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# Characterisation of IL-1 family members in Sweet syndrome highlights the overexpression of IL-1β and IL-1R3 as possible therapeutic targets

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## Abstract

Sweet syndrome (SS) as a prototypic neutrophilic dermatosis (NDs) shares certain clinical and histologic features with monogenic auto-inflammatory disorders in which interleukin (IL)-1 cytokine family members play an important role. This has led to the proposal that NDs are polygenic auto-inflammatory diseases and has fuelled research to further understand the role of IL-1 family members in the pathogenesis of NDs. The aim of this study was to characterise the expression of the IL-1 family members IL-1β, IL-36γ, IL-33 and IL-1R3 (IL-1RaP) in SS. The expression profile of IL-1β, IL-33, IL-36γ and their common co-receptor IL-1R3 was analysed by immunohistochemistry, in situ hybridisation and double immunofluorescence (IF) in healthy control skin (HC) and lesional skin samples of SS. Marked overexpression of IL-1β in the dermis of SS ($p < 0.001$), and a non-significant increase in dermal ($p = 0.087$) and epidermal ($p = 0.345$) IL-36γ expression compared to HC was observed. Significantly increased IL-1R3 expression within the dermal infiltrate of SS skin samples ($p = 0.02$) was also observed, whereas no difference in IL-33 expression was found between SS and HC ($p = 0.7139$). In situ hybridisation revealed a good correlation between gene expression levels and the above protein expression levels. Double IF identifies neutrophils and macrophages as the predominant sources of IL-1β. This study shows that IL-1β produced by macrophages and neutrophils and IL-1R3 are significantly overexpressed in SS, thereby indicating a potential pathogenic role for this cytokine and receptor in SS.

**KEYWORDS**
autoinflammatory disorders, cytokines, immunology, interleukin-1 family, neutrophilic dermatoses

Laura Calabrese, Francesca Ney and Rui Aoki contributed equally.

## 1 | INTRODUCTION

Sweet syndrome (SS), or acute febrile neutrophilic dermatosis, is a rare inflammatory skin disease considered as a prototypic neutrophilic dermatosis (ND).

First described in 1964 by Dr. Robert Douglas Sweet,[1] SS is characterised by the abrupt appearance of asymmetrical, painful, tender erythematous papules, nodules and plaques, usually affecting the face, neck and upper extremities. Cutaneous manifestations are often accompanied by systemic symptoms, including fever, malaise and leucocytosis with neutrophilia. SS is frequently associated with various diseases, with myeloid dyscrasias (leukaemias and myelodysplasias) being the primary association. Additionally, there have been reports of associations with autoimmune diseases, inflammatory or infectious diseases and with pregnancy.[2]

The pathogenesis of SS is multifaceted and still to be completely understood. It most likely derives from a complex interplay between genetics, immunological and environmental factors and may be variable according to the associated conditions. However, one unifying mechanism is the aberrant activation, proliferation, maturation and skin homing of neutrophils.[3–5]

Therapeutic options for SS are limited and systemic corticosteroids remain the mainstay of treatment. Alternative therapies include colchicine, dapsone, cyclosporine and potassium iodide.[2] Additionally, some reports regarding the efficacy of anti-TNF-α and interleukin (IL)-1β antagonists in refractory cases of SS have been published.[3] Interestingly, like other NDs, SS seems to share some of the hallmarks of auto-inflammatory disorders (AIDs), including innate immune system activation leading to an hyperproduction of IL-1β and a 'sterile' neutrophil-rich cutaneous inflammation.[3] Almost 20 years ago, the discovery of monogenic AIDs and their causative mutations in genes of effectors of innate immunity, has shed light on the processes by which a dysfunction of the innate immune system can initiate a disease. Later, the concept of autoinflammation has extended to polygenic diseases that are more frequent in daily clinical practice, such as SS and other NDs.[6]

The IL-1 family, including the IL-1 and IL-36 sub-family members as well as IL-33, is involved in a myriad of immunological responses, primarily orchestrating innate immunity.[7] In order to be biologically active, IL-1β requires proteolytic activation by a cytoplasmic innate immune complex named inflammasome. Every cytokine of the IL-1 family interacts with its cognate receptor to transmit intracellular signals. Interestingly, six cytokines of this family (IL-1α/β, IL-33, IL-36α/β/γ) require the binding to a common co-receptor, namely IL-1R3, to enable such signal transduction, upon constitution of a cytokine/receptor/co-receptor ternary complex.[8]

