Expression of cytokines, chemokines and other effector molecules in two prototypic autoinflammatory skin diseases, pyoderma gangrenosum and Sweet's syndrome

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Summary

Pyoderma gangrenosum (PG) and Sweet's syndrome (SS) are two inflammatory skin diseases presenting with painful ulcers and erythematous plaques, respectively; both disorders have a debilitating clinical behaviour and PG is potentially life-threatening. Recently, PG and SS have been included among the autoinflammatory diseases, which are characterized by recurrent episodes of sterile inflammation, without circulating autoantibodies and autoreactive T cells. However, an autoinflammatory pattern clearly supporting this inclusion has never been demonstrated. We studied 16 patients with PG, six with SS and six controls, evaluating, using a sandwich-based protein antibody array method, the expression profile of inflammatory effector molecules in PG, SS and normal skin. The expressions of interleukin (IL)-1 beta and its receptor I were significantly higher in PG ($P = 0.0001$ for both) and SS ($P = 0.004$–0.040) than in controls. In PG, chemokines such as IL-8 ($P = 0.0001$), chemokine (C-X-C motif) ligand (CXCL) 1/2/3 ($P = 0.002$), CXCL 16 ($P = 0.003$) and regulated upon activation normal T cell expressed and secreted (RANTES) ($P = 0.005$) were over-expressed. In SS, IL-8 ($P = 0.018$), CXCL 1/2/3 ($P = 0.006$) and CXCL 16 ($P = 0.036$) but not RANTES were over-expressed, suggesting that chemokine-mediated signals are lower than in PG. Fas/Fas ligand and CD40/CD40 ligand systems were over-expressed in PG ($P = 0.0001$ for Fas, $P = 0.009$ for Fas ligand, $P = 0.012$ for CD40, $P = 0.0001$ for CD40 ligand), contributing to tissue damage and inflammation, while their role seems to be less significant in SS. Over-expression of cytokines/chemokines and molecules amplifying the inflammatory network supports the view that PG and SS are autoinflammatory diseases. The differences in expression profile of inflammatory effectors between these two disorders may explain the stronger local aggressiveness in PG than SS.

Keywords: autoinflammation, chemokines, cytokines, pyoderma gangrenosum, Sweet's syndrome

Introduction

Pyoderma gangrenosum (PG) and Sweet's syndrome (SS) are two rare inflammatory cutaneous diseases that present with recurrent skin ulcers having undermined violaceous borders and erythematous plaques, respectively. Both PG and SS can occur alone or in association with different conditions, notably inflammatory bowel diseases, rheumatological disorders, haematological malignancies and intake of triggering drugs [1–5]. However, the two diseases differ in that PG is associated more frequently with gut autoinflammatory disorders, while SS occurs commonly in combination with haematological disorders and immunostimulatory drug intake [3]. The histopathological pattern of PG and SS consists mainly of neutrophilic inflammatory infiltrates involving the skin and, rarely, internal organs, giving rise to the term 'neutrophilic dermatoses' [6–8]. Systemic corticosteroids are considered the

Table 1. Clinical findings in 16 patients with pyoderma gangrenosum.

N	Sex	Age (years)	Lesion number	Sites	Associated conditions	Treatment	Course/follow-up
1	M	65	3	Legs	Klinefelter	Pred; Cyc; Infliximab	Chronic-relapsing/exitus due to sepsis
2	F	70	3	Legs	–	Pred	Single episode/complete remission
3	M	15	9	Trunk	–	Pred	Chronic-relapsing/complete remission
4	M	61	2	Legs	–	Pred	Chronic-relapsing/complete remission
5	F	28	3	Legs	Cystic fibrosis	Pred	Chronic-relapsing/complete remission
6	M	30	3	Legs	IBD	Pred; Adalimumab	Single episode/complete remission
7	F	72	2	Legs	–	Pred	Single episode/complete remission
8	F	35	4	Legs	–	Pred	Chronic-relapsing/complete remission
9	F	30	7	Legs	IBD	Pred; Aza	Chronic-relapsing/complete remission
10	M	67	3	Trunk	–	Pred; Dapsone	Chronic-relapsing/partial remission
11	F	78	3	Scalp	–	Pred; Cyc	Chronic-relapsing/partial remission
12	M	62	4	Legs	–	Pred	Single episode/partial remission
13	M	57	1	Legs	–	Pred	Chronic-relapsing/complete remission
14	M	42	8	Legs, Arms	–	Pred; Cyc	Chronic-relapsing/complete remission
15	F	44	4	Legs	–	Pred; Dapsone	Chronic-relapsing/complete remission
16	M	18	12	Trunk, Legs	–	Pred	Chronic-relapsing/complete remission

Aza = azathioprine; Cyc = oral cyclosporin; IBD = inflammatory bowel diseases; Pred = oral prednisone; M = male; F = female.

