Characterization of the immune response in the synovitis, acne, pustulosis, hyperostosis, osteitis (SAPHO) syndrome

M. Hurtado-Nedelec1,2, S. Chollet-Martin1,2, P. Nicoise-Roland1, S. Grootenboer-Mignot1, R. Ruimy3, O. Meyer4 and G. Hayem4

Objective. The aetiology of SAPHO (synovitis, acne, palmoplantar pustulosis, hyperostosis, osteitis) syndrome seems to involve genetic, infectious and immunological components. We examined innate and adaptive immune responses in SAPHO syndrome, as compared with PsA and RA. We also studied the effect of etanercept on immunological parameters.

Methods. We studied 29 patients with SAPHO syndrome, as well as 22 patients with RA, 21 patients with PsA and 15 healthy controls. Adaptive immune responses were investigated by assaying total serum immunoglobulins and several autoantibodies. Innate immunity was studied by quantifying blood PMN functions and plasma cytokine levels. PMN responses to Propionibacterium acnes were tested ex vivo. Eight patients who received etanercept for refractory rheumatic disorders were tested before and after 28 days of treatment.

Results. SAPHO syndrome was associated with elevated IL-8 and IL-18 plasma levels. IL-8 and TNF-α production by purified PMN was higher in the three patient groups than in the healthy controls, but the oxidative burst and IL-18 production were normal. No autoantibodies were detected in SAPHO patients. Induction of PMN IL-8 and TNF-α production by P. acnes was impaired in the SAPHO group as compared with the RA and PsA groups. After 28 days of etanercept therapy, PMN IL-8 and TNF-α production was down-regulated and TNF-α plasma levels were increased.

Conclusions. These results support the view that the SAPHO syndrome may be triggered by an infectious state involving P. acnes, contributing to the strong humoral and cellular pro-inflammatory responses. Etanercept modulation of PMN activation status emphasizes these new immunological findings.

Key words: SAPHO syndrome, Innate immunity, Tumour necrosis factor-α, Polymorphonuclear neutrophil, Propionibacterium acnes, Interleukin-8.

Introduction

The SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis and osteitis) is a distinct form of inflammatory sSpA that shares several features with PsA [1–3]. Chronic and frequently multifocal osteitis is the main diagnostic sign. A paediatric subset of the SAPHO syndrome is referred to as chronic recurrent multifocal osteomyelitis (CRMO) [4, 5]. No validated specific diagnostic criteria have been established since the initial description of the SAPHO syndrome by Chamot et al. in 1987 [6] and Benhamou et al. [7] in 1988.

Several studies suggest a genetic predisposition involving loci outside the MHC, as the HLA-B27 haplotype is far less frequent in the SAPHO syndrome than in other forms of SpA [1]. A susceptibility gene located on chromosome 18 (cma locus) has been identified in a mouse model of chronic multifocal osteomyelitis (CMO) [8], and is located on 18q21.3-18q22 in patients with CMO [9]. Fine analysis of the cma locus revealed mutations in the proline serine threonine phosphatase-interacting protein 2 (psip2) gene [10, 11].

The LPIN2 gene has also been implicated in the SAPHO syndrome, as three variants have been described during the Majeed syndrome, an autoimmune inflammatory syndrome combining CMO, anaemia and neutrophilic dermatosis [12]. Finally, the high prevalence of chronic inflammatory bowel disease in the SAPHO syndrome might be related to abnormalities in the NOD2 gene, leading to altered bacterial recognition [1, 3].

A triggering effect of Propionibacterium acnes in the SAPHO syndrome has been suspected for years, as this bacterium has been recovered from osteoarticular lesions of the anterior chest wall, spine and appendicular skeleton [13, 14], and as SAPHO syndrome sometimes responds in some patients to chronic antibiotic therapy [13, 15, 16].

