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Th17 gene expression in psoriatic arthritis synovial fluid and peripheral blood compared to osteoarthritis and cutaneous psoriasis

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ABSTRACT

Objective. *The IL-23/IL-17 axis is central to the pathogenesis of psoriatic arthritis (PsA). We aimed to identify Th17 signalling genes that are dysregulated in synovial fluid of PsA compared to osteoarthritis (OA) patients and to determine if differences in peripheral blood can distinguish PsA from psoriasis patients and controls.*

Methods. *Synovial fluid cells (SFCs) from 14 PsA and 9 OA patients were obtained and stored in TRIzol reagent. RNA was isolated by phenol-chloroform extraction and purified with RNeasy miniprep kits. Total RNA was extracted from PAXgene whole blood from 20 PsA, 20 psoriasis without arthritis (PsC) and 11 controls. Quantitative RT-PCR arrays were used to profile expression of 84 genes related to the Th17 regulatory network. Fold change differences were compared by Mann-Whitney U-test with false discovery rate (FDR) correction (FDR<0.05).*

Results. *In PsA compared to OA SFCs, a total of 33 genes were up-regulated and 27 genes were down-regulated. Signalling molecules (such as* STAT3, FOXP3*) were highly expressed in PsA SFCs, while cytokines (such as* IL17F, IL6*) were more predominant in OA SFCs after non-supervised hierarchal clustering. Nine genes* (MMP3, CCL1, IL17C, CCL20, IL17F, IL3, CXCL5, IL6 *and* CX3CL1*) had concordant expression in SFCs and in peripheral blood cells (PBCs) of PsA compared to PsC and/or controls.*

Conclusion. *We identified expression differences in Th17 signalling genes in PsA compared to OA SFCs, with an elevation of signalling molecules and attenuation of cytokine expression in PsA. A subset of genes was concordant in PBCs; these may thus be potential biomarkers of PsA.*

Introduction

Psoriasis is a chronic inflammatory skin condition which is prevalent in 2–3% of the population. Psoriatic arthritis (PsA), a seronegative inflammatory arthritis, develops in up to thirty percent of psoriasis patients (1, 2). A combination of genetic, environmental and immunological factors likely contributes to the progression of psoriasis to PsA. The exact mechanisms involved in the development of PsA have yet to be clearly defined.

The IL-23/IL-17 axis has been established as central to the pathogenesis of psoriasis and PsA (3, 4). Th17 cells are a subset of $CD4^+$ T cells which produce IL-17, IL-21 and IL-22. The initial differentiation of Th17 cells is dependent on IL-6 and TGF-β, while the maintenance of these cells has been linked to IL-23, primarily produced by activated dendritic cells and macrophages (5). $CD8^+$ T cells and innate immune cells, such as γδT cells, NK cells and mast cells are other sources of IL-17 in psoriasis and PsA. Interaction of IL-17 with its receptor results in activation of several signalling pathways such as signal transducer and activator of transcription (STAT), and the production of pro-inflammatory cytokines (IL-1β, IL-6, TNFα, CCL2), antimicrobial peptides (β-defensin), and matrix metalloproteinases (6).

In this study, we sought to further characterise the gene expression signature of the IL-23/IL-17 axis in PsA. Our objective was to determine if gene expression is dysregulated in the synovial fluid of patients with PsA as compared to osteoarthritis (OA). We also compared the expression of these genes in peripheral blood of subjects with PsA compared to psoriasis without arthritis (PsC) to identify genes which are altered by joint inflammation in PsA and are potential blood transcriptomic biomarkers.

Materials and methods

Study subjects

Fourteen PsA patients with synovial fluid samples from the knee joint were identified from a cohort of patients followed prospectively from 2004 at the University of Toronto PsA clinic. OA patients (n=9) were used as controls due to the lack of availability of normal synovial samples. Synovial fluid was obtained during routine joint aspirations or knee replacement surgery at Toronto Western Hospital. A separate cohort of twenty PsA patients not receiving treatment with biologic agents was recruited for peripheral blood RNA analysis. All PsA patients were diag-

Table I. Demographics of study subjects.

