Reduction of peripheral natural killer cells in patients with SAPHO syndrome

D. Xu[1], X. Liu[2], C. Lu[3], J. Luo[1], C. Wang[1], C. Gao[4], J. Xie[1], X. Li[1]

[1]Department of Rheumatology, the Second Hospital of Shanxi Medical University, Taiyuan, China; [2]The Second Clinical Medical College of Shanxi Medical University, Taiyuan, China; [3]Department of Rheumatology and Immunology, West China Hospital, Sichuan University, Chengdu, China; [4]Department of Pathology, Joint Program in Transfusion Medicine, Brigham and Women's Hospital/Children's Hospital Boston, Harvard Medical School, Boston, USA.

Abstract

Objective

Little is known about the roles of peripheral immune cell subsets in synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) syndrome. Up to now, just a few studies have focused on this issue. We aimed to analyse the distribution and phenotype of T cell subsets and natural killer (NK) cells in the peripheral blood of patients with SAPHO syndrome.

Methods

The proportion and absolute counts of circulating immune cells were assessed in 19 patients diagnosed as SAPHO syndrome and 19 healthy controls. CD4+T cell subsets were also analysed in 9 untreated SAPHO patients and 9 healthy volunteers by flow cytometry.

Results

The proportion and absolute counts of NK cells were significantly reduced in SAPHO patients in comparison with the controls (proportion, 10% vs. 18%, p<0.001; absolute counts, 231/μl vs. 307/μl, p=0.014). Conversely, the proportion and absolute counts of Th17 cells in untreated SAPHO patients were significantly higher than that in the healthy controls (proportion, 1.49% vs. 0.93%, p=0.004; absolute counts, 14.36/μl vs. 5.14/μl, p<0.001). Similarly, Th17/Th1 cells were significantly increased (proportion, 0.45% vs. 0.33%, p=0.024; absolute number, 5.47/μl vs. 1.98/μl, p<0.001), but there was no significant difference between the percentage and number of Treg cells in patients with SAPHO syndrome and healthy controls. Thus, the ratio of Th17/Treg was increased in SAPHO patients (0.68 vs. 0.17, p=0.004).

Conclusion

Our data suggested that the immune inflammation in SAPHO patients may be related to the depletion of NK cells and the imbalance of Th17 and Treg cells. A reduction of peripheral NK cells may exacerbate the disease progression by not being inhibited Th17 cells.

Key words

SAPHO syndrome, NK cell, Th17 cell, regulatory T cell

Dan Xu, MD
Xiaoyu Liu, BS
Chenyang Lu, MD
Jing Luo, PhD
Caihong Wang, PhD
Chong Gao, PhD
Jianfang Xie, MD
Xiaofeng Li, PhD

Please address correspondence to:
Dr X. Li,
Department of Rheumatology,
the Second Hospital of
Shanxi Medical University,
382 Wuyi Road,
Taiyuan 030001, China.
E-mail: lxf_9859@sxmu.edu.cn

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Introduction

SAPHO is an acronym for synovitis, acne, pustulosis, hyperostosis and osteitis, which was first coined by the French rheumatologist Chamot in 1987 (1). It is used to describe a combination of inflammatory condition involving both skin and bones. Although the pathogenesis of SAPHO syndrome is still not clearly elucidated, the co-occurrence of other immune-mediated manifestations such as psoriasis vulgaris, inflammatory bowel disease (IBD) and pyoderma gangrenosum strongly suggests a self-amplifying inflammatory response, possibly involving autoimmunity (2-3). Besides, many SAPHO syndrome patients also meet the classification criteria for spondyloarthritis (SpA), so SAPHO syndrome is considered as a special subtype of SpA. Several studies have investigated the possible role of natural killer (NK) cells in the pathogenesis of inflammatory arthropathies including rheumatoid arthritis and SpA, and NK cells, especially $CD56^{bright}$ NK cells, have been found to accumulate in target tissues, such as in psoriatic skin lesions or in synovial fluid of RA patients. Conversely, decreased absolute number, proportion and activity of NK cells have been detected in peripheral blood of patients with inflammatory arthropathies (4-5).