SAPHO syndrome has been likened to a form of ‘reactive osteitis’, both because of the inconsistent efficacy of various chronic antibiotic regimens, and the chronic and apparently autonomous course of the disease. The co-occurrence of other immune-mediated manifestations such as psoriasis vulgaris, IBD and pyoderma gangrenosum strongly suggests a self-amplifying inflammatory response, possibly involving autoimmunity [17–22].

The aim of this study was to examine the roles of innate and adaptive immunity in the SAPHO syndrome. Given the existence of borderline forms of SAPHO associated with chronic joint diseases, we compared SAPHO patients with PsA and RA patients. We assayed circulating mediators and examined polymorphonuclear neutrophil (PMN) functions ex vivo, in particular, the P. acnes response. Adaptive immune responses were studied by measuring total immunoglobulins and testing for autoantibodies. Finally, in order to better understand the role of TNF-α in the SAPHO syndrome, the same biological parameters were analysed before and after a 28-day course of etanercept in some patients.

Patients and methods

Subjects, blood collection and study design

The subjects were patients attending the Rheumatology Department of Bichat Teaching Hospital, Paris, France (national reference center for SAPHO syndrome). We studied 29 patients with SAPHO syndrome who met the diagnostic criteria proposed by Benhamou et al. [7] and who had at least one typical osseous involvement. The control patients were 22 patients with RA (fulfilling the 1987 ARA criteria [23]) and 21 patients with PsA.
Immune response in the SAPHO syndrome

1161

Immunoglobulin and autoantibody assays

Serum levels of immunoglobulins G, A, M and RF were quantified by nephelometry (BNII, Dade Behring, Marburg, Germany). A second-generation ELISA was used to assay cyclic citrullinated peptide (CCP) (Immunoassac RA, Eurodiagnostica, AB, Malmö, Sweden).

ANAs were assayed by IF on Hep2 cells (KallestadTM, Bio-Rad Laboratories, Redwood, WA, USA). IgG anti-dsDNA antibodies were measured by ELISA (EliX, Unicap, Phadia, Uppsala, Sweden) in samples positive for ANA by IF. Antibodies to soluble ENAs were tested by ELISA (EliA Symphony, Unicap, Uppsala, Sweden) with the following antigens: Sm, RNP, SSA, and SSB.

Antigen-paired cell antibodies, ASMA, anti-mitochondria antibodies and anti-liver–kidney-microsome antibodies were tested by IF on rat liver, kidney and stomach sections (KallestadTM, Bio-Rad). Anti-thyroid peroxidase (TPO) antibodies, anti-thyroglobulin (TG) antibodies, and anti-TSH receptor antibodies were tested with a radioimmunoassay (Brahms, Saint Ouen, France).

Circulating levels of inflammatory mediators

CRP was measured in serum by nephelometry (BNII). The following cytokines were assayed in plasma by ELISA: IL-8, IL-10 and TNF-α (R&D systems, Minneapolis, MN, USA), IL-18 (MBL, Nagoya, Japan) and RANKL (Biomedica, Vienna, Austria). The detection limits were 5 pg/ml for IL-8, IL-10 and TNF-α, 12 pg/ml for IL-18 and 0.08 pmol/l for RANKL.

Blood PMN isolation

PMNs were purified from the blood by using a three-step procedure developed in our laboratory allowing a purity >99.9% [25]. Briefly, leukocytes were isolated in endotoxin-free conditions by sedimentation on a separating medium containing 9% Dextran T-500™ (Phadia) and 38% Radiosolaceptan™ (Schering, Lys-lez-Lannoy, France) followed by Ficoll-Paque density gradient centrifugation (Sigma, St Louis, MO, USA). Monocytes, B lymphocytes and activated T lymphocytes were removed by 30-min incubation with pan-anti-human HLA class II-coated magnetic beads (Dynabeads M-450, Dynal AS, Oslo, Norway); we cannot exclude the possibility that some HLA class II-positive PMN may also be removed during this procedure. Flow cytometry showed the absence of CD14+, CD3+, CD19+ cells, confirming the recovery of highly purified PMN.