	Synovial fuid			Peripheral blood			
	PsA (n=14)	OA (n=9)	*p*-value[#]	PsA (n=20)	PsC (n=20)	Controls (n=11)	*p*-value[#]
Females (%)	4 (29%)	5 (71%)	0.06	11 (55%)	10 (50%)	6 (55%)	0.97
Age[a]	48.8 (16.6)	65.3 (16.2)	0.04	48.1 (10.4)	44.4 (11.8)	42.3 (16.1)	0.42
Age of diagnosis of psoriasis[b]	25.0 (19.3-39.3)	--	--	24.0 (17.5-30.8)	25.0 (16.5-32.3)	--	0.79
Age of diagnosis of PsA[b]	34.0 (25.8-47.0)	--	--	29.0 (22.3-38.0)	--	--	--
Duration of psoriasis[a]	22.4 (17.1)	--	--	23.2 (11.5)	19.8 (14.1)	--	0.39
Duration of PsA[a]	13.7 (10.2)	--	--	16.2 (7.9)	--	--	--
PASI[b]	1.8 (1.1-3.0)	--	--	4.7 (2.7-6.4)	3.8 (2.4-6.1)	--	0.74
Number of swollen joints[b,c]	0 (0-1.0)	--	--	3.0 (2.0-5.0)	--	--	--
Number of tender joints[b,c]	0 (0-1.5)	--	--	4.0 (1.0-8.8)	--	--	--

[#]One-way ANOVA, Mann Whitney *U*-test or Student's *t*-test (continuous variables); Pearson's chi square test (categorical variables). [a]Mean (standard deviation); [b]Median (Interquartile range); [c] Tender joints were determined clinically in 68 joints, swollen joints in 66 (excluding hips). PASI: psoriasis area and severity index.

Table II. Th17 signalling genes with differential expression in SFCs obtained from patients with PsA compared to patients with OA (FDR<0.05; minimum fold change ± 2) that were also significantly altered in PBCs of PsA patients (n=20) compared to PsC patients (n=20) or controls (n=11).

Symbol	Description	PsA *vs*. OA SFCs		PsA *vs*. Controls PBCs		PsA *vs*. PsC PBCs	
		FC	*p*-value	FC	*p*-value	FC	*p*-value
IL8	Interleukin 8	10.8	0.002	0.92	0.990	0.46	0.001
ISG20	Interferon stimulated exonuclease gene 20	8.56	0.004	0.72	0.001	0.66	0.008
IL6R	Interleukin 6 receptor	3.42	0.005	0.54	0.001	1.18	0.530
SYK	Spleen associated tyrosine kinase	3.39	0.002	0.46	0.0006	0.57	0.004
IL12RB1	Interleukin 12 receptor subunit beta 1	3.12	0.0002	0.62	0.003	2.09	0.365
CD8A	CD8a molecule	3.12	0.013	0.55	0.010	1.46	0.375
CCL7	C-C motif chemokine ligand 7	2.91	0.09	1.03	1.000	0.73	0.008
NFKB1	Nuclear factor kappa B subunit 1	2.65	0.0006	0.72	0.004	1.02	0.581
ICAM1	Intercellular adhesion molecule 1	2.63	0.003	0.61	0.004	1.24	0.591
JAK1	Janus kinase 1	2.56	0.001	0.69	0.001	0.89	0.779
CXCL2	C-X-C motif chemokine ligand 2	2.40	0.046	0.87	1.000	0.44	0.002
STAT3	Signal transducer and activator of transcription 3	2.39	0.004	0.50	0.0008	0.75	0.022
FOXP3	Forkhead box P3	2.38	0.12	0.56	0.0007	1.37	0.791
STAT6	Signal transducer and activator of transcription 6	2.32	0.007	0.69	0.007	0.48	0.035
TGFB1	Transforming growth factor beta 1	2.21	0.01	0.50	0.0006	0.66	0.109
TBX21	T-box 21	2.16	0.046	0.32	0.0006	0.82	0.452
IL17C	*Interleukin 17C*	*0.28*	*0.009*	*0.46*	*0.016*	*0.53*	*0.009*
CCL20	*C-C motif chemokine ligand 20*	*0.25*	*0.003*	1.00	1.000	*0.74*	*0.013*
IL6	*Interleukin 6*	*0.20*	*0.007*	0.91	0.902	*0.86*	*0.044*
CXCL5	*C-X-C motif chemokine ligand 5*	*0.18*	*0.004*	*0.43*	*0.016*	*0.68*	*0.042*
IL17F	*Interleukin 17F*	*0.09*	*0.00005*	1.11	0.790	*0.70*	*0.014*
IL3	*Interleukin 3*	*0.08*	*0.00001*	1.22	1.000	*0.83*	*0.021*
CCL1	*C-C motif chemokine ligand 1*	*0.08*	*0.000005*	0.84	0.828	*0.82*	*0.008*
MMP3	*Matrix metallopeptidase 3*	*0.05*	*0.001*	0.94	1.000	*0.77*	*0.001*
CX3CL1	*C-X3-C motif chemokine ligand 1*	*0.05*	*0.0006*	1.06	1.000	*0.78*	*0.046*