Current studies have also shown that the imbalance of T cell subsets including Th17 and regulatory T (Treg) cells contributes to the immunopathological mechanisms of SpA, whereas the role of T cell subsets still remains unclear and controversial in SAPHO syndrome (6). Notably, Th17 cells mainly exert a pro-inflammatory role and actively participate in the pathogenesis of autoimmune diseases like PsA and AS (7). On the other hand, regulatory T lymphocytes are indispensable for the maintenance of immune homeostasis (8). As a result, any imbalance of immune cell subsets may have a significant impact on autoimmune disease (9). Until now, only a few data addressing the role of Th17 cells in SAPHO pathogenesis are actually available. Firinu *et al*. have firstly described the augment of Th17 cell population in SAPHO patients with different disease activity or treatment (10). In agreement with these observations and considering the influence of different drugs on immune cells (11-12), we aimed to analyse the distribution and phenotype of immune cell subsets in a more comprehensive fashion by means of multi-parametric flow cytometry at the peripheral level in a group of SAPHO patients, including Th1, Th2, Th17 and Treg cells in some treatment-naive patients.

Material and methods

Study population

Nineteen SAPHO patients were admitted into the department of rheumatology at the Second Hospital of Shanxi Medical University between January 2015 and December 2016 were enrolled in the study. These patients were diagnosed as SAPHO syndrome according to the criteria provided by Benhamou *et al*., and 9 of them were treatment-naïve. Nineteen age-matched and gender-matched healthy volunteers, recruited from the general population of Taiyuan, China, were used as a control group. The local Ethics Committee approved the study and all participants provided written informed consent.

Clinical data collection

Medical history, laboratory data and imaging features were collected from all patients, including age, gender, age at onset of symptoms, duration of diagnosis, clinical symptoms, treatment statue, anti-nuclear antibody (ANA), rheumatoid factor (RF), immunoglobulins (IgG, IgM, IgA), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), human leukocyte antigen B27 (HLA-B27). ANA, RF, IgG, IgM and IgA were tested by enzyme-linked immunosorbent assay (ELISA), with normal ranges of 0–1:20, 0–20 Iu/ml, 7.51–15.6 g/l, 0.82–4.53 g/l, and 0.46–3.04 g/l, respectively. HLA-B27 was detected by flow cytometry. CRP was detected by Turbidimetric inhibition immunoassay. Values more than or equal to 8 mg/l were considered positive. ESR was measured by Westergren's method, and values >15 mm/h for men and >20 mm/h for women was considered abnormal. Disease activity was evaluated by using the Bath

AS Disease Activity Index (BASDAI), Ankylosing Spondylitis Disease Activity Score (ASDAS-ESR and ASDAS-CRP).

Flow cytometry

In order to determine the percentage and absolute counts of peripheral lymphocyte subsets, peripheral blood samples (2ml) from each subject was collected in EDTA-coated tubes. For immunofluorescence staining, 50 μl blood samples were placed in TruCount tubes A and B. Then, 20μl of anti-CD3-FITC/CD8-PE/CD45-PercP/CD4-APC antibody mix was added into tube A and 20μl of anti-CD3-FITC/CD16+56-PE/CD45-PercP/CD19-APC antibodies into tube B (Antibodies were purchased from BD Biosciences). After incubation at room temperature for 20 minutes in the dark, stained cells were washed with 1X FACS buffer and then incubated for 15 minutes in the dark. Fifteen thousand cells were acquired and detected on a FACScanto (BD Bioscience, San Jose, CA, USA), and the resulting data was analysed with MultiSET software. The frequency and absolute numbers of Th1, Th2, Th17, Th1/Th17 and Treg cells were examined in 9 treatment-naive SAPHO patients and 9 healthy individuals. For analysis of Th1, Th2, and Th17 cells, 80μl anti-coagulated blood were stimulated by 10μl PMA, 10μl Ionomycin and 1μl GolgiStop, and then incubated for 5 hours in 37°C. The sample were divided into Tube A and tube B both followed by staining with human anti-CD4-FITC antibodies at the room temperature in the dark for 30 minutes. Fixed and permeabilised activated cells in 4°C incubator in the dark for 30 minutes. And then the sample was stained by anti-IL-4-PE and anti-IFN-γ-APC in tube A, and by human anti-IL-17-PE in tube B at the room temperature away from light for 30 minutes. Cells were washed with PBS and examined by four-colour flow cytometry. For analysis of Treg cells, 80μl anti-coagulated blood was stained with anti-CD4-FITC and anti-CD25-APC at the room temperature in the dark for 30 minutes, and fixed and permeabilised in 4°C incubator away from light for 30 minutes by 1ml fresh Fixation/Permeabilisation followed by staining with human anti-Foxp3-PE in room temperature away from light for 30 minutes. Cells were washed and examined by flow cytometry (Calibur, BD, USA). Cell types were defined as Th1 ($CD4^+IFN\text{-}\gamma^+$), Th2 ($CD4^+$ $IL\text{-}4^+$), Th17 ($CD4^+$ $IL\text{-}17^+$), Th1/Th17 ($CD4^+$ $IFN\text{-}\gamma^+$ $IL\text{-}17^+$), Treg ($CD4^+$ $CD25^+$ $Foxp3^+$).