Studies on the genetic basis of SS have highlighted the importance of the inflammasome and the IL-1β pathway dysfunction in SS. In detail, few cases of SS have been linked to mutations in the gene MEFV,[9,10] the causative defect in familial Mediterranean fever (FMF).[11] MEFV genes encodes for the protein pyrin, an inflammasome regulator and intracellular pattern recognition receptor, and its mutations lead to an aberrant inflammasome activation and hyperproduction of IL-1β.[12] Moreover, skin manifestations reminiscent of SS, together with chronic recurrent multifocal osteomyelitis (CRMO) and congenital dyserythropoietic anaemia (CDA), constitute the symptom triad of Majeed syndrome.[13] It is an extremely rare autosomal recessive disorder caused by mutation of the LPIN2 gene that encodes lipin-2.[14] Although the physiologic role of lipin-2 remains partially understood, some cases of Majeed syndrome showed dramatical response to the IL-1β blocker canakinumab, suggesting a pathogenetic contribution of this cytokine.[15]

Experimental studies focusing on the IL-1 family in SS are limited, probably due to the rarity of the disease. On the other hand, the need to clearly decipher the role of this family of cytokines in SS becomes increasingly important as the potential of blocking one or more of the family members is now emerging as a promising therapeutic strategy for numerous polygenic AIDs.

This study aims to examine the expression of specific members of the IL-1 family, namely IL-1β, IL-36γ, IL-33 and IL-1R3, in SS. Thus, the investigation of the possible rationale for targeting their common co-receptor, IL-1R3, could pave the way for a potential therapeutic approach in the future.

## 2 | MATERIALS AND METHODS

### 2.1 | Samples from patients

This study was approved by the Medical Ethics Committee of the coordinator centre, Ludwig-Maximilians-Universität of Munich, Germany, (Project N. 22-0342).

SS lesional samples and healthy control specimens were collected from 2007 to 2021. In detail, 5 μm thick paraffin-embedded (FFPE) specimens from lesional skin of 10 patients with SS (four men and six women; mean age 66.3 years, range 53–79 years) and from 10 healthy controls (six men and four women; mean age 55.5 years, range 20–81 years) were included in the analysis. Demographic and clinical data of SS patients are summarised in Table 1.

The diagnosis was established according to clinical and laboratory diagnostic criteria of SS and histologically confirmed by an expert dermatopathologist.

The modified diagnostic criteria for SS, as proposed by von den Driesch, were used.[16] Major criteria for diagnosis included the abrupt onset of tender skin lesions and histopathologic evidence of neutrophilic infiltrate without evidence of leukocytoclastic vasculitis. Minor criteria were a rapid response to systemic corticosteroids, fever, abnormally laboratory values, and an association with an inflammatory or infectious disease. Both major criteria and at least two minor criteria were required to confirm the diagnosis of SS.[17] Healthy control skin samples were obtained from patients who underwent excision of sentinel lymph node for cutaneous melanoma, following informed patient consent.

Furthermore, three specimens of clinically and histologically confirmed pyoderma gangrenosum (PG)[18] were collected and included in the study as disease control of neutrophilic skin diseases.

**TABLE 1** Clinical and demographic findings in 10 patients with Sweet syndrome.

| N. | Sex | Age (years) | Lesion number | Biopsy year | Sites | Associated conditions | Treatment | Course/follow-up |
|---|---|---|---|---|---|---|---|---|
| 1 | M | 79 | 5 | 2007 | Trunk; upper limbs; hands | None | Prednisone | Single episode/complete remission |
| 2 | F | 61 | 3 | 2009 | Face; trunk | Behçet's disease; fibromyalgia | Prednisone | Single episode/complete remission |
| 3 | F | 53 | 5 | 2011 | Trunk; upper limbs | Erythema nodosum | Prednisone | Single episode/complete remission |
| 4 | F | 62 | 5 | 2012 | Trunk; lower limbs | None | Prednisone | Single episode/complete remission |
| 5 | M | 71 | 4 | 2012 | Face; Trunk | None | Prednisone | Single episode/complete remission |
| 6 | M | 75 | 9 | 2012 | Face; trunk; upper limbs | None | Prednisone | Chronic-relapsing/complete remission |
| 7 | F | 53 | 5 | 2013 | Trunk; upper limbs | Mixed connective tissue disease | Prednisone | Chronic-relapsing/complete remission |
| 8 | M | 78 | 9 | 2013 | Face; trunk; upper limbs | None | Prednisone | Single episode/complete remission |
| 9 | F | 75 | 9 | 2015 | Upper limbs; lower limbs | None | Prednisone | Chronic-relapsing/complete remission |
| 10 | F | 56 | 9 | 2019 | Trunk; upper limbs; lower limbs | None | Prednisone | Single episode/complete remission |