Blood PMN culture

Purified PMNs were resuspended in complete RPMI 1640 culture medium, then adjusted to 10⁶ cells/ml and cultured for 24 h at 37°C in 5% CO₂-air, with or without 100 ng/ml lipopolysaccharide (LPS) from Escherichia coli 055:B5 (Sigma) and 250 U/ml IFN-γ (R&D Systems). Cell-free supernatants were then harvested and stored at −70°C until IL-8, TNF-α and IL-18 ELISA measurements, as described above.

Effect of P. acnes on PMN cytokine production in vitro

The reference type strain of P. acnes (53.11 7T) was obtained from the collection of the Pasteur Institute, Paris, France. Bacteria were grown on a chocolate plate agar under anaerobic conditions and suspended at a final concentration of 10⁶ colony-forming units (CFUs)/ml for millilitre. Propionibacterium acnes was inactivated for min at 95°C, then immediately frozen at −80°C until use.

Highly purified PMNs were resuspended in complete RPMI medium and stimulated with increasing concentrations of P. acnes (10-10⁵ CFU/ml) for 24 h before assaying IL-8, TNF-α and IL-18 in cell-free supernatants. PMN viability was checked by the trypan blue exclusion test at the end of the culture period.

Reactive oxygen species quantification

Reactive oxygen species (ROS) production by PMN was measured by chemiluminescence in whole blood after phorbol-12-myristate-13-acetate (PMA) stimulation, according to the manufacturer’s instructions (ABEL®, Cell Activation Test, Knight Scientific, Plymouth, UK). As PMN numbers in whole blood were determined (Sysmex XE-2100, Kobe, Japan), the results were expressed in 10⁵ cpm/10³ PMN.

Statistical analysis

Results were expressed as means ± S.E.M. Mean values in the four groups were compared by using analysis of variance followed by multiple comparison of means with Fisher’s least significance difference procedure; P-values of < 0.05 were considered statistically significant.

In the SAPHO group, relationships between the different biological parameters and between biological and clinical variables (number of bone foci and articular lesions) were identified with the Spearman rank correlation test. For binary variables, we used the Mann–Whitney test to compare marker levels between the two subgroups.

Results

Clinical characteristics

SAPHO group consisted of 24 women and 5 men with a mean age of 53.6 ± 15.3 yrs; RA group of 20 women and 2 men with a mean age of 58.7 ± 12.2 yrs; and PsA group of 11 women and 10 men with a mean age of 47.8 ± 9.8 yrs. In SAPHO group, the mean disease duration was 7.7 ± 1.6 yrs.

In SAPHO group, osteitis was multifocal in 12 patients (41.4%) and unifocal in 17 (58.6%) (bone involvement is described in Table 1). Twenty patients had at least two joint foci (Table 1). Skin lesions were present in 15 patients: palmoplantar pustulosis in 15 (51.7%), severe acne in 5 (17.2%) and psoriasis vulgaris in 15 (51.7%).

Immunoglobulin and autoantibody serum levels

As shown in Table 2, levels of IgA were significantly higher in the three patient groups than in the healthy controls, whereas IgM and IgG levels were normal.

None of the SAPHO patients had detectable RF, anti-CCP antibodies, ANA or ENA antibodies. Concerning organ-specific autoantibodies, only one SAPHO patient had significant levels of anti-thyroid antibodies (anti-TPO and anti-TG). As expected, RF was detected in 50% (11/22) of the RA patients and in 5% (1/21) of the PsA patients; anti-CCP antibodies were detected in 68% (15/22) of the RA patients and in none of the PsA patients.
wall lesions (16 pg/ml) were not different among the four groups (0.18 ± 0.01 pmol/l in SAPHO patients, 0.17 ± 0.01 pmol/l in RA patients, 0.24 ± 0.05 pmol/l in PsA patients and 0.17 ± 0.01 pmol/l in healthy controls). However, pelvic joint involvement was significantly associated with higher RANKL levels (0.2 pmol/l vs 0.1 pmol/l, \( P = 0.004 \)). TNF-\( \alpha \) was detected in 6% of SAPHO patients, 27% of RA patients and 14% of PsA patients; in these patients, the levels were 20 ± 15 pg/ml (SAPHO), 62 ± 24 pg/ml (RA) and 57 ± 30 pg/ml (PsA), respectively; TNF-\( \alpha \) was always below the detection limit in the healthy controls. Plasma levels of IL-10 were below the detection limit in all the patients and healthy controls (data not shown).