Ten thousand cells in gate were detected and analysed by Cell Quest software to acquire the frequencies of $CD4^+$T subsets. And the absolute number of $CD4^+$T subsets were calculated by the percentage of $CD4^+$T subsets multiplying by the absolute number of total $CD4^+$T cells.

Statistical analysis

All statistical analyses were performed with SPSS v. 22.0 (SPSS, Chicago, IL). Continuous variables were reported as mean ± standard deviation (SD) and median [25^{th}–75^{th} percentile range]; categorical variables were reported as number of occurrences and percentages. For all study variables, comparison among controls and SAPHO subjects was based on the non-parametric Wilcoxon Mann-Whitney exact test. For all analyses, we used two-sided tests, with p-values <0.05 denoting statistical significance.

Table I. Demographic and clinical characteristics of the 19 patients with SAPHO syndrome

Items	
Sex, female/male	11/8
Age, mean (SD), years	42.37 (13.27)
Age at onset of symptoms, mean (SD), years	37.32 (13.91)
Duration of diagnosis, mean (SD), years	5.11 (7.14)
Clinical characteristics	
Osteoarticular symptoms	
Anterior chest pain	15/19 (78.95%)
Lumbosacral region pain	14/19 (73.68%)
Peripheral arthritis	10/19 (52.63%)
Skin manifestations	
PPP only	12/19 (63.16%)
SA only	5/19 (26.32%)
PPP and SA	1/19 (5.26%)
Disease activity	
BASDAI	3.18 (1.42)
ASDAS-ESR	2.20 (1.20)
ASDAS-CRP	2.15 (1.07)
Medication	
Non-steroidal anti-inflammatory drugs	10/10 (100%)
Steroids (prednisone 10-30mg/d)	5/10 (50.%)
DMARDs (SZP, MTX or LEF)	9/10 (90%)
Tumour necrosis factor-alpha inhibitors	3/10 (30%)
Antibiotics	4/10 (40%)

PPP: palmoplantar pustolosis; SA: severe acne; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS: Ankylosing Spondylitis Disease Activity Score. DMARDs: disease-modifying anti-rheumatic drugs. SZP: salazosulfapyridine. MTX: methotrexate. LEF: leflunomide.

Results

Basic information

Nineteen patients (11 women and 8 men) who met the inclusion criteria were recruited to our study. The mean age of the patients was 42.37 years and the mean age at onset of symptoms was 37.32 years. The mean duration of diagnosis was 5.11 years. Among them, 18 patients had both osteoarticular symptoms and dermatological manifestations, while one patient diagnosed by bone biopsy had only osteoarticular symptoms. Clinical features of osteoarticular symptoms were insidious and variable, from slight to severe inflammatory pain in different locations with or without swelling. In our study, 15 (78.95%) patients suffered from pain in the sternoclavicular joints and/or sternocostal joints, and 14 (73.68%) patients in the low back, sacroiliac joints and/or hip joints. In addition, 10 (52.63%) patients had pain in peripheral joints, such as the shoulder, wrist, hip, knee and ankle. Among those

Table II. Laboratory characteristics of the 19 patients with SAPHO syndrome.

