reparative process. An MRI study of bone ankylosis in the hands of a cohort of consecutive RA patients could help understand the true frequency and significance of this finding.

Rheumatology key message
- Bone ankylosis of the wrist joints in RA may be a reparative process.

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References

Dysregulation of P2X7 receptor-inflammasome axis in SAPHO syndrome: successful treatment with anakinra

Sir, The syndrome of Synovitis Acne Pustulosis Hyperostosis Osteitis (SAPHO) is a rare condition characterized by a variable combination of osteoarticular and cutaneous manifestations [1]. Although often related to the SpAs, emerging evidence suggests that SAPHO might be a primitive inflammatory osteitis, probably related to polygenic auto-inflammatory disorders [2]. In this report, we describe a dysregulation of extracellular ATP-dependent P2X7-IL1β axis in a case of SAPHO syndrome effectively treated with the IL-1 receptor antagonist (IL-1Ra) anakinra.

A 47-year-old female, was admitted to our unit in July 2007 with a 3-year history of remitting pain and swelling of anterior chest wall (ACW) structures, and a 10-year history of severe palmpoplantar pustulosis (PPP). During adolescence she suffered from acne conglobata. Total white blood cell (WBC) count was 14.5 × 10⁹/l; ESR was 35 mm/h (normal range <20) and CRP was 1.8 mg/dl (normal range <0.6). HLA-B27 antigen was negative. A CT scan of ACW revealed massive osteitis with periostitic and erosive aspects. A diagnosis of SAPHO syndrome was made, and therapy with SSZ (3 g/day) was set up for 6 months without any significant improvement. The patient was further evaluated in February 2008, after appearance of intermittent right knee arthritis. A slight leucocytosis and elevated acute-phase markers were still present (ESR and CRP were 28 mm/h and 1.1 mg/dl, respectively). At that time, the patient also referred low-grade fever and asthenia. Joint SF showed 7800 cells (65% of monocytes). SF cultures for Propionibacterium acnes resulted negative and so did the PCR for 16S ribosomal RNA and lipase genes. Technetium 99m (99mTc) bone scan revealed hypercapta- tion at the manubriosternalis syncondrosis and at the right sternoclavicular joint. As increasing evidence suggests that IL-1β might be involved in chronic inflammatory diseases of unknown origin, at the time of this second hospital admission we investigated whether a dysfunction in the processing and release of this cytokine was present [3].

Peripheral blood mononuclear cells (PBMCs) were purified by Ficoll gradient and IL-1β secretion measured in the presence of stimulators or blockers of the P2X7 receptor, a major activator of the inflammasome complex, and therefore of IL-1β processing and release [4]. Spontaneous IL-1β release from SAPHO as well as healthy control PBMCs was negligible; however, SAPHO PBMCs were significantly more responsive to lipopolysaccharide (LPS) alone or LPS plus the P2X7 agonist benzoyl-ATP (BzATP).

IL-1β release from SAPHO PBMCs averaged 130 (0.15) pg/ml (n = 3) after a 2 h incubation in the presence of LPS, whereas in healthy controls it never exceeded 54 (8.9) pg/ml (n = 12; P < 0.001). Furthermore, IL-1β secretion stimulated by appropriate doses of BzATP reached ~1910 (7.14) pg/ml, compared with 1324 (32.8) pg/ml in healthy control PBMCs (P < 0.05; Fig. 1A). Converging evidence from different laboratories points to the P2X7 receptor as the main activator of IL-1β maturation and release via the inflammasome, whether by endogenously released or exogenously added ATP [5, 6]. The P2X7 blocker oxidized-ATP fully abolished IL-1β secretion triggered by LPS or LPS plus BzATP (Fig. 1A). Real-time PCR and western blot analysis of P2X7 receptor expression revealed a level of expression of the P2X7 receptor about 1.75-fold higher in SAPHO than in healthy control leucocytes (net intensity ratio: 0.26 (0.005) and 0.45 (0.005) for healthy...
and SAPHO P2X7 receptor, respectively; Fig. 1B and C). To understand the molecular basis of the higher P2X7-stimulated IL-1β release in SAPHO PBMCs, we measured the level of expression of the inflammasome constituents NLRP3 and ASC. While NLRP3 expression did not differ, ASC was expressed at higher level in SAPHO than in healthy controls [net intensity ratio: 3.4 (0.12) and 4.54 (0.35) for the expression of ASC in healthy and SAPHO subjects, respectively; Fig. 1D]. Finally, measurement by firefly luciferin-luciferase assay showed that plasma ATP level in SAPHO patient was much higher than that in 13 healthy controls [1689 (11.54) and 1016 (160) nM, respectively, average (S.D.) of three determinations from SAPHO patient and healthy controls].