first-choice treatment for both PG and SS, while other immunosuppressants are reserved for refractory cases [6,7]. Recently, neutrophilic dermatoses have been classified within the spectrum of autoinflammatory diseases [1,7,9], which includes genetically determined forms caused by mutations of genes regulating innate immunity [10]. Autoinflammatory diseases manifest clinically as relapsing episodes of sterile inflammation in the affected organs, in the absence of high titres of circulating autoantibodies and autoreactive T cells [11–15]. PG was the first to be considered an autoinflammatory disease when associated with arthritis and acne in the so-called PAPA (pyogenic arthritis, pyoderma gangrenosum and acne) syndrome. In PAPA syndrome, specific mutations involving the *PSTPIP1* (proline–serine–threonine phosphatase-interacting protein 1) gene, via an increased binding affinity to pyrin, induce the assembly of inflammasomes. These are molecular platforms responsible for the activation of the caspase 1, an enzyme inducing the proteolytic cleavage of the inactive pro-interleukin (IL)-1 beta to its functionally active form, IL-1 beta, which is overproduced in PAPA syndrome [16]. IL-1 beta overproduction can also occur in non-genetically determined neutrophilic dermatoses, in which it may trigger the synthesis and release of several proinflammatory cytokines and chemokines. Chemokines could, in turn, induce further neutrophil recruitment and activation [17], leading to a neutrophil-mediated inflammation regarded as the pathophysiological hallmark of the neutrophilic dermatoses [1,18,19]. However, the actual occurrence of all these pathogenic pathways in neutrophilic dermatoses has never been demonstrated clearly. Thus, to support the inclusion of nutrophilic dermatoses within the spectrum of the autoinflammatory diseases, we have evaluated the cytokine expression profile in the lesional skin of PG and SS by a protein array method.

Patients and methods

Patients

Lesional skin biopsies taken from 16 patients with PG (nine men and seven women; mean age 48 years, range 15–78 years) and six patients with SS (three men and three women; mean age 44 years, range 26–60 years) were studied by a cytokine array method. All PG patients presented with the classic ulcerative variant. All patients with SS had the papulonodular presentation. The diagnosis of PG as well as SS was established on the basis of clinical, histopathological and laboratory criteria [1]. Two patients with PG had IBD as associated disease, one patient had Klinefelter's syndrome and one patient had cystic fibrosis; the other 12 PG cases were idiopathic. Only one of six patients with SS had an associated disease, namely chronic B cell lymphatic leukaemia. The clinical findings of patients with PG and those of patients with SS are summarized in Tables 1 and 2, respectively.

Skin biopsies were obtained from patients with PG and patients with SS before both systemic and topical treatment. In PG patients, skin specimens were taken from the undermined edge surrounding the ulcerative lesion to the centre of the ulcer. In SS cases, specimens were obtained from lesional skin. The controls were normal skin tissue specimens taken from six patients who underwent excision of benign skin tumours.

Table 2. Clinical findings in six patients with Sweet's syndrome.

N	Sex	Age (years)	Lesion number	Sites	Associated conditions	Treatment	Course/follow-up
1	F	44	5	Face; trunk	–	Pred	Single episode/complete remission
2	M	30	3	Face	–	Pred	Single episode/complete remission
3	M	58	9	Face; trunk Legs; arms	cB-CLL	Pred	Chronic-relapsing/exitus due to sepsis
4	M	26	4	Face; hands	–	Pred	Chronic-relapsing/complete remission
5	F	60	3	Face; neck	–	Pred	Single episode/complete remission
6	F	46	6	Neck; hands	–	Pred	Chronic-relapsing/complete remission

cB-CLL = chronic B cell lymphatic leukaemia; Pred = oral prednisone; M = male; F = female.

The protocol was approved by our Institutional Review Board and all the subjects gave their informed consent before participating in the study.

Protein array

Each tissue sample was weighed and diced into very small pieces using a clean razor blade. Frozen tissue were sliced very thinly and thawed in radioimmunoprecipitation assay (RIPA) buffer (sc-24948) containing protease- and phosphatase-inhibitors using 3 ml of ice-cold RIPA buffer per gram of tissue. Samples were incubated on ice for 30 min, transferred to microcentrifuge tubes and centrifuged at $10\,000\,g$ for 10 min at 4°C. The supernatant was collected and the sample was centrifuged again. The new supernatant fluid was added to the previous one, this mixture representing the total cell lysate. In order to standardize the cell lysate of each tissue sample, we measured the total proteins in each sample by a microBCA kit (ThermoScientific, Waltham, MA, USA). For each sample, we loaded a volume containing 100 μg of proteins in a glass-slide format of cytokine antibody array (RayBio®, Norcross, GA, USA). The volume to be loaded was calculated by the following formula: volume (expressed in μl) = 100 μg/protein concentration (expressed in μg/μl). Each glass-slide array contained 14 subarrays and was suitable for 14 samples. Each subarray allowed the evaluation of cytokine expression levels in a sample. Normalization of data at the end of the experiment provided semiquantitative results. The subarray was composed of specific antibodies against target molecules coated on the glass-slide. After hybridization of the tissue lysate, each antibody bound its target molecule and unbound proteins were washed out. The slide was then incubated with biotin-conjugated antibodies against the same target cytokines, washed and then incubated with cyanin 3 (Cy3)-conjugated streptavidin, creating a biotin–streptavidin–Cy3 complex detectable using a microarray laser scanner. Using a data extraction software we could convert fluorescent signals into numerical data and, after normalization, we obtained an expression value of signal intensity for each molecule in each sample. The molecules tested were the following: IL-1 beta, IL-1RI (IL-1 receptor I), IL-1RII, tumour necrosis factor (TNF)-alpha, TNF-receptor I (TNF-RI), TNF-RII, IL-17, IL-17R, leucocyte selectin (L-selectin), IL-8, regulated on activation, normal T cell expressed and secreted (RANTES), [chemokine (C-X-C motif) ligand 1,2,3, (C = cysteine, X = any amino acid) (CXCL 1,2,3)], CXCL 16, matrix metalloproteinase-2 (MMP-2), MMP-9, tissue inhibitor of metalloproteinase 1 (TIMP-1), TIMP-2, sialic acid-binding immunoglobulin-type lectin 5 (Siglec 5), Siglec 9, Fas protein also known as CD95 (Fas), Fas ligand also known as CD178 (FasL); cluster of differentiation 40 (CD40) and CD40 ligand (CD40L).