## 2.2 | Immunohistochemical (IHC) staining

Immunostaining for IL-1β, IL-36γ, IL-33 and IL-1R3 was performed on 5 μm thick FFPE tissue sections of lesional SS skin and healthy skin. Ten different sections were stained for IL-1β, IL-36γ, IL-33 and six for IL-1R3. Furthermore, sections from three PG samples were stained for IL-1β and IL-1R3. All sections were also stained with an appropriate isotype control antibody. The alkaline phosphatase detection system was used. Briefly, FFPE tissue sections were deparaffinised, rehydrated and pre-treated in either citrate buffer (pH 8; IL-36γ, IL-1R3) for 5 min or in citraconic anhydride (CCA) for 15 min (IL-1β, IL-33) in a pressure cooker.

A blocking step was performed by incubating the sections with 0.1% cow milk powder in Tris-buffered for 10 min in the case of IL-36γ and IL-1R3 staining, with 1% donkey serum in PBS solution (Phosphate buffered saline) and 5% goat serum in PBS in the case of IL-1β and IL-33, respectively.

After blocking, the sections were incubated with primary antibodies overnight at 4 degrees, followed for 1 h by biotinylated goat anti-mouse secondary antibodies for IL-36γ and IL-33, donkey anti-goat and goat anti-rabbit secondary antibodies for IL-1β and IL-1R3, respectively. All antibodies used are shown in Table 2. The sections were developed with Vector® Red Substrate Kit, Alkaline Phosphatase (AP) SK-5100. Endogenous alkaline phosphatase was blocked by adding levamisole. Finally, the sections were counterstained with haematoxylin and mounted.

## 2.3 | Immunofluorescence

Double IF staining was performed for IL-1β/MPO and IL-1β/CD68 on 5 μm thick FFPE tissue sections of representative SS samples. All sections were stained with appropriate isotype control antibodies. FFPE tissue sections were deparaffinised, rehydrated and pre-treated in citraconic anhydride (CCA) for 15 min in a pressure cooker. Blocking step consisted in incubating the sections with 1% donkey serum in PBS solution. After blocking, the sections were incubated with primary antibodies overnight at 4 degrees, followed for 1.5 h by secondary antibodies (Table 2). Finally, the sections were counterstained with DAPI (MBD0015; Sigma-Aldrich).

## 2.4 | Sample analysis

A semi-quantitative analysis of proteins expression was performed on IHC stained sections using ImageJ Fiji software. IL-36γ and IL-1R3 expression were analysed both in the epidermal and dermal areas. Conversely, only the dermal and epidermal district was respectively considered for IL-1β and IL-33 analysis, as IL-1β was predominantly expressed within the dermal immune infiltrate and IL-33 in basal keratinocytes. IL-36γ and IL-1R3 expression in the epidermis was estimated by calculating the proportions between the positive and the total epidermal area at a 200× magnification. IL-33 expression in the epidermis was estimated by manual counting and calculating proportions between IL-33 positive basal keratinocytes and total keratinocytes at a 200× magnification.

For all pathological examinations, the investigators were blinded to the clinical information or study groups associated with the samples.

Finally, IL-1β, IL-36γ and IL-1R3 expression in the dermis was evaluated by manual counting and calculating proportions between positive immune cells and total immune cells at a 400× magnification. For each section, three different regions of the dermis and epidermis were analysed, the investigator being blinded

TABLE 2 List of primary and secondary antibody used in the study.

| | |
|---|---|
| **Primary antibodies** | |
| IL-1β | AF-201-NA; R&D Systems; Polyclonal Goat IgG |
| IL-36γ | ab156783; Abcam; mouse IgG1 |
| IL-33 | (Nessy-1): sc-517600; Santa Cruz biotechnology; mouse IgG1 |
| IL-R3 | ab232950; Abcam; Polyclonal Rabbit IgG |
| MPO | MAB3174; R&D Systems; monoclonal mouse IgG2B |
| CD68 | M0876; Agilent Dako; monoclonal mouse IgG3 |
| **Isotype controls** | |
| IL-33, IL-36γ Isotype control | MAB002; R&D Systems; Monoclonal Mouse IgG1 |
| IL-1β Istoype control | AB-108-C; R&D Systems; Polyclonal Goat IgG |
| IL-1R3 Isotype control | ab172730; Abcam; Rabbit IgG, monoclonal |
| MPO Isotype control | MAB0041; R&D Systems; monoclonal mouse IgG2B |
| CD68 Isotype control | 14–4742-82; Agilent; monoclonal mouse IgG3 |
| **Secondary antibodies (IHC)** | |
| Goat anti-mouse IgG (Biotin) | ab6788; Abcam |
| Goat anti-rabbit IgG (Biotin) | ab6720; Abcam |
| Donkey anti-goat IgG (Biotin) | Ab6884; Abcam |
| **Secondary antibodies (IF)** | |
| Donkey anti-goat IgG (Alexa Fluor® 594) | Ab150132; Abcam |
| Goat anti-mouse IgG (CF®488A) | 20010; Biotium |

