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

Keywords: Cytokine, Psoriatic Arthritis, Spondyloarthritis, Enthesitis

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 enthesis samples, as the enthesis represent the primary inflammatory lesion 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=8118pg/ml) when compared to OA patients (median=4720pg/ml). We found that entheseal stromal cells were the dominant producer of IL-41 from the enthesis. 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 enthesis. 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]. Metoerin-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 enthesis, 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 enthesis 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 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 erythematous[10]. However, no study to date has considered IL-41 in the allied psoriatic arthritis.

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°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 enthesis 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 enthesis samples as before, digested enthesis cells were subsequently placed in T75 flasks in StemMACS^{TM} 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[15]. 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.

1x10^5 EMCs, or Enthesis stromal cells, synovial fibroblasts or PBMCs were plated in 96 well plates in relevant growth media for 24 hr. For cytokine stimulation, enthesis stromal cells or synovial fibroblasts at a concentration of 1x10^5 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 hr. All cytokine proteins were from Peprotech (Rocky Hill, New Jersey, United States). IL-41 was subsequently measured by ELISA as described previously.

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 Cytoflex S (Beckman Coulter, San Diego, CA) and data further processed using FlowJo software (Tree Star Software, San Carlos, California, USA).

Patient Demographics

A table containing patient gender, age and disease diagnosis can be found in the supplementary materials.

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 (figure 1A). IL-41 was significantly higher in PsA (median 8118 pg/ml, P=0.0011) and RA (median 8057 pg/ml, P=0.0003) when compared to OA (median 4720 pg/ml). In gout synovial fluid, IL-41 levels were higher (median 6054 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 (figure 2B).

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

Given that the human enthesis is regarded as the primary inflammatory lesion in PsA, we investigated IL-41 production by ELISA from healthy human enthesis samples. Minimal IL-41 was produced from enthesis mononuclear cells (EMCs) or PBMCs. (Figure 2A). However, entheseal 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 (Figure 2B). This finding was also replicated when stimulating synovial fibroblasts (Figure 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 (Figure 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 septicaemia[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[16]. Further work is required to confirm the specific cellular lineage source of IL-41 in the PsA synovium.

The 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 entheseal 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 [17-19]. We report that IL-17A/F and TNF synergistically induce IL-41 from entheseal stromal cells and synovial fibroblasts. The synergistic effect of TNF and IL-17A in activating stromal cells has previously been reported [20]. The exact mechanism behind IL-17 and TNF synergy has recently been deciphered at a pathway level[21].

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.

Acknowledgements

This article/paper/report presents independent research funded/supported by the National Institute for Health Research (NIHR) Leeds Biomedical Research Centre (BRC). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

Conflict of 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.

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Figure 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 < 0.05, **p < 0.01, and ***p < 0.001).

Figure 2. A) EMCS (enthesis mononuclear cells), enthesis stromal cells, synovial fibroblasts or PBMCS were cultured for 24 hr 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 hr and the supernatant was analysed for IL-41 secretion. Paired t test. (*p < 0.05, **p < 0.01, ***p < 0.001 and **** p < 0.0001). IL-17 receptor (D) and TNF receptor (E) expression was confirmed on enthesis stromal cells by flow cytometry.