Statistics

Because the signal intensity data were positively skewed, they were log-transformed before analysis. The results are reported as anti-log values of means with standard deviation (s.d.). Student's *t*-test for unpaired values was used to assess statistical significance of differences between normal controls, patients with PG and patients with Sweet's syndrome. The significance level was set at $P < 0.05$.

Results

Cytokine expression

Pyoderma gangrenosum. IL-1-beta and its receptors (IL-1RI and -RII) were significantly more expressed in PG lesional skin (34.74 ± 26.89, 3.68 ± 2.67 and 11.00 ± 8.79, respectively) than in normal skin (3.42 ± 1.87, 2.00 ± 0.82 and 4.49 ± 1.73; $P = 0.0001$ for all) (Fig. 1). The proinflammatory cytokine TNF-alpha was also over-expressed in PG (3.24 ± 0.64 *versus* 2.60 ± 0.19; $P = 0.01$), as well as its receptors TNF-RI (12.51 ± 7.83 *versus* 5.87 ± 3.43; $P = 0.015$) and TNF-RII (13.58 ± 7.83 *versus* 6.25 ± 3.31; $P = 0.023$) (Fig. 2). Finally, we observed an overproduction of IL-17 (5.80 ± 3.62 *versus* 2.17 ± 0.44; $P = 0.008$) and its receptor IL-17R (5.47 ± 1.24 *versus* 3.98 ± 1.33; $P = 0.010$) in PG (Fig. 3).

Sweet's syndrome. The expression of IL-1 beta was significantly higher in SS lesional skin (15.70 ± 7.88) than in
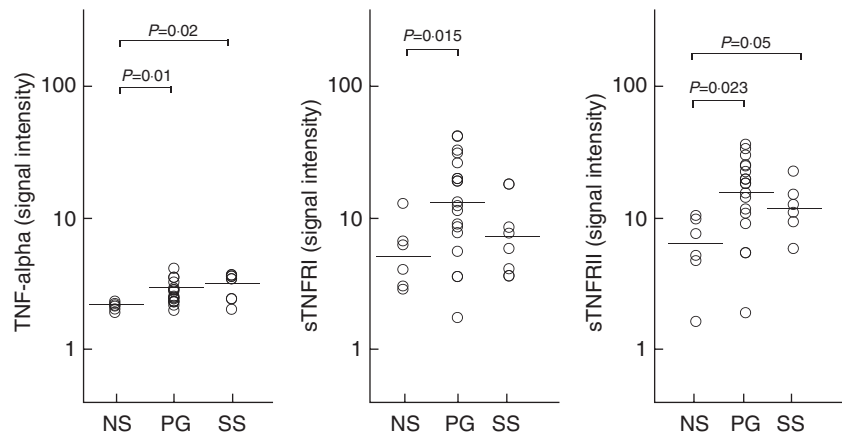
Fig. 1. Expression of interleukin (IL)-1 beta and its soluble receptors I and II (sIL-1RI and sIL-1RII) in homogenate samples of lesional skin from 16 patients with pyoderma gangrenosum (PG) and six patients with Sweet's syndrome (SS). Six normal subjects (NS) served as controls. Numerical values represent signal intensity in a cytokine array assay.
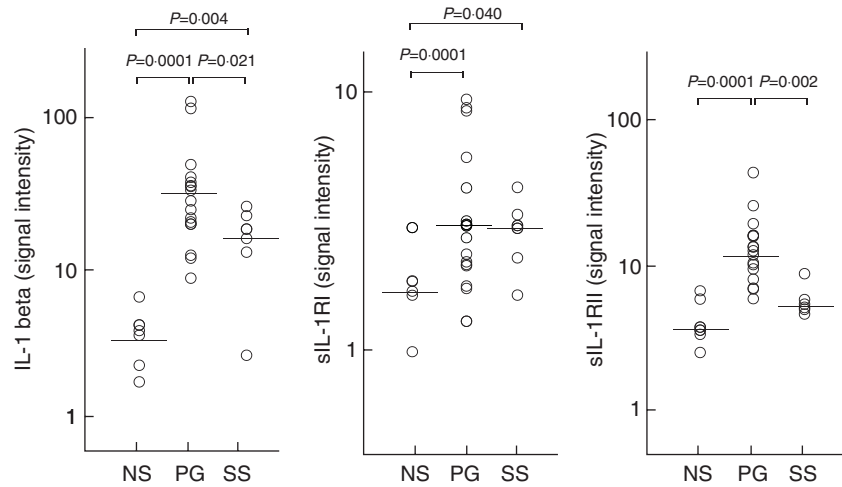
normal skin (3.42 ± 1.87; $P = 0.004$), but significantly lower than in PG lesional skin ($P = 0.021$) (Fig. 1). IL-1RI (3.59 ± 1.76) was significantly over-expressed compared to normal skin (2.00 ± 0.82; $P = 0.040$); a trend towards an increase in IL-1RII expression was also observed (5.06 ± 2.51 versus 4.49 ± 1.73). Statistically significant over-expression of TNF-alpha (3.46 ± 0.85 versus 2.60 ± 0.19)

