cytokine with potential pathogenetic relevance. Therefore, we aimed to investigate skin-relevant effects of this mediator. Because perilesional and lesional HS skin did not differ in IL-1β receptor (IL-1R1) expression (Figure 2a), and our interest was focused on the progression of HS lesions, we decided to use primary human cells not influenced by inflammation for the following investigations. Because IL-1R1 was robustly expressed in immune cells, microvascular dermal endothelial cells, dermal fibroblasts, and keratinocytes (Figure 2b), we analyzed the response of all of these cells toward 24-hour IL-1β stimulation using a comprehensive RNA deep-sequencing (RNA-seq) approach. The transcriptional changes initiated in all investigated cell types mainly consisted of up-regulations (Figure 2c). The overall number of regulations was highest in fibroblasts, matching the outstanding IL-1R1 expression in these cells (Figure 2b). Nevertheless, all investigated cell types showed a high degree of overlap when considering the individual top 15 up-regulated (Figure 2d) and down-regulated (see Supplementary Figure S2 online) transcripts. Based on these data and using the PANTHER (i.e., Protein ANalysis THrough Evolutionary Relationships) classification scheme, we identified the following five major functional groups within the IL-1β—up-regulated transcripts (log2-fold change ≥ 1): molecules involved in remodeling of the extracellular matrix (6.5%–15.8%), chemokines (6.1%–21.1%), adhesion molecules (3.0%–11.5%), cytokines (6.4%–10.5%), and signal transduction elements (9.5%–15.8%) (Figure 2e).

IL-1β induces expression of manifold molecules involved in extracellular matrix destruction and immune cell infiltration, as well as of specific cytokines

We next examined the IL-1β influence on molecules representative of the identified functional groups in detail. Regarding molecules involved in extracellular matrix remodeling, transcripts, whose expression was up-regulated by IL-1β, comprised enzymes including matrix metalloproteinase (MMPs) (e.g., MMP3, MMP10) and other enzymes (e.g. ADAM12), proteinase inhibitors (e.g. serpinA1), and matrix components (e.g. collagen type III (COL3A1), COL10A1) (see Supplementary Table S1 online). The group of chemokines included numerous mediators known to recruit neutrophilic granulocytes (e.g., CXCL1, CXCL6),
Figure 1. IL-1β is the prominent element of differential cytokine composition in HS versus psoriasis skin lesions. (a) Pictures of affected skin of HS patients (left, axillary; right, gluteal). (b) Cutaneous cytokine expression was analyzed in healthy donors (n = 10–11), HS patients (lesional, n = 11–13), and psoriasis patients (lesional, n = 11–13) by RT-qPCR. (c) Cutaneous expression of IL-1β in patients with HS (paired perilesional and lesional, n = 7) and atopic dermatitis (lesional, n = 8) was assessed by RT-qPCR. (d) Cutaneous expression of inflammasome components was analyzed in healthy donors (n = 12) and HS patients.
myeloid cells (CCL7), T cells (e.g., CXCL10), natural killer T cells and innate lymphoid cells (CXCL16), B cells (CXCL13), and eosinophilic and basophilic granulocytes (CCL24), as well as those with a broader chemotactic profile (e.g., CCL2, CCL8, CCL20) (see Supplementary Table S1). The unique pattern of cytokines showed growth factors (e.g., M-CSF, GM-CSF) and interleukins (e.g., IL-1β, IL-6, IL-32, IL-36) (see Supplementary Table S1).

From these three groups, we chose MMP1, MMP3, MMP10 (representative of collagens and stromelysins, two major classes of extracellular matrix-degrading enzymes with different substrate specificities), CXCL1, CXCL6, CXCL10, CCL2, CCL8, CCL20, IL-6, IL-32, and IL-36β. The expression of these molecules in resting and IL-1β-stimulated skin-relevant cell populations was quantified by means of RT-qPCR and ELISA. Already resting fibroblasts showed high expression of many MMPs compared with the other analyzed cell types (see Supplementary Figures S3a and S4a online, and data not shown). IL-1β further up-regulated these molecules in these cells, whereas the response in immune cells, endothelial cells, and keratinocytes was more restricted. This is in line with the known dominant role of fibroblasts in extracellular matrix reorganization. A strong IL-1β-dependent up-regulation of the chemokines was mainly evident in immune cells, endothelial cells, and fibroblasts (see Supplementary Figures S3b and S4b, and data not shown). Furthermore, IL-1β-induced expressional increase of the cytokines IL-6, IL-32, and IL-36β (see Supplementary Figures S3c and S4c, and data not shown) was obvious. It was most evident for endothelial cells and fibroblasts in the case of IL-6. In contrast, robust increase of IL-32 transcript levels was observed for endothelial cells, fibroblasts, and keratinocytes, whereas fibroblasts were the main source of IL-36β upon IL-1β stimulation.