### II-8, TNF-\( \alpha \) and IL-18 productions by purified blood PMN

As shown in Fig. 2A, spontaneous IL-8 production by PMN from RA patients was significantly increased as compared with healthy controls, but only slightly increased in the patients with the SAPHO syndrome and PsA. However, in the SAPHO group, palmoplantar pustulosis was associated with increased spontaneous IL-8 production (1100 pg/ml/10⁷ PMN vs 395 pg/ml/10⁷ PMN; \( P = 0.04 \)). Unstimulated TNF-\( \alpha \) production was low or undetectable in all the patients and controls (data not shown). The spontaneous IL-18 production by PMN was also low and similar among controls and the three patients groups (Fig. 2E).

As shown in Fig. 2A and B, IL-8 and TNF-\( \alpha \)-production by PMN stimulated ex vivo with LPS+IFN-\( \gamma \) was significantly increased in the SAPHO group compared with the healthy controls. Similar results were observed in the RA and PsA patients, with no significant difference between SAPHO and RA or SAPHO and PsA. Together, these results suggest that PMNs from SAPHO patients are as hyperresponsive as PMN from RA and PsA patients in terms of pro-inflammatory cytokine production. Figure 2C shows that LPS+IFN-\( \gamma \) was only a weak IL-18 inducer, whatever the patient group.

### Propionibacterium acnes effect on IL-8 and TNF-\( \alpha \) production by purified blood PMN

Given the above results obtained with LPS, we searched for the effect of \( P. \) acnes, potentially involved in SAPHO

---

**Table 1. Clinical features of 29 SAPHO patients**

<table>
<thead>
<tr>
<th>Bone lesions</th>
<th>Articular lesions</th>
<th>Skin lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACW</td>
<td>Vertebrae</td>
<td>Pelvis</td>
</tr>
<tr>
<td>1</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>2</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>4</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>5</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>6</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>8</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>9</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>10</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>11</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>12</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>13</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>14</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>15</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>16</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>17</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>18</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>19</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>20</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>21</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>22</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>23</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>24</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>25</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>26</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>27</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>28</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>29</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

Total (%) 21 (72.4) 5 (17.2) 8 (27.6) 6 (20.7) 13 (45) 6 (20.7) 5 (17.2) 15 (51.7) 15 (51.7)

ACW: anterior chest wall. Includes sternal and/or clavicular manifestations; Pelvis: includes sacroiliac and/or pubic manifestations; PPP: palmoplantar pustulosis; NA: not available.

**Table 2. Serum immunoglobulin levels (g/l)**

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls (n = 15)</td>
<td>11.1 ± 0.5</td>
<td>1.8 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>SAPHO (n = 29)</td>
<td>10.1 ± 0.5</td>
<td>2.7 ± 0.3</td>
<td>1 ± 0.8</td>
</tr>
<tr>
<td>RA (n = 22)</td>
<td>10.5 ± 0.7</td>
<td>2.6 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>PsA (n = 21)</td>
<td>9.8 ± 0.5</td>
<td>2.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are presented as mean ± s.e.m. *\( P < 0.05 \) vs healthy controls.

ANA was detected by IF in 36% (8/22) of the RA patients and in 14% (3/21) of the PsA patients, without anti-DNA or anti-ENA specificity. Anti-thyroid antibodies (anti-TPO and anti-TG) were detected in 13% (3/22) of the RA patients and in 5% (1/21) of the PsA patients.
pathophysiology. *P. acnes* stimulated *ex vivo* production of IL-8 and TNF-α by PMN from both healthy controls and patients with SAPHO, RA and PsA in a concentration-dependent manner (Fig. 2D and E).