for diagnosis and antibody, and the mean value was subsequently calculated.

## 2.5 | Statistical analysis

The D'Agostino-Pearson test was used to assess whether our data were normally distributed. Differences between two means were evaluated using t-test and Mann–Whitney *U*-test in case of normally and non-normally distributed variables, respectively. A *p*-value lower than 0.05 was considered significant, with $p<0.05=^{*}$, $p<0.01=^{**}$ and $p<0.001=^{***}$. All the analysis were performed with GraphPad Prism software, version 6 for Windows, San Diego, CA, USA.

# 3 | RESULTS

Protein expression of three members of the IL-1 family (IL-1β, IL-36γ, IL-33) and of their shared co-receptor IL-1R3 (IL-1RAcP, IL-1R Accessory Protein) was studied by IHC on lesional SS as well as on healthy skin. Semi-quantitative analysis of the IHC staining for each molecule in SS and healthy controls is shown in (Figure 1). In detail, the most striking difference between the two groups was the expression level of IL-1β, which was particularly high in SS samples and hardly detected in healthy controls ($p<0.001$; Figure 2). Lesional SS skin showed a trend towards elevated protein levels of IL-36γ, also expressed in healthy skin, albeit minimally. Nevertheless, the difference between SS samples and healthy controls was not statistically significant in the dermis ($p=0.0865$) and epidermis ($p=0.3453$; Figure S1).

Furthermore, no significant difference was found between SS samples and healthy skin in terms of IL-33 expression in basal keratinocytes ($p=0.7139$; Figure S2).

With regard to IL-1R3 expression, the comparison between SS samples and healthy skin showed a statistically significant difference in the dermal area exclusively ($p=0.02$), but not in the epidermis ($p=0.2104$; Figure 3).

Interestingly, whereas IL-33, IL-36γ and IL-1R3 were expressed in a rather uniform pattern in the regions analysed, IL-1β expression was found to be restricted to specific areas within the dermis, with nearly no involvement of the surrounding tissue. These areas were represented by intense active immune infiltrate, mainly consisting of macrophages and neutrophils (Figure S3). Furthermore, in a few SS samples, IL-1β expression was not detected at all.

In order to elucidate the specific cell type responsible for IL-1β production in SS, representative samples underwent double immunofluorescence (IF) staining for CD68/IL-1β and MPO/IL-1β. The aim was to determine the cellular co-expression patterns of IL-1β in the context of SS. The results showed that, within the immune infiltrate of SS, IL-1β was secreted mostly by neutrophils, but also to a lesser extent by macrophages (Figure 4).

To confirm the IHC results, in situ hybridisation (ISH) for the above-mentioned members of the IL-1 family was performed on