**IL-1β effects are not shared by other HS-typical cytokines in fibroblasts**

Next, we were interested in the specificity of the identified IL-1β effects. To this aim, we compared the IL-1β effect on the protein production of identified molecules with the effects of a range of other HS-relevant cytokines through in vitro stimulation assays. We focused on dermal fibroblasts, because these cells were the broadest and most potent producers of IL-1β target molecules among the analyzed cell types (Figures 2c, and see Supplementary Figures S3 and S4). Keratinocytes were included for comparison. Among the different cytokines tested, IL-1β turned out to be by far the strongest inducer of analyzed molecules in fibroblasts (Figure 3a). In keratinocytes, in contrast, other cytokines such as IL-17A (induction of MMP10 and CXCL1) or TNF-α and IFN-γ (induction of MMP10) partly shared the IL-1β effects (Figure 3b).

Because IL-17A and TNF-α also showed elevated expressions in HS lesions (Figure 1b) and both are well known for their cooperative actions with other mediators (Carrier et al., 2011; Liang et al., 2006; Wolk et al., 2009), we next tested for the synergistic potential of these cytokines with IL-1β. Although IL-17A had virtually no impact on the IL-1β effects in fibroblasts (Figure 3c), it strengthened the action of IL-1β in keratinocytes (Figure 3d). Clear amplification of the IL-1β effect by TNF-α was especially observed for fibroblast IL-6 production (Figures 3c and d).

**The IL-1β-induced transcriptome is highly recapitulated in HS skin lesions**

According to our hypothesis that HS lesions harbor strong IL-1β activity that influences local cells, we investigated the expression of in vitro-identified IL-1β target molecules in HS lesions. First, we compared the transcripts found to be up-regulated by IL-1β in the different cutaneous cell types in vitro (Figure 2d and e) with the up-regulations in HS lesions over healthy control skin identified by our preliminary RNA-seq analyses. As shown in Figure 4a, only 5% of transcripts that were not up-regulated by IL-1β in vitro were found to be up-regulated in HS lesions. Approximately 40% of transcripts that were up-regulated by IL-1β in vitro were also up-regulated in HS lesions. Furthermore, the probability that a transcript was up-regulated in HS lesions increased when it was induced by IL-1β in more than one cell type in vitro (Figure 4a). The consideration of individual cutaneous cell types showed that 39%–63% of transcripts induced by IL-1β in these cells were also found to be up-regulated in HS lesions (Figure 4b). Many of these transcripts concern molecules involved in extracellular matrix remodeling, chemokines, or cytokines. In line with that, RT-qPCR analyses showed markedly up-regulated expression of MMP1, MMP3, MMP9, MMP10, CCL2, CXCL1, IL-6, and IL-32 in lesional HS skin compared with healthy control skin (Figure 4c). The levels of these molecules were even considerably higher in HS lesions compared with psoriatic lesions (Figure 4c). The increased expression of matrix-degrading enzymes in HS skin lesions was paralleled by down-regulation of TIMP4, an important inhibitor of MMP activity. This resulted in strongly increased MMP/TIMP4 ratios in HS (Figure 4d), indicating an extraordinary activity of these enzymes in HS, which is in line with the destructive character of the disease. The strong up-regulation of chemokines attracting neutrophilic granulocytes (like CXCL1 or CXCL6) compared with other chemokines (e.g., CCL2) suggests a contribution of the IL-1β pathway to the massive infiltration of these cells and pus production observed in HS. In line with the mRNA data, up-regulation of IL-1β target molecules in HS lesions was also observed at the protein level: ELISA-quantified proteins diffused from cultured skin biopsy samples showed that MMP3, CXCL1, and IL-6 were strongly present in HS lesional
Figure 2. IL-1β–induced transcriptomes in different skin cell types harbor similar groups of molecules related to inflammation and extracellular matrix remodeling. (a) IL-1R1 expression was analyzed in paired perilesional (HS-PL) and lesional (HS-L) skin of seven HS patients by RT-qPCR. (b) Immune cells (PBMC), microvascular dermal endothelial cells (EC), dermal fibroblasts (FB), and keratinocytes (KC) were analyzed for IL-1R1 expression by RT-qPCR (mean ± standard error of the mean, n = 3). (c–e) PBMCs, ECs, FBs, and KCS were treated with IL-1β or left without stimulation (control) for 24 hours. Analyses of the induced transcriptome (n = 3) was performed by RNA sequencing. Up- and down-regulations were defined based on a log2 fold change of 1 or more and −1 or less, respectively. (c) Total numbers of regulated transcripts are shown. (d) Color-formatted data of individual top 15 up-regulated transcripts in each cell population are given as the mean of log2 fold change values. (e) Pie charts of up-regulated transcripts in each cell population are shown according to PANTHER classification. Numbers of transcripts per group are indicated. PANTHER, Protein ANalysis Through Evolutionary Relationships; PBMC, peripheral blood mononuclear cell; RT-qPCR, quantitative real-time PCR on reverse transcribed RNA.