Laboratory test	
ESR ↑	14/19 (73.68%)
CRP ↑	10/17 (58.82%)
HLA-B27 positive	1/17 (5.88%)
ANA positive	3/19 (15.79%)
RF positive	0/19
IgG ↑	6/19 (31.58%)
IgA ↑	1/19 (5.26%)
IgM ↑	1/19 (5.26%)

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HLA-B27: human leukocyte antigen B27; ANA: anti-nuclear antibody; RF: rheumatoid factor; Ig: immunoglobulins.

Table III. Absolute counts and percentage of PB lymphocytes in the study participants (n=19).

Variable	SAPHO patients (n=19)	Healthy donors (n=19)	*p*-value
PB lymphocyte (cells/μl)	median (quartile range)	median (quartile range)	
T	1629.00 (1542.00-2111.00)	1144.00 (843.00-15031.00)	*p*<0.001
B	238.00 (171.00-331.00)	173.00 (147.00-260.00)	0.146
$CD4^+T$	987.00 (801.00-1298.00)	554.00 (484.00-719.00)	*p*<0.001
$CD8^+T$	554.00 (425.00-734.00)	341.00 (281.00-495.00)	0.004
NK	231.00 (171.00-310.00)	307.00 (243.00-406.00)	0.014
$CD4^+T/CD8^+T$, ratio	1.85 (1.08-2.41)	1.61 (1.37-2.34)	0.863
PB lymphocyte %			
T%	76.00 (72.00-82.00)	66.00 (63.00-72.00)	*p*<0.001
B%	11.00 (9.00-14.00)	12.00 (8.00-16.00)	0.686
$CD4^+T$%	44.00 (37.00-49.00)	37.00 (32.00-40.00)	0.02
$CD8^+T$%	24.00 (21.00-33.00)	22.00 (18.00-28.00)	0.201
NK%	10.00 (8.00-12.00)	18.00 (15.00-22.00)	*p*<0.001

with dermatological manifestations, 1 (5.26%) patient had both palmoplantar pustulosis (PPP) and severe acne (SA), 12 patients (63.16%) only have PPP and 5 patients (26.32%) only had SA, respectively. Furthermore, two of them had IBD. Concerning the treatment among 10 patients, non-steroidal anti-inflammatory drugs (NSAIDs) were used in all of them, prednisone at a dosage between 10 to 30mg/day in 5 patients, and disease-modifying anti-rheumatic drugs (DMARDs) were used in 9 patients, of whom 4, 2, 1 and 2 patients took methotrexate (MTX) + salazosulfapyridine (SZP), SZP, SZP + leflunomide (LEF) and LEF, respectively. In addition, three patients had received anti-TNF-α agents, and antibiotics were used in 4 patients. Demographics, clinical characteristics and treatment of the study population are presented in Table I.

Among the 19 patients, 14 (73.68%) had elevated ESR levels and 11 (58.82%) showed increased CRP levels. Only 3 patients were ANA positive and 1 patient was HLA-B27 positive. IgG level raised in 6 patients (31.58%). IgA or IgM was elevated in one patient (5.26%), respectively. Laboratory characteristics of the patients are shown in Table II.

CT scans of the thoracic and lumbar vertebrae were performed in 7 patients, of whom 6 patients (85.71%) had lesions in costosternal, sternoclavicular or manubriosternal joints. Fourteen patients who had lumbosacral region pain received CT scans of sacroiliac joints, and sacroiliitis could be found in 78.57% (11/14) of patients. Bone scintigraphy was carried out in 7 patients, and all of them had anterior chest wall involvement. The vertebrae and peripheral joints were involved in 4 and 3 patients, respectively.

