



## Brief communication

# The novel cytokine Metrnl/IL-41 is elevated in Psoriatic Arthritis synovium and inducible from both enthesal and synovial fibroblasts



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## ABSTRACT

Meteorin-like(IL-41), is a novel cytokine that is thought to be immunoregulatory and is highly expressed in psoriatic skin. We investigated IL-41 protein expression in synovial tissue in RA(Rheumatoid Arthritis), PsA(Psoriatic Arthritis) and OA(Osteoarthritis) patients and evaluated IL-41 production from healthy entheses samples, as the entheses represent the primary inflammatory site in PsA. IL-41 was measured in synovial fluid from PsA, RA and OA patients. Synovial biopsies were stained for IL-41 by immunohistochemistry. IL-41 was highly expressed in the synovial fluid and synovial tissue of PsA patients (median = 7722 pg/ml) when compared to OA patients (median = 5044 pg/ml). We found that enthesal stromal cells were the dominant producer of IL-41 from the entheses. Moreover, stromal derived IL-41, could be further induced by IL-17A/F and TNF. In conclusion, IL-41 is expressed in PsA synovium and is present and inducible at the entheses. Its functional effect in psoriatic inflammation remains to be fully elucidated.

## 1. Introduction

IL-41, also known as METRNL or Meteorin-like, is a novel immunoregulatory cytokine. IL-41 shares 40% sequence homology with another protein, METRN (Meteorin). METRN was discovered in 2004 and has a role as a neurotrophic factor [1]. Meteorin-like was initially tentatively named IL-39, but this number, has now been allocated to an unrelated IL-12 family member cytokine [2]. Unlike METRN, which is expressed in the CNS, IL-41 has been shown to be highly expressed in numerous human tissues including the skin and mucosa [3]. The cellular sources of IL-41, the cells it targets or the signalling pathways it activates are still being deciphered. Initial mouse studies revealed that IL-41 is induced in muscles and plays a role in attenuating insulin resistance and is also involved in the induction of white adipose browning [4–7]. Away from muscle, IL-41 is also emerging as crucial immunoregulatory cytokine. Studies of the IL-41 KO mouse have provided several interesting observations. IL-41 KO mice, appear to be immuno-

compromised, have reduced serum Igs, develop spontaneous inflammatory lesions in uterus, kidney and liver, and also have increased mortality to septicaemia [8].

Psoriatic arthritis is a chronic seronegative disease that is a member of the wider Spondyloarthritis (SpA) family, which also includes Ankylosing Spondylitis and reactive arthritis [9]. SpA diseases are associated with inflammatory lesions at the entheses, the attachment site of tendon and ligaments to bone [10]. MRI studies and animal models suggest enthesitis is the primary lesion in SpA [11]. The entheses contains numerous immune cell populations and stroma, all of which could potentially be a cellular source of IL-41 [12]. Psoriasis is genetically linked to PsA, with up to a third of patients developing PsA [13]. IL-41 was recently found to be highly elevated in psoriasis skin lesions, when compared to other inflammatory skin diseases such as atopic dermatitis, and cutaneous lupus erythematosus [10]. However, no study to date has considered IL-41 in the allied psoriatic arthritis.

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**Table 1**  
Demographics of patients used in the study.

Gender	Age	Disease
F	54	RA
F	88	RA
M	59	RA
F	77	RA
F	75	RA
M	50	RA
M	60	RA
M	63	RA
M	60	RA
F	66	RA
M	52	PsA
M	49	PsA
M	63	PsA
F	59	PsA
M	55	PsA
M	70	PsA
F	45	PsA
F	34	PsA
M	44	PsA
M	34	PsA
M	49	PsA
M	65	PsA
M	51	OA
M	69	OA
F	88	OA
F	71	OA
M	74	OA
M	76	OA
M	25	OA
M	78	OA
M	67	OA
F	79	OA
F	58	OA
F	78	OA
M	74	OA
F	75	OA
M	56	OA
F	42	GOUT
M	70	GOUT
M	63	GOUT