skin compared with healthy control skin and perilesional HS skin (Figure 4e).

IL-1β target molecule expression in HS skin lesions is dependent on IL-1β activity

To further support the role of local IL-1β for the high presence of the identified target molecules in HS skin lesions, we first wondered whether there is a statistical relationship between the cutaneous expression of these molecules. Indeed, expression data obtained from HS lesions, psoriatic lesions, and healthy donor skin showed a significantly strong positive correlation for most IL-1β target molecules, as shown for MMP1 and CXCL1 (Figure 4f). In a second approach, we treated biopsy samples obtained from the same HS lesion in vitro for 1–3 days with or without antagonistic IL-1RA. As shown in Figure 4g and h, decreased expression of selected IL-1β targets on IL-1RA exposure was observed. Altogether, these results emphasize the functional link between IL-1β...
and discovered target molecules and thereby identify IL-1β as a driver of a pathogenetic cascade that leads to immune cell infiltration and local tissue destruction in HS.

Systemic levels of SAA reflect the activity of the lesional IL-1β pathway in HS

Finally, we asked whether the cutaneous abundance of IL-1β and its targets is reflected by systemic levels of some of these molecules. For this purpose, blood samples of HS patients and healthy control participants were analyzed by ELISA. MMP3 levels were detectable but did not differ between healthy control donors and HS patients (Figure 5a). Levels of IL-1β itself and CXCL6 were elevated in HS patients by trend without reaching significance. Furthermore, blood levels of IL-6 were significantly increased in HS patients but were by far too low to establish this as a routine blood biomarker. Therefore, we searched for molecules, which are cooperatively induced in large quantities by the action of IL-1β and
Figure 4. Expression of IL-1β target molecules is elevated in HS skin lesions and depends on cutaneous IL-1 activity. (a, b) The transcriptome of HS skin (three axillary lesions from different patients) and healthy donor skin (four axillary samples from different donors) was analyzed by RNA sequencing, and transcripts up-regulated in HS were compared with the transcriptome induced in IL-1β-stimulated skin-relevant cell types (immune cells [PBMC], dermal microvascular endothelial cells [EC], dermal fibroblasts [FB], and keratinocytes [KC]) in vitro (Figure 2c and d). (a) The totality of analyzed transcripts was divided into the following three groups: (i) those not found to be up-regulated by IL-1β, (ii) those up-regulated in a single cell population, and (iii) those up-regulated in more than one cell population. The percentages of transcripts in each of these groups that was found to be up-regulated in HS lesions are shown. (b) Given are the numbers of transcripts up-regulated by IL-1β in vitro in the different cell populations and the number of them also found to be up-regulated in HS lesions. (c, d) Cutaneous expression was analyzed in healthy donors (n = 8–9), HS patients (lesional, n = 10), and psoriasis patients (lesional, n = 8–9) by RT-qPCR. (e) Skin explant culture.
IL-6 and which are easily detectable in blood. It is known that hepatocytes (i.e., epithelioid cells) respond to small systemic increases of these cytokines by strong production of acute phase proteins. Testing the influence of IL-1β and IL-6 on serum amyloid A (SAA) expression in hepatic HepG2 cells in vitro, we observed increased mRNA expression of both SAA isoforms, SAA1 and SAA2, in response to single cytokine stimulation, with IL-1β being most potent (Figure 5b). Moreover, a synergistic action was found with both cytokines. Protein measurement capturing both SAA isoforms confirmed the strong cooperative action of IL-1β and IL-6 (Figure 5b). IL-22, a mediator known for its acute phase effect on the IL-1 pathway. Fourth, by use of IL-1RA application, we showed the significant positive correlation with the disease severity index (Sartorius score) (Figure 5f). More detailed analyses showed the specific association of SAA with the number of involved skin regions containing nodules, whose significance exceeded even that with the Sartorius score (Figure 5f). These results suggest the suitability of SAA as an easy-to-monitor blood biomarker, to indicate inflammatory disease activity in HS patients. Furthermore, we detected a significant inverse correlation with blood HDL cholesterol but not with patients’ age (Figure 5g), suggesting the age-independent involvement of the IL-1β pathway in cardiovascular alterations in these patients.