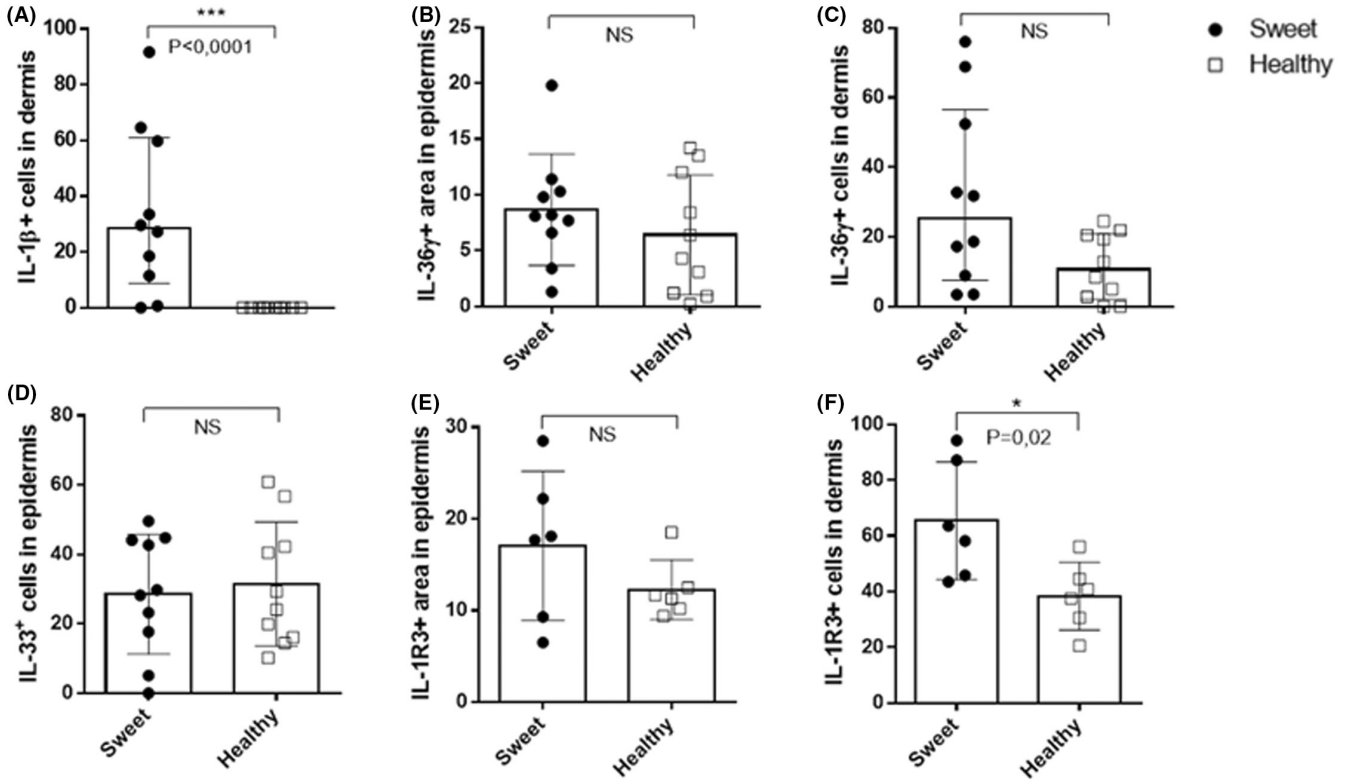


**FIGURE 1** Semi-quantitative analysis of the IHC stainings for IL-1 family cytokines. Scatter plot with bar graphs illustrate the semi-quantitative analysis of the IHC stainings for each molecule analysed (A–F). Differences between means were evaluated using *t*-test and Mann–Whitney *U*-test in case of normally and non-normally distributed variables, respectively. Plots showing means and standard deviations (SD) were used in case of *t*-test comparisons whereas plots showing median and interquartile ranges in case of Mann–Whitney *U*-test comparisons. Comparisons between SS samples and healthy controls showed statistically significant difference in IL-1β ($p > 0.0001$) (A) and IL-1R3 expression (F) in the dermis ($p = 0.02$). No statistically significant differences were found in IL-36γ expression in epidermis (B) or dermis (C), nor in IL-33 expression (D), nor in IL-1R3 expression in epidermis (E). IHC, immunohistochemistry; SS, Sweet syndrome.

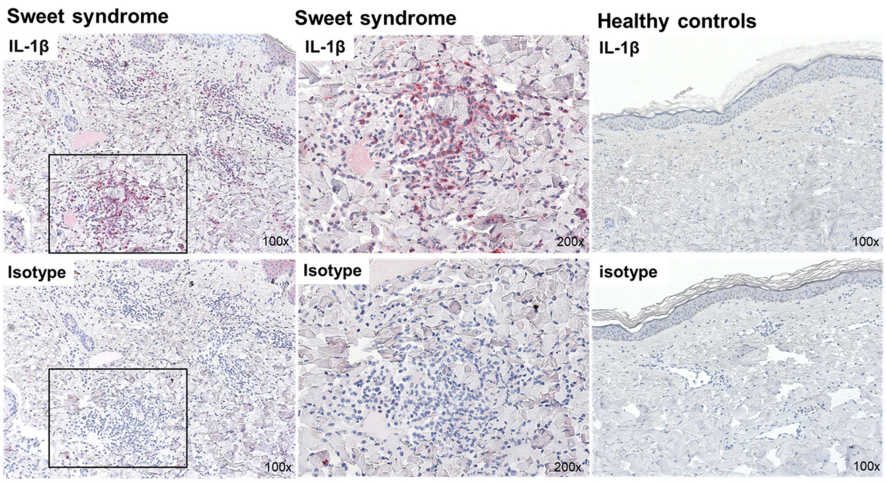


**FIGURE 2** Expression of IL-1β in lesional SS skin. These panels show representative examples of FFPE skin sections immunohistochemically stained for IL-1β in lesional SS and healthy skin. Sections were counterstained with haematoxylin; magnification is specified in the bottom right-hand corner of each picture (100×–200×). SS, Sweet syndrome.