## 2. Materials and methods

### 2.1. Synovial fluid and tissue biopsies

All samples were collected following informed written consent with relevant ethical approval (Medical Research Ethics Committee:04/Q1205/65; Human Research Ethics Committee, Southern Adelaide Local Health Network; 396.10, 199.10). All patients were treatment naïve. For synovial fluid, all cells and debris were pelleted at 16,000 RCF for 5 mins. The cell free synovial fluid was stored at  $-80^{\circ}\text{C}$  until use. IL-41 was measured by ELISA (R&D systems, Abingdon, UK), according to the manufacturer's protocol. Reproducibility of synovial fluid results were confirmed by triplicate testing, with  $< 10\%$  error.

### 2.2. Immunohistochemistry

Paraffin embedded synovial biopsies were prepared for histologic analysis using standard protocols and subsequently stained with rabbit anti-human METRNL (Novus, Centennial, Colorado, United States) (1:50) and horseradish peroxidase stain (Dako, Santa Clara, California, United States) and DAB (1:50). Slides were then counterstained with haematoxylin.

### 2.3. Samples and cell culture

Normal spinous process entheses was obtained from patients who

were undergoing spinal decompression or surgery for scoliosis correction of thoracic or lumbar vertebrae using previously described methods [12]. The study protocol of the current investigation was approved by North West - Greater Manchester West Research Ethics Committee (16/NW/0797). Enthesis samples were digested with collagenase as previously described [12]. Enthesis mononuclear cells (EMCs) were subsequently isolated by Lymphoprep (STEMCELL Technologies, Vancouver, British Columbia, Canada) density gradient centrifugation and cultured in RPMI containing 10% FCS and 1% Penicillin Streptomycin. Enthesis stromal cells were isolated by digesting entheses samples as before, digested entheses cells were subsequently placed in T75 flasks in StemMACS™ MSC Expansion Media (Miltenyi Biotec, Bergisch Gladbach, Germany) containing 1% Penicillin Streptomycin. Non-adherent cells were removed during media changes. Stromal cells were allowed to reach confluency and passaged for further downstream applications.

Synovial Fibroblasts were isolated from OA patients as previously described [14]. All fibroblast cells were cultured in DMEM media (GIBCO, Life Technologies, Paisley UK) containing 10% FCS and 1% Penicillin Streptomycin. PBMCs were isolated from healthy volunteers using lymphoprep, and cultured using the same method as EMCs described above.

$1 \times 10^5$  EMCs, Enthesis stromal cells, synovial fibroblasts or PBMCs were plated in 96 well plates in relevant growth media for 24 h. For cytokine stimulation, entheses stromal cells or synovial fibroblasts at a concentration of  $1 \times 10^5$  were plated out in 96 well plates and stimulated with combinations of TNF (10 ng/ml), IL-17A (100 ng/ml) or IL-17F (100 ng/ml) for 24 h. All cytokine proteins were from Peprotech (Rocky Hill, New Jersey, United States). IL-41 was subsequently measured by ELISA as described previously.

### 2.4. Receptor analysis by flow cytometry

Enthesis stromal cells were incubated with 10% mouse serum and 1% IgG and subsequently stained with either Alexa Fluor 647 mouse anti-human IL-17AR or APC mouse anti-human TNFR1 (both Biolegend, San Diego, CA, USA) or relevant isotype control (BD Biosciences, San Jose, CA, USA). Cells were analysed using the Cytotflex S (Beckman Coulter, San Diego, CA) and data further processed using FlowJo software (Tree Star Software, San Carlos, California, USA).

### 2.5. Patient demographics

A table containing patient gender, age and disease diagnosis can be found in the supplementary materials (See Table 1).