**DISCUSSION**

The type of cytokine pathways active in inflamed tissue not only determines the kind of tissue response but also the clinical picture. This knowledge is especially important for chronic diseases when interventional concepts are considered. Although the terminal hair follicle-plugging hypothesis of how HS skin alterations initially develop has been widely accepted, the involved cytokine pathways, which lead to the massive purulent secretion and destruction of skin architecture, are largely unknown.

With our study, we showed that the IL-1β pathway is highly active in HS patients, contributing to local and systemic inflammation. This is suggested based on the following observations. First, IL-1β mRNA and protein levels were strongly elevated in lesional HS skin compared with healthy control skin. Moreover, the IL-1β elevation was even more pronounced than in lesional psoriatic skin. It was not accompanied by an IL-1RA increase, resulting in a high IL-1β/IL-RA ratio in HS lesions (0.849) compared with healthy skin (0.008) and psoriatic lesions (0.070). Immune cells, microvascular dermal endothelial cells, dermal fibroblasts, and keratinocytes responded similarly to IL-1β activation, with the most prominent up-regulations comprising molecules regulating the extracellular matrix remodeling, chemokines (particularly those attracting neutrophilic granulocytes), and immune-modulatory cytokines. Numerous IL-1β target molecules seem to be predominantly induced by IL-1β, although TNF-α and IL-17A were able to strengthen the IL-1β effects, particularly on keratinocytes. However, further investigations of these aspects are necessary. Second, “footprints” of the IL-1β action on cutaneous cells were found in HS lesions. In fact, about 39%–63% of the transcripts, up-regulated by IL-1β in the different cutaneous cell types, were also up-regulated in HS lesions, as shown by our RNA-seq data. Furthermore, a significantly increased cutaneous expression of MMP1, MMP3, MMP9, MMP10, CCL2, CXCL1, IL-6, and IL-32 compared with healthy control skin and psoriasis lesions was clearly evident for HS. Third, evidence of an active IL-1β pathway in HS was also found systemically in the blood of patients. In fact, levels of IL-6, which was induced in dermal endothelial cells and fibroblasts by IL-1β, significantly exceeded those in blood of healthy participants. Moreover, the elevated blood levels of SAA in HS patients might result from the synergistic action of IL-1β and IL-6 on hepatocytes, as observed in vitro. The positive correlation of blood IL-6 levels with systemic SAA concentrations further underpins the possible involvement of these molecules in the IL-1β pathway. Fourth, by use of IL-1RA application, we showed the dependence of high IL-1β target molecule expressions in HS skin lesions on the local IL-1 receptor activity.