SAPHO patients display a significant depletion of peripheral NK cells

The levels of immune cell subsets in peripheral blood are displayed in Table III. As shown, when compared with healthy controls, SAPHO patients had higher percentage and absolute counts of T cells (proportion, 76% *vs*. 66%, *p*<0.001; absolute number, 1629/μl *vs*. 1144/μl, *p*<0.001), $CD4^+$ T cells (proportion, 44% *vs*. 37%, p=0.02; absolute number, 987/μl *vs*. 554/μl, *p*<0.001), and a large number of $CD8^+$ T cells (554/μl *vs*. 341/μl, *p*=0.004), but not the proportions and absolute numbers of NK cells (proportion, 10% *vs*. 18%, *p*<0.001; absolute number, 231/μl *vs*. 307/μl, *p*=0.014). In addition, the proportion of NK cell was positively correlated with disease duration (r=0.677, *p*=0.001) while it was negatively correlated with the percentage of T cell (r=-0.717, *p*<0.001). No difference was reported when comparing the percentage and absolute number of B cell, the percentage of $CD8^+$T cells and the ratio of $CD4^+/CD8^+$ T cells between SAPHO patients and healthy group. Furthermore, although there were trends towards increased in the percentage of T cells (*p*=0.095) and B cells (*p*=0.065) in treatment-naive patients compared with treated patients, no difference was found between two groups in terms of NK cells, $CD4^+$T cells and $CD8^+$T cells (data are available in the Supplementary Table). Among SAPHO patients, 10 (52.63%) had peripheral arthritis and 9 (47.37%) had axial articular involvement, but no difference was observed in immune cells parameters between them. Besides, there patients who had BASDAI score higher than 4 were defined as active. Also, there was no difference between active *versus* inactive patients (data not shown).

Percentage and absolute counts of Th17 and Th17/Th1 cells increased and the imbalance of Th17/Treg cell was found in treatment-naïve SAPHO patients

There was no statistically significant difference in the Th1 and Th2 cells population between SAPHO patients and healthy controls. Higher percentage and numbers of Th17 cells were observed in patients with SAPHO syndrome compared with healthy controls (proportion, 1.49 *vs*. 0.93, *p*=0.004; absolute number, 14.36/μl *vs*. 5.14/μl, *p*<0.001). Similarly, Th17/Th1 cells demonstrated significantly elevated (proportion, 0.45 *vs*. 0.33, *p*=0.024; absolute number, 5.47/μl *vs*. 1.98/μl, *p*<0.001). The percentage and number of Treg cells were also investigated according to FoxP3 expression. But no significant difference was observed in patients with SAPHO as compared to healthy controls. Interestingly, the ratio of Th17/Treg increased in SAPHO patients in comparison with healthy individuals (0.68 *vs*. 0.17, *p*=0.004), indicating an imbalance of Th17/Treg. Data

Table IV. Absolute counts and percentage of $CD4^+$ T cells in the study participants (n=9).

Variable	SAPHO patients (n=9)	Healthy donors (n=9)	*p*-value
$CD4^+$T subsets (cells/μl)	median (quartile range)	median (quartile range)	
Th1	139.49 (72.82-164.88)	77.80 (29.07-159.88)	0.258
Th2	14.08 (9.78-25.85)	8.16 (5.73-18.07)	0.113
Th17	14.36 (10.28-30.94)	5.14 (3.70-6.33)	<0.001
Treg	37.42 (23.31-49.40)	36.21 (25.23-38.36)	0.546
Th1/Th17	5.47 (3.56-6.96)	1.98 (1.92-2.22)	<0.001
Th1/Th2, ratio	9.35 (4.28-11.30)	6.82 (4.24-15.68)	1.000
Th17/Treg, ratio	0.68 (0.29-0.92)	0.17 (0.13-0.20)	0.004
$CD4^+$T subsets %			
Th1%	12.48 (7.83-18.32)	14.99 (4.09-23.54)	1.000
Th2%	1.57 (1.07-2.52)	1.22 (0.93-2.21)	0.730
Th17%	1.49 (1.17-2.73)	0.93 (0.48-1.00)	0.004
Treg%	3.72 (2.13-4.54)	5.39 (3.89-5.79)	0.113
Th1/Th17%	0.45 (0.35-0.74)	0.33 (0.30-0.34)	0.024

were shown in Table IV. Meanwhile, we found no statistically significant association between all above parameters of immune cells and BASDAI, ASDAS-ESR, or ASDAS-CRP.

Discussion

SAPHO syndrome is a rare clinical entity and several previous studies indicated that disturbance of autoimmune system may play an important role in its pathogenesis.