Interestingly, PMN from SAPHO patients had a significantly lower response to *P. acnes* (10⁶ and 10⁷ CFU/ml) than those from RA and PsA patients, suggesting desensitization to *P. acnes* in the SAPHO syndrome.

**Reactive oxygen species production by blood PMN after ex vivo PMA stimulation**

ROS production by blood PMN tended to be lower in the SAPHO group (15 ± 2 cpm/10⁵/10³ PMN) than in the healthy controls (22 ± 2 cpm/10⁵/10³ PMN) and in the PsA group (20 ± 4 cpm/10⁷/10³ PMN), which could be related to a deactivation of oxidative burst. Significant increased ROS production was observed during RA (30 ± 8 cpm/10⁷/10³ PMN, *P* < 0.05).

**Effect of etanercept treatment**

Eight patients (three SAPHO, two RA and three PsA) started etanercept treatment during the study period and could be studied before and after 28 days of therapy. All eight patients felt better after the first 4 weeks of etanercept therapy, but not sufficiently for any other ongoing treatments to be reduced (particularly NSAIDs and prednisone).

As shown in Fig. 3A, basal *ex vivo* IL-8 production by PMN fell between day 0 and day 28 in five of the eight patients (one SAPHO, two RA and two PsA). Likewise, after stimulation with LPS+IFN-γ, IL-8 and TNF-α production also fell in six patients (three SAPHO, two RA and one PsA) (Fig. 3B and C). Plasma levels of IL-8 fell in four patients (one SAPHO and three PsA) (Fig. 3D). Plasma levels of TNF-α increased in six patients (Fig. 3E), while plasma levels of RANKL fell significantly in all the patients on day 28 (Fig. 3F).

Autoantibody results on day 28 were similar to those on day 0.

**Discussion**

The SAPHO syndrome is rare, although its incidence is probably underestimated because of the broad spectrum of clinical manifestations and the lack of validated diagnostic criteria. We found that the SAPHO syndrome was consistently associated with pronounced circulating and cellular inflammatory responses, in particular to *P. acnes*, but we found no circulating signs of autoimmunity.

The possible link between autoimmunity and the SAPHO syndrome is controversial. We screened our 29 patients for numerous autoantibodies, and found that RA markers (RF and anti-CCP2 antibodies) were absent in the SAPHO syndrome, while their prevalence in our control patients with RA and PsA were in keeping with published data [26]. ANAs were also absent in the SAPHO syndrome and, as expected, were present in some patients with RA and PsA, in particular, anti-SSa (60 + 52 kDa). In a recent study, Jansson *et al.* [4] detected ANA in 30% of the patients with CRMO without specific identification. This discrepancy might be due to technical factors. Similarly, no organ-specific autoantibodies were detected in SAPHO patients, apart from the one who had anti-TPO anti-TG antibodies. Taken together, our findings argue against a main role of autoimmunity in the SAPHO syndrome, at least in the first stages of the disease. In particular, the autoantibody patterns classically associated with RA and PsA were not found in studied SAPHO patients.