Taking all these results into account, we propose that the following hypothetical cascade contributes to the clinical picture of HS (see Supplementary Figure S6 online): IL-1β might be secreted by keratinocytes (Hotz et al., 2016; Witte et al., 2014) and the clearly more potent macrophages/monocytes (our current study), probably activated by sebum and/or bacterial components in a caspase-1-dependent manner (Kelly et al., 2015). This is in line with the suggested importance of these cells in HS (Shah et al., 2017). Large amounts of IL-1β then induce the strong production of

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**Biopsy samples from healthy donors (n = 5) and HS patients (perilesional and lesional, n = 8 obtained from four donors) were cultured without any stimulation for 4 hours, followed by MMP3, CXCL1, and IL-6 quantification in supernatants by ELISA. (f) Cutaneous gene expression was analyzed in healthy donors (n = 10–11), psoriasis patients (PsO; lesional, n = 12), and HS patients (HS-L; lesional, n = 12–13) by RT-qPCR. Individual data and their correlations are shown. (g, h) Paired biopsy samples of lesional HS skin (n = 5–6) were cultured in the presence or absence (control) of IL-1RA for (g) 72 hours or (h) 24 hours. Gene expression was analyzed by RT-qPCR. (c–e, g, h) Mean data ± standard error of the mean are shown. *P < 0.05, **P < 0.01, ***P < 0.001. HS, hidradenitis suppurativa; HS-L, lesional hidradenitis suppurativa skin; HS-PL, lesional hidradenitis suppurativa skin; PBMC, peripheral blood mononuclear cell; PsO, psoriasis; r_s, Spearman rank correlation coefficient; RT-qPCR, quantitative real-time PCR on reverse transcribed RNA.
chemokines, which in turn contributes to the massive infiltration of immune cells, in particular neutrophilic granulocytes. This finally leads to purulent discharge, a clinical key feature of HS. Furthermore, IL-1β enhances the secretion of a range of matrix-degrading enzymes, which not only further supports tissue immune cell infiltration but also contributes to tissue destruction, another major feature of HS (see Supplementary Figure S6 online). Locally produced IL-1β and IL-6 also seem to reach the bloodstream and induce the hepatic production of SAA, which is reflected by markedly increased blood SAA levels.

Figure 5. Blood SAA is associated with local IL-1β pathway activity in HS. (a) Levels of IL-1β and its target molecules in the blood from 19–26 healthy donors and 20–21 HS patients were analyzed by ELISA. (b) HepG2 hepatocytes were treated with IL-1β, IL-6, IL-22, and combinations thereof or were left without stimulation (control) for 42 hours. SAA1 and SAA2 expressions were analyzed by RT-qPCR (upper two panels), and concentration of SAA (covering variants 1 and 2) in culture supernatants was analyzed by ELISA (lower panel). Data from one experiment are shown. (c) SAA levels in the blood from 37 healthy donors and 47 HS patients were analyzed by ELISA. Mean data ± standard error of the mean are shown. (d, e, g) SAA blood levels of HS patients were tested for possible correlation with (d) IL-6 blood levels, (e) HS disease duration, and (g) blood levels of HDL cholesterol, as well as patients’ age. (f) Aspects of clinical disease manifestation were tested for possible correlation with SAA blood levels in HS patients. rs and P-values are indicated. **P ≤ 0.01, ***P ≤ 0.001. HDL, high density lipoprotein; HS, hidradenitis suppurativa; rs, Spearman rank correlation coefficient; SAA, serum amyloid A.
increased blood levels of this acute phase mediator. Elevated IL-1β, IL-6, and SAA blood levels are indications of systemic inflammation, which can be frequently observed even clinically in HS patients (Hessam et al., 2015).

We also found a significant inverse relationship of blood SAA and HDL cholesterol levels. In the blood, SAA is known to associate with HDL cholesterol. SAA-loaded HDL cholesterol shows an increased catabolism (Hoffman and Benditt, 1983), which may explain the negative correlation between SAA and HDL cholesterol observed in our HS patients. Moreover, the integration of SAA into HDL cholesterol during inflammation converts these a priori protective lipoproteins into proatherogenic and inflammatory particles (Khovidhunkit et al., 2000; Salazar et al., 2000; Vaisar et al., 2015; Van Lenten et al., 1995). Both mechanisms suggest that the IL-1β pathway, via SAA induction, contributes to the cardiovascular comorbidities in HS patients (see Supplementary Figure S6). Furthermore, elevated blood SAA levels might lead to amyloid A amyloidosis (Girouard et al., 2012; Schandorff et al., 2016), a very rare, life-threatening complication of HS.