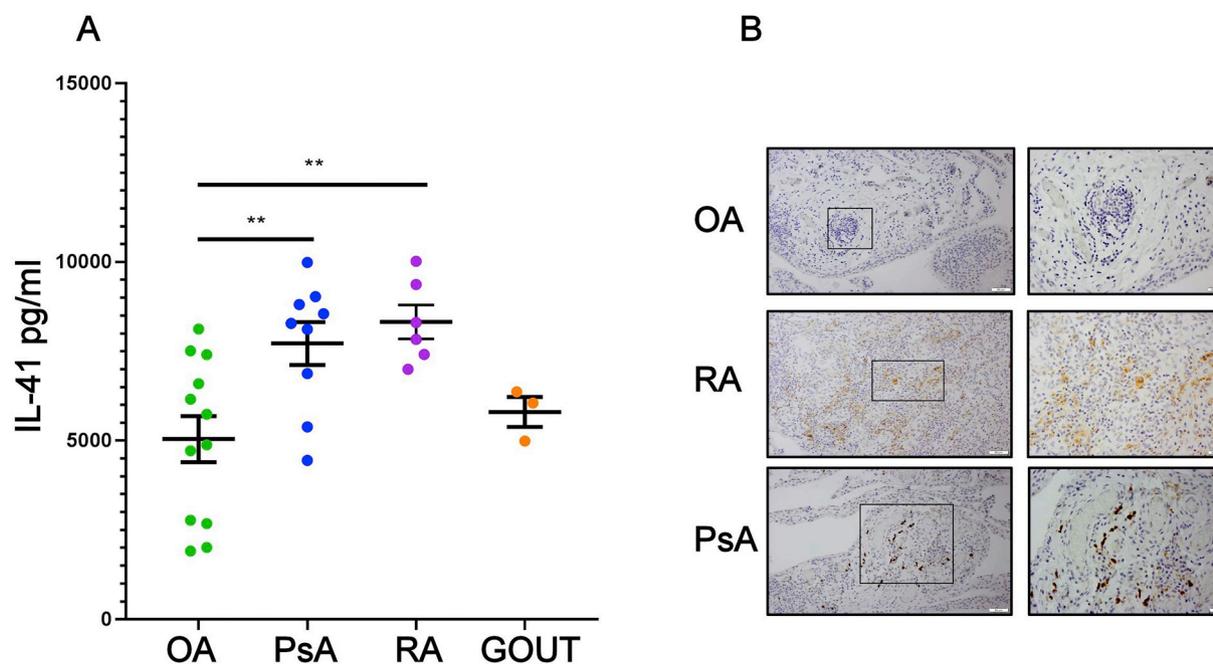
## 3. Results

### 3.1. IL-41 is elevated in the synovium and synovial fluid of PsA patients

IL-41 was detectable in the synovial fluid samples from all disease types (Fig. 1A). IL-41 was significantly higher in PsA (median 7722 pg/ml,  $P = .0011$ ) and RA (median 8325 pg/ml,  $P = .0003$ ) when compared to OA (median 5044 pg/ml). In gout synovial fluid, IL-41 levels were higher (median 5804 pg/ml) when compared to OA, but this did not achieve statistical significance. IHC staining of synovial biopsies showed strong positive staining in both RA and PsA (Fig. 2B).

### 3.2. Stromal cells are the primary source of IL-41 at the entheses, and this is enhanced by synergy between TNF and IL-17A/F stimulation

Given that the human entheses is regarded as the primary inflammatory site in PsA, we investigated IL-41 production by ELISA from healthy human entheses samples. Minimal IL-41 was produced from entheses mononuclear cells (EMCs) or PBMCs. (Fig. 2A). However,



**Fig. 1.** A) IL-41 levels were measured in the synovial fluid of OA, PsA, RA and gout patients by ELISA. OA  $n = 12$ , PsA  $n = 9$ , RA  $n = 6$ , Gout  $n = 3$ . B) Synovial biopsies from OA, RA or PsA patients were stained for IL-41 ( $n = 3$ ). Positive cells are stained brown. Right hand panel is enlarged image of indicated area. Mann Whitney  $U$  test ( $*p < .05$ ,  $**p < .01$ , and  $***p < .001$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enthesial stromal cells produced significantly more IL-41 than EMCs. Other stromal cells tested, (synovial fibroblasts) also produced comparable amounts of IL-41 to enthesis stromal cells.