Interestingly, we found that both the percentage and absolute number of NK cells were significantly reduced in SAPHO syndrome and there was no difference between patients with axial and peripheral articular involvements, with receiving treatment and those not, or with active and inactive disease. By far, there is no information concerning the role and distribution of NK cells in SAPHO patients. NK cells are prominent components of the innate immune response that not only can exert cell-mediated cytotoxicity against tumor cells or infected cell, but also play regulatory role through promoting or suppressing functions of other immune cells by secretion of cytokines and chemokines. For instance, NK cells induce IL-27 production from DCs by secreting IFN-γ, which diminishes the Th17 inflammatory response in autoimmune uveitis (13). In the same study, Conigliaro *et al*. investigated NK cells in the PB from patients with PsA and found a reduction of this subtype of lymphocytes (both as percentages and in absolute numbers) as compared to healthy individuals. Moreover, a positive correlation between NK cells cytotoxicity *in vitro* and disease activity has been described in PsA (14). Similarly, the involvement of NK cells in pathogenesis of such common autoimmune diseases as systemic lupus erythematosus, juvenile rheumatoid arthritis and multiple sclerosis was suggested by other studies (15-17). Different mechanisms may explain the reduction of peripheral NK cells observed in SAPHO syndrome, such as their recruitment into the inflamed target tissues (synovium and skin) and altered apoptosis. Although our data showed that treatment and disease activity had no impact on NK cell percentage and that longer disease duration was related to higher percentage of NK cells in peripheral blood, it is hard to draw the conclusions since a small number of patients were involved. Further studies with larger numbers of patients are needed to investigate this issue. And the detailed mechanism of this condition including the clear role of NK cell dysfunction in SAPHO syndrome still requires elucidation.

We also found that both the frequency and absolute number of blood $CD4^+$T cells were significantly increased in SAPHO cases when compared to normal subjects. In order to better understand the role of $CD4^+$T subsets in the SAPHO syndrome, the Th1, Th2, Th17 and Treg cells were analysed in 9 untreated patients. In healthy individuals, approximately 1% of peripheral blood $CD4^+$ T lymphocytes is represented by Th17 cells. Interestingly, we found that the frequency and absolute number of IL-17 expressing $CD4^+$ helper cells was dramatically increased in SAPHO patients in comparison with the control group. Similar results were reported by Firinu *et al*. (10). IL-17, secreted from Th17 cells, amplifies the recruitment of neutrophils and monocytes by increasing the local production of chemokines, most notably IL-8, IL-23 and synergising with various other cytokines, in particular TNF-α (18). It has been reported that TNF-α was highly expressed in the affected bone tissue of SAPHO syndrome patients. And abnormal expression of IL-8 and IL-18 was found in the serum of patients with SAPHO syndrome. High expression of these cytokines can change the neutrophil response and induce the up-regulation of TNF-α and its related products. Also, *in vitro*, Etanercept can inhibit the neutrophil response and alter the expression of these cytokines. Several studies

Supplementary Table. Absolute counts and percentage of lymphocyte in untreated and treated participants.

Variable	Treated (n=10)	Untreated (n=9)	*p*-value
PB lymphocyte (cells/μl)	median (quartile range)	median (quartile range)	
T	1858.50 (1349.25-2259.00)	1629.00 (1604.00-2013.50)	0.968
B	322.00 (186.00-429.25)	209.00 (165.50-267.50)	0.182
$CD4^+$T	1027.00 (789.75-1345.75)	951.00 (783.00-1280.00)	0.720
$CD8^+$T	536.00 (421.25-1017.25)	554.00 (497.50-678.50)	1.000
NK	276.50 (175.00-339.00)	218.00 (157.50-278.00)	0.243
$CD4^+$T/$CD8^+$T, ratio	1.57 (0.92-2.73)	1.87 (1.22-2.33)	0.661
PB lymphocyte %			
T%	72.50 (70.75-78.25)	78.00 (75.50-83.00)	0.095
B%	13.00 (10.75-16.00)	9.00 (8.00-12.50)	0.065
$CD4^+$T%	39.00 (33.75-50.00)	46.00 (39.00-48.50)	0.720
$CD8^+$T%	26.00 (19.00-36.00)	24.00 (21.00-28.00)	0.842
NK%	10.00 (9.00-13.25)	9.00 (7.50-12.00)	0.315

have reported that TNF-α inhibitors could improve the skin and joint symptoms of SAPHO patients (19).