representative SS samples and showed a good correlation between the protein and RNA expression levels (Figure S4). Noteworthy, in one of the SS sections depicting an intraepidermal blister, IL-33 mRNA expression was found to be located around and not within the blister area (Figure S4).

Additionally, to compare our results with another prototypic ND, we conducted an examination of IL-1β and IL-1R3 in PG lesions ($n = 3$) by IHC, and representative images are shown in Figures S5 and S6. PG lesions showed strong overexpression of IL-1β (Figure S5) and IL-1R3 (Figure S6) in the dermal infiltrate. Interestingly, the distribution pattern of IL-1β positive cells was patchy, similar to what we observed in some SS samples.

Based on our results, we can infer that the increased expressions of IL-1β and IL-1R3 in the inflammatory cells are not specific to SS,

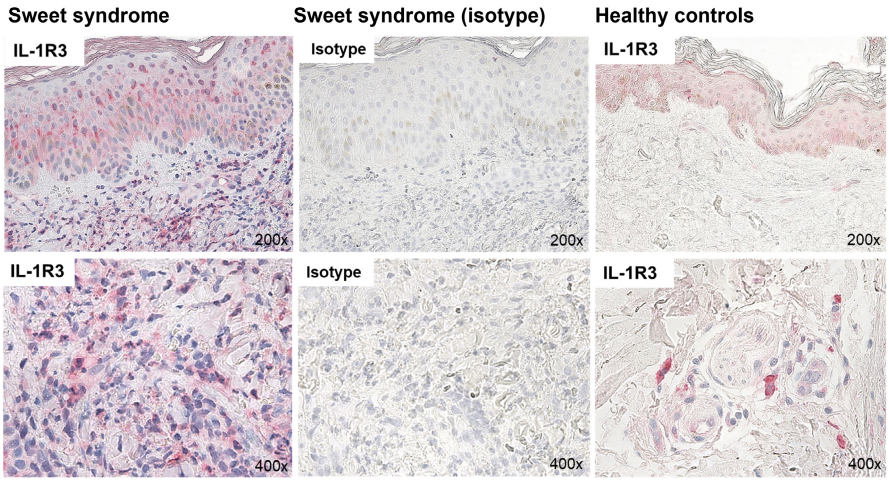


FIGURE 3 Expression of IL-1R3 in lesional SS skin. These panels show representative examples of FFPE skin sections immunohistochemically stained for IL-1R3 in lesional SS and healthy skin. Sections were counterstained with haematoxylin; magnification is specified in the bottom right-hand corner of each picture (200×–400×). SS, Sweet syndrome.