and its receptor TNF-RII (11.83 ± 4.55 versus 6.25 ± 3.31), but not its receptor TNF-RI (7.50 ± 4.50 versus 5.87 ± 3.43), was found ($P = 0.02$ and $P = 0.05$, respectively) (Fig. 2). An expression significantly higher than in normal skin was detected for both IL-17 (4.93 ± 1.02 versus 2.17 ± 0.44; $P = 0.011$) and its receptor IL-17R (4.54 ± 0.72 versus 3.98 ± 1.33; $P = 0.025$) (Fig. 3).

Fig. 2. Expression of tumour necrosis factor (TNF)-alpha and its soluble receptors I and II (sTNF-RI and sTNF-RII) in homogenate samples of lesional skin from 16 patients with pyoderma gangrenosum (PG) and six patients with Sweet's syndrome (SS). Six normal subjects (NS) served as controls. Numerical values represent signal intensity in a cytokine array assay.

Fig. 3. Expression of interleukin (IL)-17, its soluble receptor (sIL-17R) and leucocyte selectin (L-selectin) in homogenate samples of lesional skin from 16 patients with pyoderma gangrenosum (PG) and six patients with Sweet's syndrome (SS). Six normal subjects (NS) served as controls. Numerical values represent signal intensity in a cytokine array assay.
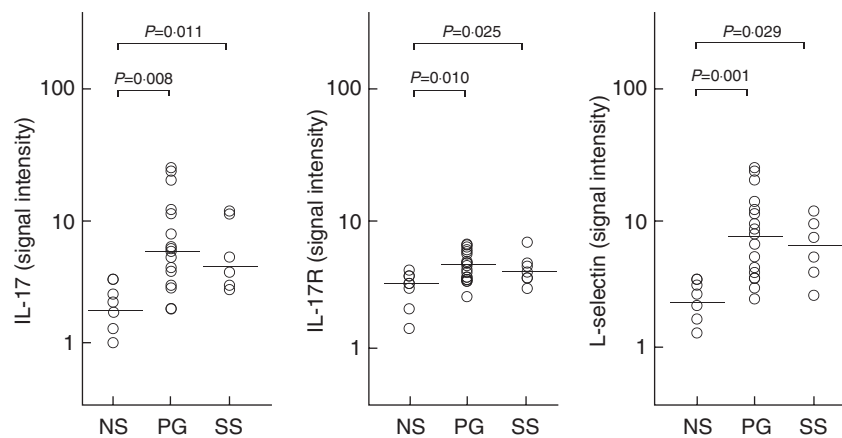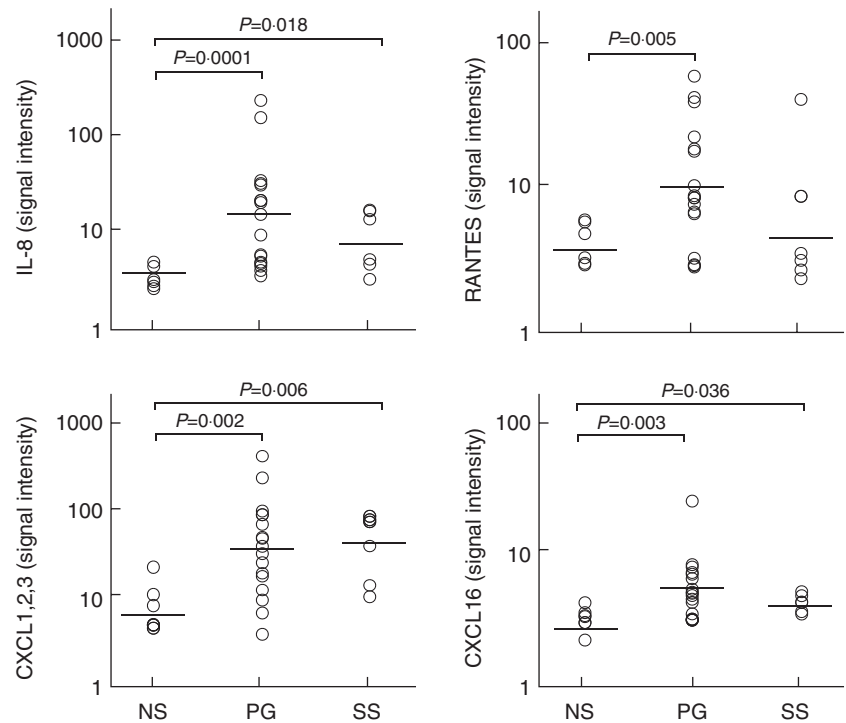
Fig. 4. Expression of interleukin (IL)-8, regulated on activation, normal T cell expressed and secreted (RANTES), chemokine (C-X-C motif) ligand (CXCL) 1,2,3 (C = cysteine, X = any amino acid) and CXCL 16 in homogenate samples of lesional skin from 16 patients with pyoderma gangrenosum (PG) and six patients with Sweet's syndrome (SS). Six normal subjects (NS) served as controls. Numerical values represent signal intensity in a cytokine array assay.