We also assayed several cytokines, for the first time in this setting, in order to document the concept of inflammasome during SAPHO syndrome [27]. IL-8 plasma levels were moderately increased in patients with the SAPHO syndrome, to an extent similar to that observed in the other patient groups. However, the significantly higher levels observed in SAPHO patients with anterior chest wall lesions suggests that this major chemokine may
FIG. 2. IL-8, TNF-α and IL-18 production by purified blood PMN isolated from the patients (SAPHO, RA and PsA) and from the healthy controls, in response to various stimuli for 24 h of culture. Three cytokines were assayed in the cell-free supernatants. (A), (B) and (C) IL-8, TNF-α and IL-18 production by PMN from the four groups, at rest (open square) and after LPS+IFN-γ stimulation ex vivo (filled square). (D) and (E) IL-8 and TNF-α production after ex vivo, P. acnes stimulation at increasing concentrations (from 10 to $10^7$ CFU/ml) in patients with SAPHO ($n=4$), RA ($n=5$), PsA ($n=5$) (filled square) and healthy controls ($n=10$) (open square). Values are presented as mean ± S.E.M. *P < 0.05 vs healthy controls.
orchestrate sequential cell recruitment to inflamed bones and joints. RANKL-mediated osteoclast differentiation, activation, survival and migration might play a role in the bone damage associated with the SAPHO syndrome [28, 29]. The normal RANKL plasma values found point to a local rather than a systemic role of RANKL in bone destruction. TNF-α also plays an important role in chronic osteitis, synergistically with RANKL [28, 30]. We detected TNF-α in the plasma of only 2 of our 29 patients with the SAPHO syndrome, whereas Jansson et al. [4] recently reported that 66% of patients with CRMO had

![Fig. 3. Cytokine levels in patients with the SAPHO syndrome (n = 3), RA (n = 2) or PsA (n = 3), before (open square) and after 28 days of etanercept therapy (filled square). (A) and (B) IL-8 production by 24-h cultured purified blood PMN from the eight treated patients, at baseline (A) and in response to LPS+IFN-γ stimulation (B). (C) TNF-α production by 24-h cultured purified blood PMN from the eight treated patients, in response to LPS+IFN-γ stimulation. Cytokine plasma levels before (open square) and after 28 days of etanercept therapy (filled square): IL-8 (D), TNF-α (E) and RANKL (F). Values are presented as mean ± S.E.M.]
detectable plasma TNF-α. This discrepancy underlines the difficulty of defining homogeneous patient groups at comparable stages of the disease. IL-18 is another important mediator of both innate and adaptive immune responses. High IL-18 circulating levels have been found in RA and PsA [31, 32], promoting Th1 cell maturation and proliferation in arthritis, and also local neutrophil activation in the synovia. Moreover, IL-18 gene polymorphism is associated with RA [31]. The increased IL-18 plasma levels observed in the studied SAPHO patients could support a new role for this cytokine in this setting. Finally, the failure to detect circulating IL-10 suggests a possible imbalance between pro- and anti-inflammatory mediators. In conclusion, the profile of circulating cytokines that we found in SAPHO patients was close to that observed in RA and PsA patients with similar anti-inflammatory and immunomodulatory therapies. This supports the potential use of anti-TNF-α therapy, which we found affecting both plasma cytokine levels and neutrophil activation status.

We also investigated cellular inflammatory status by conducting PMN functional studies, given the important role of PMN in RA and inflammatory SpAs [33]. We observed normal ROS production in patients with the SAPHO syndrome, in response to both PMA and _P. acnes_ (data not shown). This suggests that the oxidative burst of circulating PMN might not play a prominent role in the pathophysiology of the SAPHO syndrome. In addition to the phagocytic and killing properties, PMN can launch immune responses by recruiting, activating and programming antigen-presenting cells (APCs). Moreover, PMN helps to drive proliferation and maturation of B cells and T cells, as well as macrophages. These tasks are mainly driven by PMN-derived cytokines, the main source of human cytokines such as IL-8 or IL-18, linking innate and adaptive immune responses [34, 35]. This production includes IL-8 that has a key role in recruiting circulating phagocytes to inflammatory sites [34, 36, 37]. We found that the baseline capacity of PMN to produce IL-8 _ex vivo_ was slightly increased in SAPHO patients as IL-18 production was normal. When we exposed PMN to LPS+IFN-γ, which we have shown to be the optimal stimulus [25], we observed marked up-regulation (up to 10-fold) of IL-8 and TNF-α production in the SAPHO patients. This PMN priming might be related to the increased circulating levels of IL-8 and IL-18. It might also be due to continuous stimulation by infectious agents, leading to chronic inflammation and tissue damage.