Concordant results between SFCs and PBCs are in italics. FC: Fold change; FDR: False discovery rate.

nosed by a rheumatologist and satisfied CASPAR classification criteria (7). Twenty patients with psoriasis without arthritis (PsC) with plaque psoriasis and not undergoing treatment were recruited from the psoriasis cohort at Toronto Western Hospital. PsC patients were diagnosed by a dermatologist and examined by a rheumatologist to exclude PsA. PsA and PsC patients were matched for psoriasis duration and for skin severity using the psoriasis area severity index (PASI). Eleven healthy volunteers were recruited as controls. All subjects were Caucasian and were matched for age and sex. This study was approved by the University Health Network Research Ethics Board according to principles of the Declaration of Helsinki and all participants provided written consent.

RNA extraction and gene expression analysis

RNA from synovial fluid cells (SFCs) was stored in TRIzol reagent, extracted using phenol-chloroform and purified with RNeasy miniprep kits. Blood RNA was collected in PAXgene tubes and extracted using the PAXgene Blood RNA Kit (Life Technologies, USA). RNA was compared using the human Th17 Response PCR Array (SABiosciences, USA) which profiles the expression of 84 genes related to the Th17 regulatory network. Gene expression differences were determined by the Mann-Whitney *U*-test with a false discovery rate (FDR) correction. In SFCs, genes with a mini-

mum ± 2-fold change difference and FDR corrected $p<0.05$ were accepted as significant. Next, all differentially expressed genes in peripheral blood cells (PBCs) between PsA patients and controls or PsC patients were identified (FDR corrected $p<0.05$ accepted as significant).

Results

Demographics

A summary of the demographics and clinical characteristics of study subjects is shown in Table I. PsA patients with synovial fluid samples were significantly younger than patients with OA ($p=0.04$, Student's *t*-test). No significant differences in clinical or demographic characteristics were found for patients included in the peripheral blood analysis.

Differential gene expression in synovial fluid and peripheral blood

In PsA compared to OA SFCs, a total of 33 genes were up-regulated and 27 genes were down-regulated. In PBCs, 28 and 22 genes were differentially expressed in PsA compared to controls and PsC patients, respectively. Among these genes, 15 genes in PsA compared to controls and 18 genes in PsA compared to PsC PBCs overlapped with expression in SFCs and are listed in Table II. Nine genes that were differentially expressed in PsA SFCs (compared to OA) and PsA PBCs (compared to PsC) showed concordant fold changes (*MMP3, CCL1, IL17C, CCL20, IL17F, IL3, CXCL5, IL6* and *CX3CL1*). Two of these genes (*IL17C* and *CXCL5*) also had concordant fold changes in PsA SFCs (compared to OA) as in PsA PBCs compared to controls.

Hierarchal clustering of gene expression in synovial fluid cells

To better visualise the Th17 gene expression differences between PsA and OA SFCs, we performed non-supervised hierarchal clustering to generate a heat map of these results (Fig. 1). Distinct clusters of low and high expressing genes were formed and PsA and OA patients tended to cluster separately. Signalling molecules and transcription factors were highly expressed in PsA SFCs, while cytokines were more predominant in OA SFCs.