These findings suggested a possible dysregulation of the IL-1β processing machinery, which prompted us to start off label treatment with anakinra 100 mg/day, in late March 2008 after local ethics committee approval from The Ethical Committee of Azienda Ospedaliero – Universitaria Sant’Anna, Ferrara, and patient’s written informed consent was obtained. By June 2008, the painful osteoarticular symptomatology, the cutaneous lesions and the systemic symptoms had disappeared. Peripheral synovitis at the right knee remitted, and laboratory parameters were within the normal range. A 99mTc bone scintigraphy showed complete resolution of previous uptake abnormality at the manubrium sterni and a considerable reduction of tracer uptake in the right sternoclavicular joint. The dosage of anakinra was then gradually reduced to 100 mg every 2 days and the patient is still symptom free.

In conclusion, we wish to suggest that these findings and response to anakinra should be taken as the criteria to include SAPHO syndrome in the growing family of auto-inflammatory disorders. However, since a positive

Fig. 1 (A) Measurement by ELISA of IL-1β release in the supernatant of SAPHO (closed bars) and healthy control (open bars) PBMCs. Cells were left untreated (control) or primed for 2 h in the presence of 1 μg/ml LPS. Treated PBMCs were also incubated for 30 min with increasing concentrations of BzATP. To block P2X7, PBMCs were incubated for 2 h in the presence of 600 μM oxidized ATP (oATP) and then washed before addition of stimulators. Data from the SAPHO patients are averages (s.e.) of the same experiment, performed in triplicate. Control data are averages (s.e.) of three experiments performed with PBMCs from three different controls. (B–D) Expression of inflammasome components in healthy controls (H) and SAPHO (S) PBMCs. Quantitation of P2X7 transcript was performed by real-time PCR (B). Primers and probes were selected from Applied Biosystems Taqman® Gene Expression Custom Assay (Foster City, CA, USA). Data are averages (s.e.) of expression of P2X7 evaluated in two different controls and in the SAPHO PBMCs. Amplifications were performed in triplicate for each sample. P2X7 (C). NLRP3 and ASC (D) protein expression levels were measured by western blot. For statistical analysis: *P < 0.05, **P < 0.005, ***P < 0.001, vs healthy subjects.
response to anti-TNF-\(\alpha\) agents has also been observed [7, 8] the precise pathogenetic role of IL-1\(\beta\) and TNF-\(\alpha\) is a matter for further investigations.

**Rheumatology key message**

- IL-1 is involved in the pathogenesis of SAPHO syndrome.

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**References**


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**Comment on: Monitoring Achilles enthesitis in ankylosing spondylitis during TNF-\(\alpha\) antagonist therapy: an ultrasound study**

Sir, I read with great interest the recent report by Aydin et al. [1] highlighting the potential role of ultrasound including grey scale and power-Doppler imaging. I would appreciate commenting on some issues raised by the authors.

At baseline, both grey scale and power-Doppler ultrasound features were recorded, which were re-evaluated after 2 months of TNF-\(\alpha\) antagonist therapy. In addition to the outcome measures used by the authors, one should consider the validated Victorian Institute of Sports Assessment-Achilles (VISA-A) questionnaire, which quantifies the pain and activity level with scores ranging from 0 (worse) to 100 (perfect) [2]. It has been applied in a number of publications dealing with Achilles tendinopathy and, thus, might be applicable for patients suffering from AS.

As far as gender is concerned, the authors found that men had higher baseline ultrasound abnormality values than females, albeit similar Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), ESR and CRP levels. Treatment response, however, was similar, but not displayed by the authors. From a microcirculatory perspective, tendon microcirculatory changes between symptomatic females and males suffering from Achilles tendinopathy have been described previously [3]. Although there were similar pain levels on a visual analogue scale [VAS 0–10, \(\ddagger\): 5.3 (2.2) vs \(\ddagger\): 5.4 (2); \(P = 0.864\)], females had a superior tendon oxygenation and reduced post-capillary venous filling pressures than symptomatic males. Notably, treatment response is not necessarily the same for both genders, at least in Achilles tendinopathy, which might be different to AS. As far as painful eccentric training is concerned in Achilles tendinopathy, symptomatic females do not benefit as much as symptomatic males from eccentric training alone over 12 weeks of treatment [4].