Following the discovery that stromal cells were the dominant producer of IL-41 at the enthesis, we investigated whether stromal IL-41 could be induced by disease relevant mediators. TNF, IL-17A and IL-17F, failed to induce a significant increase in IL-41 when used alone, however, TNF with IL-17A or IL-17F resulted in a significant increase in IL-41 production (Fig. 2B). This finding was also replicated when stimulating synovial fibroblasts (Fig. 2C). As research into human enthesis stromal cells is in its infancy, we also sought to confirm the presence of IL-17 and TNF receptors on these cells. Flow cytometry analysis confirmed that enthesis stromal cells express both TNFR1 and IL17RA (Fig. 2D&E).

#### 4. Discussion

IL-41 is a novel immunoregulatory cytokine, which has been implicated in both adipose function and insulin resistance [4]. The IL-41 KO mouse has also revealed several fascinating features. IL-41 KO mice are generally immune-compromised, develop inflammatory lesions, in the uterus, kidneys and liver, and have increased susceptibility to septicemia [8].

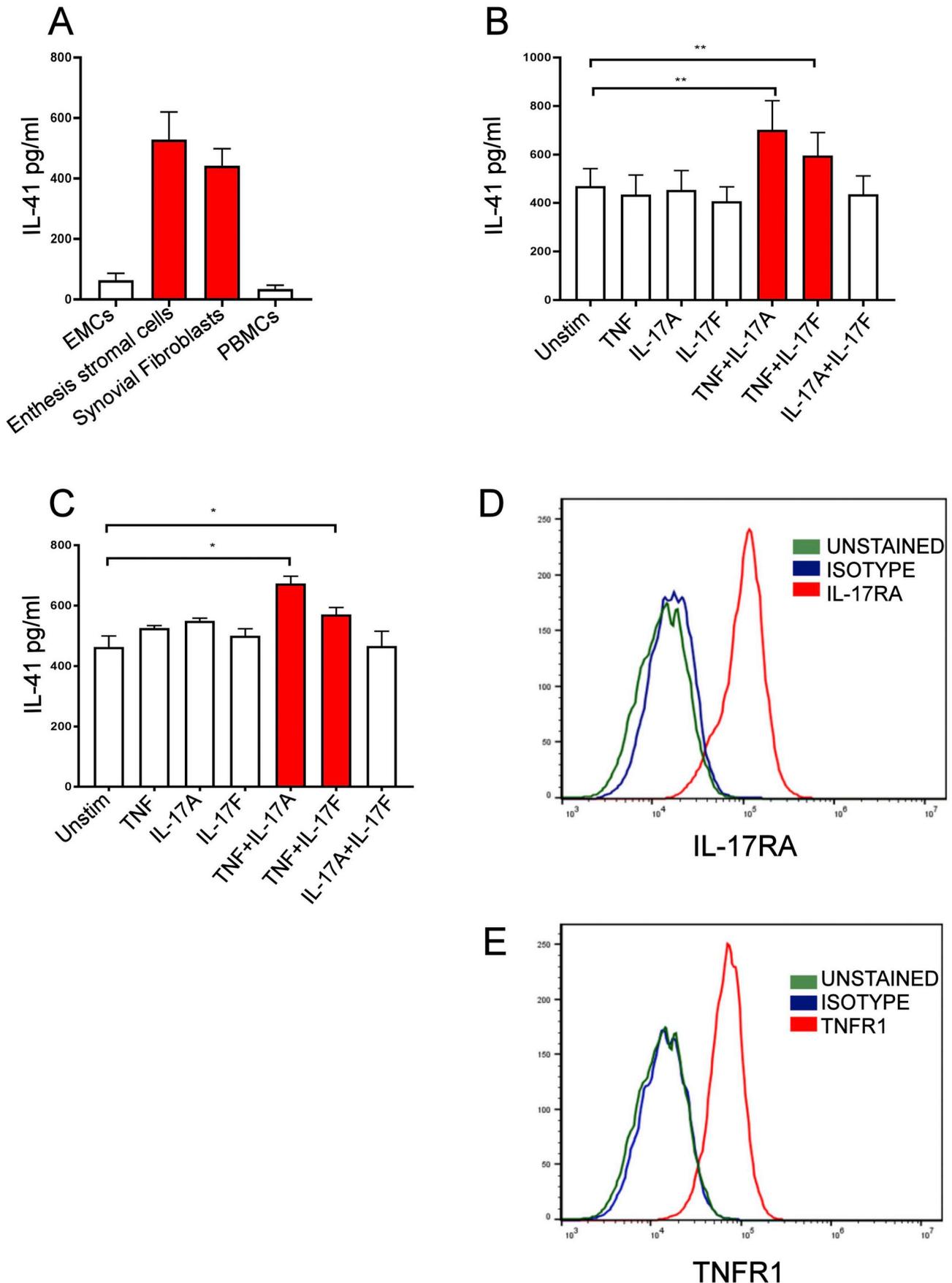
We report that IL-41 is detectable in the synovial fluid of OA, PsA, RA and gout patients, however, highest expression was detected in PsA and RA patients. Synovial biopsies also mirror this finding with both RA and PsA staining strongly for IL-41. Whilst gout synovial fluid levels of IL-41 trended upwards when compared to OA, this could be due to associated flares with this disease [15]. Further work is required to confirm the specific cellular lineage source of IL-41 in the PsA synovium.