Our study recommends the use of blood SAA as a biomarker that mainly reflects the activity of the IL-1β pathway and the grade of inflammation in HS patients. Having a blood biomarker would meet the great need for objective and easy-to-measure assessment tools for monitoring inflammation and disease activity in HS. In fact, current scoring systems either suffer from insensitivity toward modest changes of the disease stage (e.g., Hurley score) or are too complicated for a clinical routine (e.g., Sartorius score) (Zouboulis et al., 2017).

The presented role of the IL-1β pathway in local and systemic inflammation in HS also suggests IL-1β as a promising therapeutic target. In fact, a first case report documented significant clinical improvement by an anti-IL-1β antibody (canakinumab) (Jaeger et al., 2013). Furthermore, the application of IL-1RA (anakinra) was effective in most, but not all, HS patients in case reports and studies (Leslie et al., 2014; Russo and Alikh, 2016; Tzanetakou et al., 2016; van der Zee et al., 2013; Zarchi et al., 2013). A recently published double-blind, randomized, placebo-controlled clinical trial showed that 78% (7/9) of the patients treated with anakinra versus only 30% (3/10) of the patients treated with placebo achieved at least a 50% reduction in the inflammatory lesion count (sum of abscesses and inflammatory nodules) after 12 weeks and no increase in abscesses or draining fistulas compared with the baseline visit (Tzanetakou et al., 2016). Furthermore, after the end of treatment, patients of the anakinra group showed a significant delay of novel HS exacerbations (Tzanetakou et al., 2016). Thus, in the sense of a personalized therapy, we recommend the measurement of SAA blood levels and the stratification of patients according to the activity of the IL-1β pathway before starting anti-IL-1β antibody or IL-1RA treatment. This also appears to be important because—apart from the IL-1β pathway—other immunological pathways might be involved in the development and maintenance of HS lesions. According to the evidence presented here, anti-IL-1β treatment should also be started early to prevent the development of large abscesses and fistulas.

**MATERIALS AND METHODS**

**Study population**

We performed a hospital-based case-control study with a total of 78 patients with from HS (mean age ± standard deviation [SD] = 40.1 ± 11.9 years; 46 female, 32 male), 55 healthy control participants (mean age ± SD = 39.8 ± 11.2 years; 29 female, 26 male), 21 patients with psoriasis (mean age ± SD = 50.1 ± 11.1 years; 6 female, 15 male), and 8 patients with atopic dermatitis (mean age ± SD = 27.0 ± 7.2 years; 6 female, 2 male). Demographic, anamnestic, and clinical data, as well as skin and blood samples used in the study, are described in Supplementary Materials online. Data and sample collection and respective analyses were approved by the clinical institutional review board, and written informed consent was obtained from all participants. Written informed consent was also provided for photographs presented in the article. The study was conducted according to the principles of the Declaration of Helsinki.

**Isolation of cell types from skin biopsy samples**

Isolation of cell types from skin biopsy samples was performed by flow-cytometric cell sorting, as described in the Supplementary Materials.