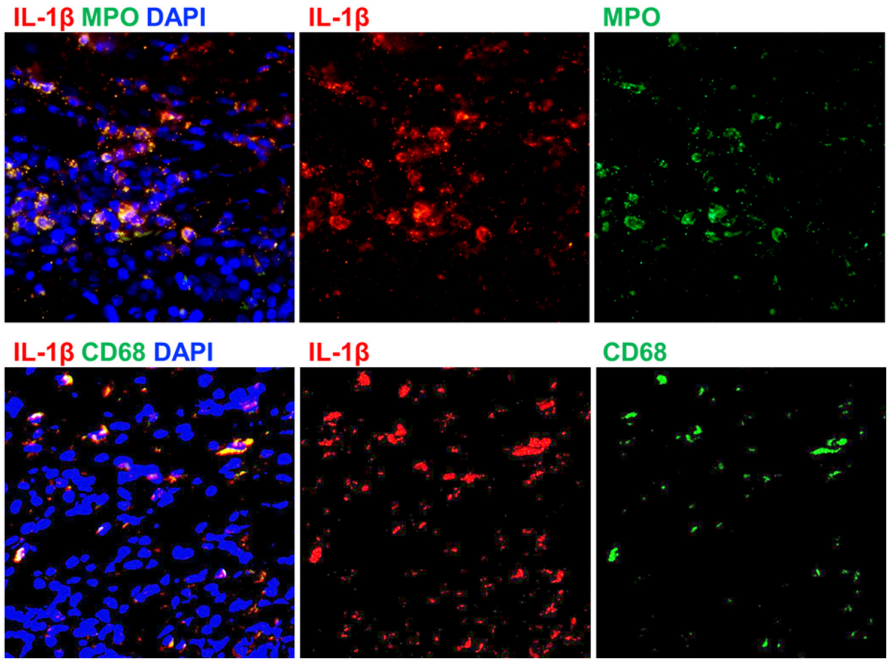


FIGURE 4 IL-1β expression in MPO+ neutrophils and CD68+ macrophages of SS skin lesion. Representative immunofluorescence staining of MPO (A) or CD68 (B) -positive cells (green) for IL-1β (red; overlay with green appears yellow). Original magnification 400×. SS, Sweet syndrome.

although the difference from healthy skins is evident. These findings may represent common characteristics of NDs.

## 4 | DISCUSSION

The IL-1 family comprises several cytokines, including those belonging to the IL-1 and IL-36 subfamilies, as well as IL-33. This huge family primarily plays a role in orchestrating innate immune responses but is also involved in adaptive immunity.

Our study, for the first time, analyses the expression of selected cytokines of the IL-1 family together with the common co-receptor IL-1R3 in lesional samples of SS.

A cardinal finding of our study is the striking over-expression of IL-1β in the majority of SS samples as compared to healthy controls. This molecule is usually not active in its pro-form and its activation is induced solely in response to danger and in pathological conditions.[19] Pro-IL-1β is mainly processed in the cytosol by the NLRP3 inflammasome/caspase-1 pathway.[20] Our study showed that IL-1β is produced not only by neutrophils but also by macrophages in SS.

Interestingly, IL-1β expression was detected only in certain areas of abundant dermal inflammation. This finding could be explained as a localised activation of the inflammasome complex within inflammatory cell types in selected dermal areas.

As early as 1987, JJ Going was the first to propose the involvement of IL-1 in SS.[21] To date, only few studies have explored the role of IL-1 in SS. In detail, one study analysed serum levels of IL-1α and IL-1β in 8 SS patients and healthy controls and found a statistically significant difference between the two groups.[22] Furthermore, Marzano et al. studied protein expression levels in six SS skin samples and six healthy controls using a protein antibody array method and found that the expression of IL-1β and IL-1R1 were significantly increased in SS relative to controls.[23]

Finally, Imhof et al. reported a case of azathioprine-associated SS in a patient suffering from Crohn's disease, in which gene expression

analysis showed very high levels of IL-1β mRNA in lesional skin, and high levels of IL-1β protein in the skin by immunohistochemistry.[24]

However, the most convincing evidence for a role of IL-1 in SS pathogenesis was provided by the reported efficacy of IL-1 blockade reported in some cases.[25–28]

Furthermore, the inhibition of downstream cytokines encoded by IL-1β-induced genes, such as TNF-α,[29] has also shown clinical efficacy in some cases of SS.[30–34]

No studies have investigated the role of IL-36γ in SS and no evidence analysing the effect of IL-36 antagonists in SS have been published to date. In our study, we found a trend-towards an overexpression of IL-36γ in lesional skin specimens, although with no statistically significant difference between SS and healthy skin. IL-36 cytokine activity is usually found at barrier sites with IL-36γ being constitutionally expressed in keratinocytes.[35] These cytokines play a first-line defensive role against exogenous insults, but they also contribute to inflammation by bridging innate and adaptive immune responses, for example, by stimulating Th cell activation and Th1-polarisation.[36] IL-36 pathway dysfunction is well known to be associated with certain inflammatory dermatoses, particularly generalised pustular psoriasis (GPP).[37] IL-36 cytokines are able to recruit neutrophils to the skin[36] and neutrophil-derived proteases process IL-36 cytokines, thus enhancing their biological activity.[38] This suggests that neutrophils are key players in enhancing IL-36 driven inflammation and that IL-36 might be central to the pathogenesis of NDs beyond GPP.

Our study found no significant difference in IL-33 expression between lesional SS skin and healthy skin.

Although it is known that the overactivation of the innate immune system and increased production of IL-1 family cytokines represent key drivers in monogenic and polygenic skin AIDs, the contribution of IL-1β has recently gained scientific attention beyond the spectrum of the diseases classically considered as AIDs.[37] For example, in an autoimmune skin disease, namely psoriasis, IL-1β, produced by macrophages, dendritic cells and keratinocytes, has been proven to be critical in Th17-cell differentiation and activation.[39] Furthermore, dysregulation of the IL-1 pathway has been described in other cutaneous disorders, ranging from inflammatory diseases such as AD,[40] vitiligo,[41] acne[42] to systemic sclerosis[43] and neoplasms.[44] Whether IL-1 acts as a primary driver in these diseases or a co-factor in the pathogenic inflammatory cascade is the subject of current research initiatives.