Enthesitis is regarded as the primary inflammatory lesion within PsA [10]. The human healthy enthesis contains a range of resident immune cell subsets and stromal cells [12]. We report that the primary source of IL-41 at the normal enthesis is enthesial stromal cells, when compared to whole enthesis mononuclear cells. We also report that other stromal cells, such as synovial fibroblasts produce similar amounts of IL-41. Thus synovial fibroblasts represent a likely candidate for the cellular source of IL-41 in PsA and RA synovium. Our data is in agreement with a previous paper which showed at mRNA level, IL-41 is most highly expressed by skin fibroblasts when compared to PBMCs and keratinocytes [3]. Therapeutic targeting of the inflammatory cytokines TNF or IL-17A has proved efficacious in SpA and there is emerging data regarding IL-17F [16–18]. We report that IL-17A/F and TNF synergistically induce IL-41 from enthesial stromal cells and synovial fibroblasts. The synergistic effect of TNF and IL-17A in activating stromal cells has previously been reported [19]. The exact mechanism behind IL-17 and TNF synergy has recently been deciphered at a pathway level [20].

In conclusion, we report that IL-41 is increased in the synovium of PsA patients and that this novel cytokine is also induced from enthesis stromal cells. The precise role of IL-41 in PsA immunopathogenesis requires further study.

#### Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



**Fig. 2.** A) EMCS (entheses mononuclear cells), entheses stromal cells, synovial fibroblasts or PBMCs were cultured for 24 h and supernatant was analysed for IL-41 secretion ( $n = 5$ ) Enthesis stromal (B) cells ( $n = 8$ ) and synovial fibroblast (C) ( $n = 3$ ) were stimulated with alone, or in combinations of TNF, IL-17A or IL-17F for 24 h and the supernatant was analysed for IL-41 secretion. Paired *t*-test. (\* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$  and \*\*\*\* $p < .0001$ ). IL-17 receptor (D) and TNF receptor (E) expression was confirmed on entheses stromal cells by flow cytometry.

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