_Propionibacterium acnes_ is an anaerobic skin saprophyte, which has been strongly implicated in the SAPHO syndrome [14]. Several authors have detected _P. acnes_ in biopsy specimens of bone and tissue lesions of the anterior chest wall, spine or appendicular skeleton of SAPHO patients [13, 15, 38, 39]. Moreover, several antibiotics have proven beneficial in some patients. We, therefore, tested the influence of _P. acnes_ on blood PMN isolated from patients with the SAPHO syndrome in comparison with the other patient groups. _Propionibacterium acnes_ had a concentration-dependent effect on both IL-8 and TNF-α productions, and this could play a role in initiating inflammation and PMN infiltration in the studied patients groups. PMNs thus represent an additional target for _P. acnes_ stimulation (10^6 and 10^7 CFU/ml) was drastically lower in SAPHO patients than in RA and PsA patients. This suggests hyporeactivity to _P. acnes_, probably related to chronic exposure to this bacterium. Indeed, similar desensitization to bacterial challenge _ex vivo_ has been reported by us during peritonitis [45] and by others during sepsis [46].

The increased circulating IgA levels found here in the SAPHO syndrome plead for an anti-infectious response, in keeping with the high IgA levels (mainly against Enterobacteriaceae and mycobacteria) observed by ourselves and others in RA and SpA patients, and reinforcing the theory of infection-induced rheumatic diseases [47].

Following the successful treatment of several rheumatic diseases with TNF-α antagonists, such drugs were tested in a few patients with the SAPHO syndrome, with promising results [21]. Four studies involved between one and four patients treated with infliximab [17, 19, 20, 22] and one study evaluated etanercept in two patients [18]. Our preliminary results in a limited patient group are in line with these clinical results and offer a tentative immunological explanation. Indeed, after 28 days of etanercept therapy, _ex vivo_ IL-8 and TNF-α productions by cultured blood PMN after LPS+IFN-γ stimulation were reduced relative to day 0. Higher TNF-α plasma levels were found on day 28 in six of the eight treated patients. This paradoxical response has previously been described in etanercept-treated patients with SpA [48], breast cancer [49] and multiple myeloma [50], without compromised clinical response [51]. One possible explanation is that etanercept could act as a decoy receptor, increasing the TNF-α half-life in the circulation and impairing interaction with the TNF receptor, thus disrupting subsequent signalling events [50]. Our findings suggest that etanercept is able to modulate the TNF-α autoinflammatory response in PMN. This finding could be relevant to the SAPHO syndrome, given the numerous effects of TNF-α on PMN functions, such as oxidative burst priming, cytokine synthesis, apoptosis regulation and differentiation [52–54].

This immunological study of 72 patients with inflammatory rheumatic disorders, including 29 patients with the SAPHO syndrome, suggests a number of pathophysiological mechanisms that may be involved in this rare syndrome. First, autoimmunity does not seem to be of major importance, despite a genetic predisposition that is under investigation by our group and others. Second, the innate immune response appears to be hyperstimulated, as in RA and SpA, as reflected by circulating mediator levels and PMN functional status. Third, _our ex vivo_ model of PMN activation by _P. acnes_ supports a complex potential implication of this bacterium, which may be a form of ‘reactive osteitis’ initially triggered by an infective agent, and chronic desensitization due to persistence of this agent or one of its components. Finally, etanercept therapy down-regulated PMN functions and cytokine production in patients refractory to conventional treatments, in line with the observed clinical effectiveness of this TNF-α antagonist in some patients.

Rheumatology key messages

- SAPHO syndrome is associated with inflammatory cytokine release and global neutrophil activation.
- Neutrophils seem to be desensitized to _P. acnes ex vivo_.
- Etanercept could be a useful therapeutic tool for patients with refractory SAPHO syndrome.

Acknowledgement

Funding: This work was supported by a grant from Wyeth Laboratories.

Disclosure statement: The authors have declared no conflicts of interest.

References

Immune response in the SAPHO syndrome