L-selectin expression

Pyoderma gangrenosum. The expression of L-selectin was significantly higher in PG lesional skin ($7 \cdot 19 \pm 6 \cdot 31$) than in normal skin ($2 \cdot 14 \pm 1 \cdot 21$; $P = 0 \cdot 001$) (Fig. 3).

Sweet's syndrome. The expression of L-selectin was significantly higher in SS lesional skin ($6 \cdot 23 \pm 3 \cdot 81$) than in normal skin ($2 \cdot 14 \pm 1 \cdot 21$; $P = 0 \cdot 029$) (Fig. 3).

Chemokine expression

Pyoderma gangrenosum. Compared to controls, PG patients showed over-expression of chemokines promoting neutrophil transendothelial migration into inflamed tissues, such as IL-8 ($14 \cdot 05 \pm 62 \cdot 85$ *versus* $2 \cdot 73 \pm 1 \cdot 05$; $P = 0 \cdot 0001$), CXCL 1,2,3 ($36 \cdot 74 \pm 118 \cdot 58$ *versus* $7 \cdot 61 \pm 7 \cdot 65$; $P = 0 \cdot 002$), CXCL 16 ($4 \cdot 91 \pm 5 \cdot 73$ *versus* $2 \cdot 50 \pm 0 \cdot 74$; $P = 0 \cdot 003$) and RANTES ($9 \cdot 75 \pm 19 \cdot 07$ *versus* $3 \cdot 41 \pm 1 \cdot 56$; $P = 0 \cdot 005$) (Fig. 4).

Sweet's syndrome. In SS patients, statistically significant over-expression was observed for IL-8 ($7 \cdot 79 \pm 7 \cdot 03$ *versus* $2 \cdot 73 \pm 1 \cdot 05$; $0 \cdot 018$), CXCL 1,2,3 ($40 \cdot 25 \pm 37 \cdot 14$ *versus* $7 \cdot 61 \pm 7 \cdot 65$; $P = 0 \cdot 006$) and CXCL 16 ($3 \cdot 62 \pm 0 \cdot 71$ *versus* $2 \cdot 50 \pm 0 \cdot 74$; $P = 0 \cdot 036$), but not for RANTES ($4 \cdot 45 \pm 17 \cdot 25$ *versus* $3 \cdot 41 \pm 1 \cdot 56$) (Fig. 4).
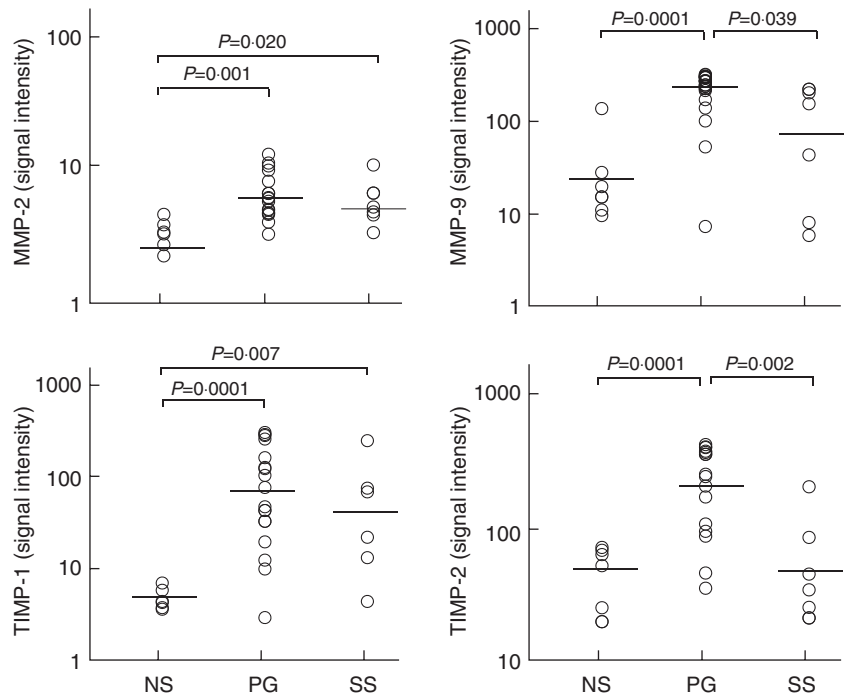
MMP and TIMP expression