**Cell and explant culture**

Peripheral blood mononuclear cells were freshly separated from the venous blood of healthy donors by density gradient centrifugation, as previously described (Wolk et al., 2014). Primary human epidermal keratinocytes isolated from skin biopsy samples of healthy donors were cultured in KGM-Gold medium (Lonza, Verviers, Belgium) as previously described (Wolk et al., 2013). Dermal fibroblasts were obtained from Provitro (Berlin, Germany) and Lonza and cultured in FGM-2 medium (Lonza) according to the manufacturer’s instructions. Dermal microvascular endothelial cells were obtained from Provitro and Life Technologies (Darmstadt, Germany) and cultured in endothelial cell proliferation medium (Provitro), as recommended by the manufacturer’s protocol. For RNA-seq and RT-qPCR analyses, cells were stimulated in the presence or absence (control) of 5 ng/ml recombinant IL-1β for 24 hours. To investigate production of soluble mediators in response to IL-1β, cells were exposed to increasing concentrations of IL-1β (0/1/10/100 ng/ml) for 24 hours. To test for the specificity of IL-1β effects and cooperative action with other cytokines, dermal fibroblasts and keratinocytes were stimulated or not (control) with the following recombinant cytokines: IL-1β (5 ng/ml), IL-6 (10 ng/ml), IL-17A (10 ng/ml), IL-19 (50 ng/ml), IL-22 (20 ng/ml), IL-24 (20 ng/ml), TNF-α (5 ng/ml), IFN-γ (10 ng/ml), or combinations of these as indicated for 24 hours. HepG2 cells were purchased from ECCC (Salisbury, UK), cultured as previously described (Wolk et al., 2007), and stimulated or not (control) with 10 ng/ml IL-1β, 10 ng/ml IL-6, 10 ng/ml IL-22, 10 ng/ml TNF-α, or combinations thereof as indicated for 48 hours.

The cell types isolated from lesional HS skin were cultured ex vivo without stimulation in (i) gentamicin/amphotericin B (Lonza)-supplemented RPMI 1640 (with 10% FCS, 2 mmol/L l-alanyl-l-glutamine) (all from Biochrom, Berlin, Germany) (immune cell subtypes) or (ii) FGM-2 medium (Lonza) (fibroblasts) each at 1–2 × 10^5 cells/ml for 12 hours.

The 3-mm punch skin biopsy samples obtained from healthy donors (taken from axiles) and from perilesional and lesional skin from HS patients were cultured without stimulation for 4 hours or 24 hours in KGM-Gold medium. Paired 3-mm punch skin biopsy samples obtained from lesional areas of HS patients were cultured in the presence or absence (control) of 2 μg/ml of recombinant IL-1RA.
for 24 hours and 72 hours in KGM-Gold medium, as previously established for psoriatic lesions (Witte et al., 2016; Wolk et al., 2013). All recombinant human cytokines mentioned were purchased from Bio-Techne (Wiesbaden-Nordenstadt, Germany).

**RNA-seq transcriptome analysis**

Isolation of total cellular RNA was done as previously described (Wolk et al., 2014). RNA-seq was performed (described in detail in the Supplementary Materials). Data for IL-1β–treated cells and lesional HS skin are deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database (GSE120784 and GSE122592, respectively).

**RT-qPCR**

Tissue homogenization, isolation of total cellular RNA, mRNA reverse transcription, and RT-qPCR analysis were done as described previously (Wolk et al., 2002). All other detection systems were purchased from Fisher Scientific (Schwerte, Germany). Analysis of the housekeeping gene HPRT was included to normalize expressions, as previously described (Weiss et al., 2004).

**ELISA**

Information about the ELISA used in the study is given in the Supplementary Materials.

**Statistics**

Standard descriptive statistics such as mean, SD, and standard error of the mean were computed. Further statistical calculations were made using SPSS 19.0 software (IBM, Ehningen, Germany). Results from control participants and patients were compared using the Mann-Whitney U test (two-tailed). Results from in vitro cultures and paired patient samples were analyzed by applying the Wilcoxon matched-pairs signed-rank test (two-tailed). Differences between frequencies in transcript groups were tested by chi-square test. Correlation analyses were performed by Spearman rank correlation test.

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**CONFLICT OF INTEREST**

SJ, NS, and W-DD are employees of Bayer AG, which partly supported the study (see Acknowledgments). EJG-B has received honoraria (paid to the University of Athens) from different companies interested in HS. KW and RS have received grants supporting the study (see Acknowledgments).

**ACKNOWLEDGMENTS**

The authors would like to acknowledge Annette Bus, Brigitte Ketel, and Anne Schulze for excellent technical assistance and Christine Zelenak for help with manuscript preparation.

Different parts of this study were partly supported by grants from the German Federal Ministry of Education and Research (http://www.bmbf.de; grant 01ZX1312A to KW and RS), Bayer AG (to Charité—Universitätsmedizin Berlin/RS), and Sanofi-Aventis Deutschland GmbH (to Charité—Universitätsmedizin Berlin/RS and KW).

**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.2018.11.018.

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