Our study, for the first time, demonstrated strong expression of the IL-1R3 receptor in the dermal infiltrate of SS, although epidermal expression of the receptor was not significantly different between SS and healthy controls. First described in 1995,[45] IL-1R3 is an accessory receptor of the IL-1 family, not directly involved in ligand binding, but crucial for the initiation of the IL-1/IL-33/IL-36-mediated intracellular pro-inflammatory signalling. Since all these above-mentioned cytokines need to bind to IL-1R3, in addition to their cognate receptors, for the establishment of the so-called ternary signalling complexes, the overexpression of the co-receptor indirectly indicates potential for over-activation of IL-1 family signalling in SS. This finding may also have therapeutic implications. In fact, targeting IL-1R3 can lead to simultaneous inhibition of signalling by different members of the IL-1 family (IL-1α/β, IL-33 and IL-36) and might be useful in the case of diseases in which multiple cytokine pathways are involved.

The role of this molecule was first identified in relation to malignancies[46] and IL-1R3 has been proposed as a novel therapeutic target for antibody-based therapy of acute and chronic myeloid leukaemia.[47,48]

Højen et al. first investigated the potential of IL-1R3 inhibition in inflammatory conditions.[49] In their study, IL-1R3 blockade was able to selectively abrogate signalling via IL-1, IL-33 and IL-36 in vitro with a much greater impact compared to single IL-1R1, IL-33R and IL-36R inhibition. Furthermore, antibody-mediated targeting of IL-1R3 resulted in remission of in vivo murine models of inflammatory diseases driven by IL-1β (peritonitis), IL-33 (allergic inflammation) and IL-36 (imiquimod model of psoriasiform inflammation).[49] These data have paved the way for a novel therapeutic approach, potentially eligible for a wide range of multiple cytokine-driven diseases.

Our finding of significantly increased expression of IL-1R3 in the immune infiltrate of SS suggests that it could be a therapeutic target.

## 5 | CONCLUSIONS

In this study analysing the expression of selected cytokines of the IL-1 family in SS, we demonstrate a significant increase in the expression of the IL-1 family members IL-1β and IL-1R3 in lesional skin samples of SS. Evidence for a contribution of IL-1 family members in SS is increasing and emanates from a few preclinical studies, reports of mutations in innate immune and IL-1 pathway-related genes, as well as encouraging results of the use of IL-1 or IL-1-downstream cytokine antagonists in patients with SS.

Our study provides supportive evidence for a role of IL-1β and IL-1R3 in the pathogenesis of SS. These could thus be interesting therapeutic targets for cases of SS that are unresponsive to systemic steroids or in which steroids are contraindicated.

### AUTHOR CONTRIBUTION

LC, FN and RA performed the research. PS and CM contributed to the execution of the experiments and the collection of patient data. LC designed the research study. LEF and TKS contributed essential reagents or tools. LC, FN and RA analysed the data. LC wrote the paper. TKS, LEF and AVM revised the manuscript. Furthermore, all authors have read and approved the final manuscript.

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## CONFLICT OF INTEREST STATEMENT

Laura Calabrese, Francesca Ney, Chiara Moltrasio, Rui Aoki, Pia-Charlotte Stadler and Takashi K. Satoh have no conflict of interest to declare. Angelo Valerio Marzano reports consultancy/advisory boards disease-relevant honoraria from AbbVie, Boehringer-Ingelheim, Novartis, Pfizer, Sanofi and UCB, outside the submitted work. Lars E. French has consulted or given lectures for AbbVie, Novartis, UCB, Galderma, UNION Therapeutics, Almirall, Janssen and Regeneron, outside the submitted work.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Data S1.** In situ hybriditzation.
**Figure S1.** Expression of IL-36γ in lesional SS skin.
**Figure S2.** Expression of IL-33 in lesional SS skin.
**Figure S3.** Representative staining of IL-1β in lesional SS skin.
**Figure S4.** mRNA expression of IL-1 family cytokines in SS lesion by ISH.
**Figure S5.** Expression of IL-1β in SS vs. PG lesions.
**Figure S6.** Expression of L-1R3 in SS vs. PG lesions.

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