Pyoderma gangrenosum. In PG patients, we observed significant over-expression of molecules involved in tissue damage such as MMP-2 ($5 \cdot 72 \pm 3 \cdot 16$ *versus* $2 \cdot 49 \pm 0 \cdot 90$; $P = 0 \cdot 001$) and MMP-9 ($230 \cdot 94 \pm 137 \cdot 95$ *versus* $24 \cdot 70 \pm 68 \cdot 32$; $P = 0 \cdot 0001$) (Fig. 5). Overproduction of molecules responsible for inhibitory signals aimed at attenuating MMP-mediated inflammation was also demonstrated: TIMP-1 ($79 \cdot 78 \pm 155 \cdot 65$ *versus* $4 \cdot 45 \pm 1 \cdot 63$; $P = 0 \cdot 0001$) and TIMP-2 ($208 \cdot 16 \pm 149 \cdot 67$ *versus* $48 \cdot 69 \pm 24 \cdot 40$; $P = 0 \ 0001$) (Fig. 5).

Sweet's syndrome. The expression of MMP-2 but not of MMP-9 was significantly higher than in normal skin ($4 \cdot 88 \pm 2 \cdot 87$ *versus* $2 \cdot 49 \pm 0 \cdot 90$; $P = 0 \ 020$) (Fig. 5); MMP-9 expression in SS was significantly lower than in PG ($60 \cdot 58 \pm 136 \cdot 89$ *versus* $230 \cdot 94 \pm 137 \cdot 95$; $P = 0 \cdot 039$) (Fig. 5). The expression of TIMP-1 but not of TIMP-2 was significantly higher than in normal skin ($41 \cdot 16 \pm 128 \cdot 93$ *versus* $4 \cdot 45 \pm 1 \cdot 63$; $P = 0 \cdot 007$) (Fig. 5); TIMP-2 expression in SS was significantly lower than in PG ($53 \cdot 59 \pm 75 \cdot 17$ *versus* $208 \cdot 16 \pm 149 \cdot 67$; $P = 0 \cdot 002$) (Fig. 5).

Siglec, FAS/FASL and CD40/CD40L expression

Pyoderma gangrenosum. Siglec 5 ($61 \cdot 21 \pm 96 \cdot 94$; $P = 0 \cdot 0001$) and Siglec 9 ($50 \cdot 33 \pm 75 \cdot 71$; $P = 0 \cdot 0001$), which carry inhibitory signals dampening inflammation, were more expressed in PG than in normal skin ($4 \cdot 74 \pm 3 \cdot 09$ and $7 \cdot 65 \pm 3 \cdot 55$, respectively) (Fig. 6). Fas and its ligand were also over-expressed in PG ($16 \cdot 47 \pm 9 \cdot 15$ *versus* $8 \cdot 75 \pm 2 \cdot 07$ with $P = 0 \cdot 0001$ for Fas; $7 \cdot 38 \pm 4 \cdot 40$ *versus* $3 \cdot 86 \pm 1 \cdot 48$ with $P = 0 \cdot 009$ for Fas ligand) (Fig. 6). CD40 and its ligand were expressed significantly more in PG than in normal skin

Fig. 5. Expression of matrix metalloproteinase (MMP) 2, MMP-9, tissue inhibitor of metalloproteinase 1 (TIMP)-1 and TIMP-2 in homogenate samples of lesional skin from 16 patients with pyoderma gangrenosum (PG) and six patients with Sweet's syndrome (SS). Six normal subjects (NS) served as controls. Numerical values represent signal intensity in a cytokine array assay.

$(6 \cdot 16 \pm 3 \cdot 57$ *versus* $3 \cdot 68 \pm 1 \cdot 04$ with $P = 0 \cdot 012$ for CD40; $5 \cdot 77 \pm 6 \cdot 27$ *versus* $2 \cdot 32 \pm 0 \cdot 78$ with $P = 0 \cdot 0001$ for CD40L) (Fig. 6).

Sweet's syndrome. Siglec 5 $(29 \cdot 21 \pm 18 \ 40; \ P = 0 \cdot 001)$ and Siglec 9 $(40 \cdot 45 \pm 38 \cdot 43; \ P = 0 \cdot 001)$ were expressed more in SS than in normal skin $(4 \cdot 74 \pm 3 \cdot 09$ and $7 \cdot 65 \pm 3 \cdot 55$, respectively) (Fig. 6). Fas and its ligand were also over-expressed in SS $(11 \cdot 92 \pm 3 \cdot 15$ *versus* $8 \cdot 75 \pm 2 \cdot 07$ with $P = 0 \cdot 047$ for Fas; $7 \cdot 20 \pm 1 \cdot 16$ *versus* $3 \cdot 86 \pm 1 \cdot 48$ with $P = 0 \cdot 008$ for FasL) (Fig. 6). CD40L was expressed more significantly in SS $(3 \cdot 75 \pm 1 \cdot 06)$ than in normal skin $(2 \cdot 32 \pm 0 \cdot 78; \ P = 0 \cdot 021)$

(Fig. 6). Differences between SS and PG were found for Fas $(11 \cdot 92 \pm 3 \cdot 15$ *versus* $16 \cdot 47 \pm 9 \cdot 15; \ P = 0 \cdot 013)$, CD40 $(4 \cdot 22 \pm 3 \cdot 77$ *versus* $6 \cdot 16 \pm 3 \cdot 57; \ P = 0 \cdot 028)$ and CD40L $(3 \cdot 75 \pm 1 \cdot 06$ *versus* $5 \cdot 77 \pm 6 \cdot 27; \ P = 0 \cdot 047)$.