Moreover, the dysregulation of the P2X7–IL-1β axis is hypothesised to lead to an increased release of IL-1β, as found in other autoimmune diseases (AIDs). Increasing evidence pointed that P2X7R is a main player in Th17 differentiation and IL-17 secretion (20). In 2014, Firinu *et al*. reported a case of SAPHO syndrome, who did not response to TNF-α antagonist treatment, improved significantly after receiving treatment with ustekinumab for six months (90 mg per day, subcutaneous injection), both in the skin and joint symptoms (21). Ustekinumab is a humanised monoclonal antibody that binds to the P40 subunit common to IL-12 and IL-23. IL-12 is a key cytokine in the Th1 inflammatory response and IL-23 is involved in the activation of Th17 cell (22). In addition, patients with SpA showed increased number of type 17 helper T cells in the peripheral blood, which is in accordance with SAPHO patients. And a study on mouse models of SpA indicated that IL-17 was involved in the development of enthesitis and ileitis (23). IL-17 is also able to promote osteogenesis under inflammatory conditions, and therefor may contribute to the osteitis and synovitis in SAPHO patients (24). Recent clinical trials on the treatment of SAPHO patients with anti-IL-17 monoclonal antibodies have provided promising results. In three cases of SAPHO syndrome treated with secukinumab for three months, two cases demonstrated significant improvement of skin lesions. (25). Interestingly, Th17/Th1 cells demonstrated significantly elevated in patients with SAPHO syndrome in this study. Th17/Th1 cells, coexpressing IL-17 and IFN-γ, have been identified in the context of the induction of inflammatory diseases such as Crohn's disease (26). Also, pathogenic bacterial infection, like propionibacterium acnes, promotes Th17/Th1 cells response (27). In this study, the elevated Th17/Th1 cells in SAPHO syndrome may response to the infection of pathogen, which plays an important role in the pathogenesis of SAPHO syndrome (28). In addition, this infectious aetiology is also supported by increased levels of circulating immunoglobulins.

We additionally observed that there was no difference between SAPHO patients and control subjects about the frequency and number of circulating $CD4^+CD25^+FoxP3^+$ Treg cells. Treg cells may be implicated in the pathogenesis of autoimmune and inflammatory conditions, and their numbers have been found altered in the peripheral blood of patients with SLE and RA (29, 30). But in SpA, the role of Treg cell is a debatable point. Previous work by Appel *et al*. indicated that the proportion of $CD4^+FoxP3^+$ T cells in the peripheral blood of established SpA patients, including 8 subjects with undifferentiated SpA, is not different from that of controls, which is consistent with our results (31). Th17 and Treg cells exert opposite effects on the pathogenesis of autoimmune diseases. An imbalance in the number of Th17 and Treg cells is suggested to be associated with the pathogenesis of SpA (32). We observed that the ratio of Th17 and Treg cells was significantly increased, indicating that the imbalance of Th17 and Treg cells also plays novel role in SAPHO syndrome.

However, this is a study with a limited number of patients. There is still a lack of a specific evaluation tool for SAPHO syndrome, a condition characterised by a large polymorphism of the clinical presentations. Further efforts in larger study populations are requested to address in a more precise fashion the contribution and dynamics of these critical players of the immune response in SAPHO syndrome. The skin distribution and function of NK cells, Th17 cells, and Treg cells also need to be studied to fully understand the immunopathological mechanisms of SAPHO syndrome.

In conclusion, our data suggested that immune inflammation in SAPHO patients may be related to the reduction of NK cells, the increased Th17 cells and Th17/Th1 cells. We suggest that the reduced number of NK cells may lead to instability of immune system and uncontrolled proliferation of effected or pathologically changed cells, such as $CD4^+$T cells, Th17 and Th17/Th1 cells in SAPHO syndrome. As a consequence, it may contribute to the development of autoimmunisation. The lack of increase in numbers of Treg cells lead to the imbalance of Th17 and Treg cells, which could further perpetuate inflammation.

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