Discussion

Although a few reports have previously shown an over-expression of cytokines such as IL-8 and IL-23 in PG [18,20,21] and of granulocyte–macrophage colony-stimulating factor (GM-CSF) in SS [22], our study, for the first time, analysed systematically the cytokine and
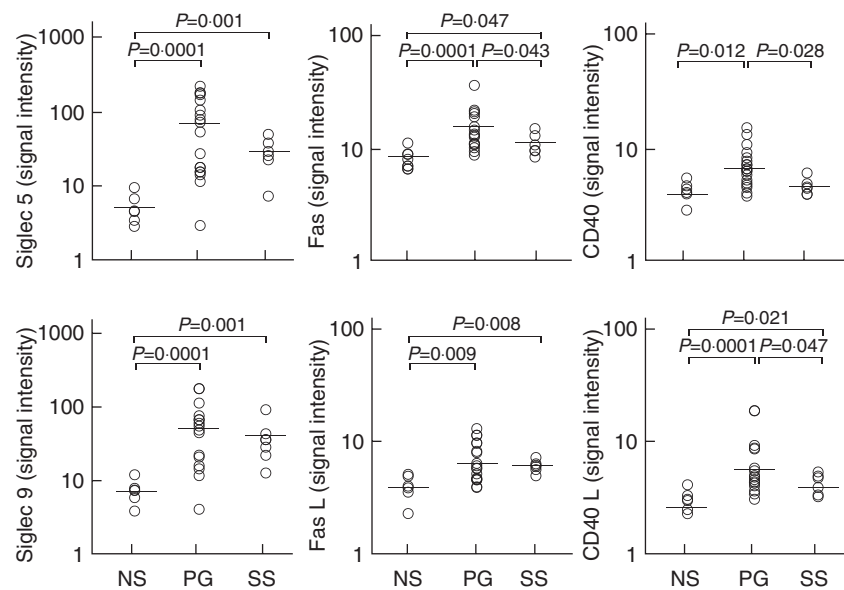
Fig. 6. Expression of sialic acid-binding immunoglobulin-type lectin (Siglec) 5, Siglec 9, Fas protein also known as CD95 (Fas), Fas ligand also known as CD178 (FasL), cluster of differentiation (CD)40 and CD40 ligand (CD40L) in homogenate samples of lesional skin from 16 patients with pyoderma gangrenosum (PG) and six patients with Sweet's syndrome (SS). Six normal subjects (NS) served as controls. Numerical values represent signal intensity in a cytokine array assay.

chemokine expression profile on lesional skin of PG and SS. A cardinal finding in our study was the over-expression of IL-1β and its receptors in both PG and SS, linked theoretically to dysregulation of inflammasome function. As emphasized previously, this highly active pleiotropic cytokine is a leading actor in the autoinflammation [12,17] and plays a pivotal role in triggering the neutrophilic inflammation of the skin. IL-1 is produced mainly by macrophages, T lymphocytes, endothelial cells and fibroblasts. In particular, T lymphocytes have been reported to be predominant in the wound edge of ulcerative PG [18], where they contribute to neutrophil recruitment paving the way to ulcer formation. Conversely, several lines of evidence suggest that activated keratinocytes are also an important source of IL-1 in the skin [23,24].

IL-1 is a well-known inducer of cytokines, notably including proinflammatory cyotokines (such as IFN-γ) and chemokines, forming a network of cytokine-induced cytokines [17]. It is of note that IL-1β over-expression was significantly higher in PG than in SS; moreover, IL-1β receptor II was significantly more expressed in PG than SS. IL-1 promotes the production and release of both classic proinflammatory cytokines, such as TNF-α and IFN-γ, and a number of chemokines, notably IL-8 and RANTES [17].

In fact, TNF-α, which also acts as a key regulator of other proinflammatory cytokines and chemokines, including the same IL-1β and IL-8 [25], was over-expressed in the present study. Overproduction of TNF-α in lesional skin of idiopathic PG has been demonstrated previously by immunohistochemistry [18], suggesting that TNF-α plays an important role in the development of the inflammatory process in this disorder and providing the rationale for the use of anti-TNF-α therapy in refractory PG or in PG associated with inflammatory bowel diseases [26,27]. In these resistant cases, on the basis of the very high levels of IL-1, the use of IL-1 blockers may be even more appropriate as in classic autoinflammatory diseases, such as familial Mediterranean fever [28].

Several chemokines, such as IL-8, CXCL 1,2,3, CXCL16 and RANTES, were over-expressed in our study, promoting neutrophil transendothelial migration into the site of inflammatory process, which was also favoured by the up-regulation of L-selectin.

Interestingly, the chemokine expression profile was different in SS, in which IL-8, CXCL1,2,3 and CXCL16 proved to be significantly more expressed than in normal skin; in contrast, RANTES expression was not significantly higher than in normal skin, suggesting that chemokine-mediated signals inducing neutrophil recruitment in SS are lower than in PG. Taken together, these findings suggest that the IL-1β-induced release of cytokines and chemokines may be lower in SS than PG, providing the physiopathological background for the different clinical presentation of these two conditions. In fact, PG presents with a skin ulcer, which is due to strong tissue damage,

while SS manifests as a plaque lesion with only a tendency to forming vesicles.

In our study we found an over-expression of IL-17 and its receptor in both PG and SS, confirming the previously hypothesized role for this T helper type 17-related cytokine in the pathophisiology of the whole spectrum of neutrophilic dermatoses, similarly to psoriasis and other autoimmune diseases [18,29,30]. IL-17 amplifies the recruitment of neutrophils and monocytes by increasing the local production of chemokines, most notably IL-8 [31], and synergizing with various other cytokines, in particular with TNF-α [32]. Moreover, IL-17, like IL-1 and TNF-alpha, induces the production of MMPs [33], a family of endopeptidases that include the so-called gelatinases MMP-2 and MMP-9. Gelatinases are major contributors to the breakdown and reconstitution of the extracellular matrix in both physiological processes, such as wound repair, and in pathological conditions, including neutrophilic dermatoses [18,34]. An improper activity of MMPs synthesized by inflammatory cells, particularly neutrophils, is known to cause the destruction of tissue via degradation of components of the extracellular matrix and to influence the production of chemokines, promoting neutrophil transendothelial migration [35].

In the present study we found an over-expression of MMP-9 in the inflammatory infiltrate of PG lesions, suggesting that this proteinase and, to a lesser degree, MMP-2 may be relevantly involved in inducing both tissue damage and repair. In contrast, the role of MMPs as tissue damage effector molecules in SS seems to be less relevant, and this fits in well with the less local aggressiveness of SS.

Interestingly, we also detected overproduction of TIMP-1 and TIMP-2, which probably represents an inhibitory pathway aimed at attenuating MMP-mediated inflammation. Other important inhibitory signals that attenuate immune responses and dampen inflammation in PG, and in autoinflammation in general, are probably carried by Siglec 5 and Siglec 9, both over-expressed in our cases. In fact, Siglecs are inhibitory receptors expressed mainly by cells of the innate immune system that regulate inflammation mediated by damage-associated and pathogen-associated molecular patterns [36]. Compared to PG, the TIMP- and Siglec-mediated inhibitory signals act to a lesser degree in SS, in which the skin inflammatory process is less strong. Our study suggests that two other important systems, the Fas/FasL system and the CD40/CD40L system, may contribute to tissue damage and inflammation in PG. The Fas/FasL system belongs to the TNF/TNF receptor superfamily and to date is the best-known pathway mediating apoptosis [37]. Apoptosis is a form of cell death involved in many inflammatory conditions, including cutaneous disorders [38,39]. The CD40/CD40L system also belongs to the TNF/TNF receptor superfamily. It represents a co-stimulatory system that amplifies the immune response and can promote inflammation via up-regulation

of adhesion molecules and inducing the production of various cytokines and chemokines such as IL-1, TNF-α, IL-8 and RANTES [40]. The pathogenetic role of these two systems (Fas/FasL and CD40/CD40L) in SS is less significant, and this could contribute to explaining the lack of evolution of the typical SS plaque-lesion into a frank skin ulcer, as in classical ulcerative PG.

Overall, our data clearly show high values of proinflammatory cytokines, chemokines and tissue damage effector molecules in patients with PG and, to a lesser degree, in patients with SS in which, however, the small number of cases may represent a limitation. To confirm the specificity of our findings, future studies are needed aimed at evaluating the cytokine expression profile on other inflammatory skin diseases. Moreover, to characterize the cells responsible for the increased secretion of the most important cytokines and chemokines, double-staining studies with specific cell markers should be performed.

Conclusions

This is the first systematic study showing an increased cytokine and chemokine expression profile on lesional skin of PG and SS. This finding supports the inclusion of these two disorders into the neutrophil-mediated autoinflammatory diseases and suggests that the different cytokine profiles may be responsible for the clinical differences between the two diseases. Accordingly, molecules contributing to tissue damage, such as MMPs, and mediating apoptosis, such as the Fas/FasL system, are expressed differently in PG and SS. Finally, inhibitory signals dampening inflammation act to a different degree. The different inflammatory scenarios explain the stronger local aggressiveness in PG than in SS and pave the way to the use of inflammatory cytokine blockers to treat severe PG cases unresponsive to corticosteroids.

Author contributions

A. V. M. and M. C. designed the study. A. V. M. followed the patients and collected clinical and laboratory data. D. F. performed laboratory investigations. M. C. and A. V. M. analysed the data and all the authors contributed to the interpretation of the results. A. V. M. and M. C. drafted the manuscript and P. L. M. contributed to the writing. All the authors critically reviewed the manuscript and approved the final version for submission.

Disclosure

The authors declare that they have no conflicts of interest.

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