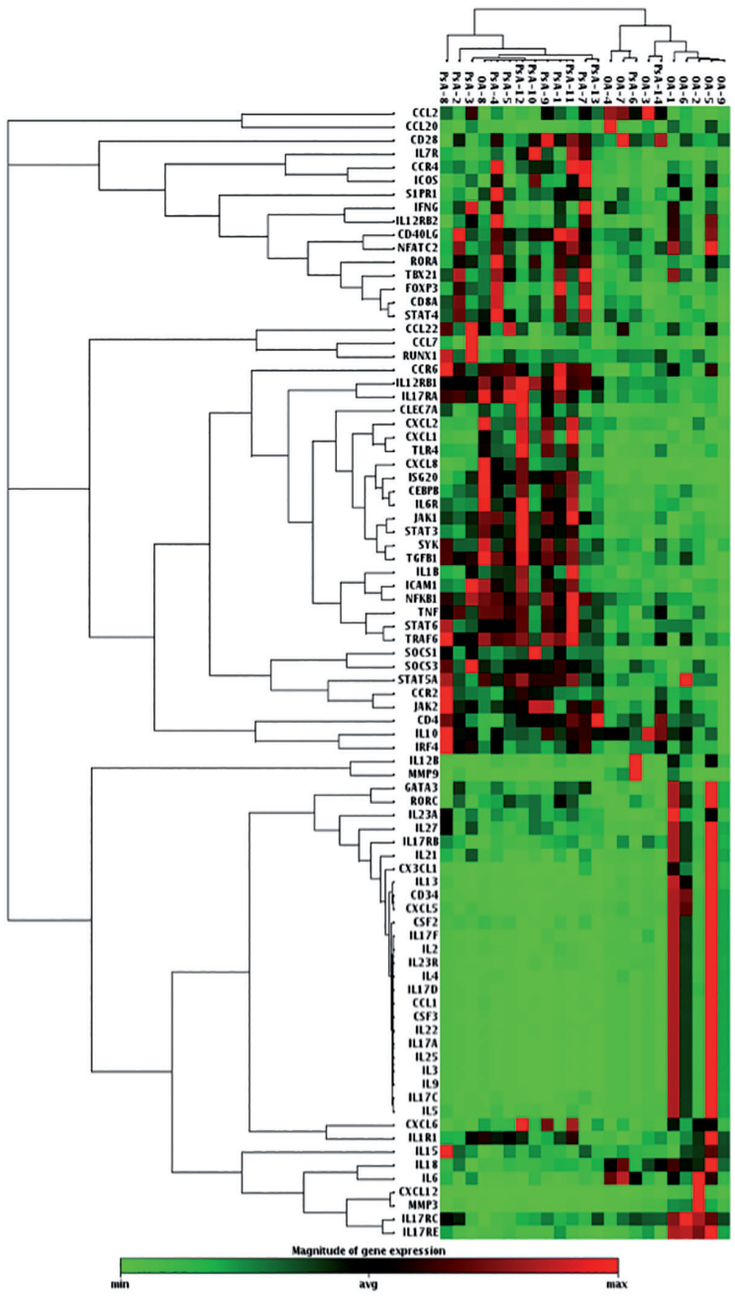

Fig. 1. Heat map of non-supervised hierarchal clustering of gene expression results from PsA and OA SFCs. Columns correspond to individual patient samples and rows correspond to genes.

Discussion

The discovery of Th17 cells in 2007 has significantly advanced our understanding of several disease pathologies, including multiple sclerosis, psoriasis and Crohn's disease. Currently, several groups have reported evidence for the involvement of the IL-17/IL-23 axis in psoriasis and PsA; however, gene expression studies have primarily focused on synovial tissue in PsA (8, 9). We sought to identify genomic differences in SFCs that were also present in PBCs in PsA. We found 60 genes from the Th17 regulatory network that were dysregulated in SFCs of PsA compared to OA patients and observed distinct clusters in Th17 genes between these two cohorts. Nine of these genes (*MMP3, CCL1, IL17C, CCL20, IL17F, IL3, CXCL5, IL6* and *CX3CL1*) also had concordant fold change differences in PBCs of PsA *versus* PsC patients and/or healthy controls.

A strong up-regulation of Th17 signalling genes was identified in SFCs of PsA patients. *IL-8*, the top up-regulated gene, is induced by IL-17 and is a strong chemoattractant for neutrophils to sites of inflammation (10). *TGFB1* and *STAT3* are involved in differentiation of naïve $CD4^+$ T cells into a Th17 lineage. Activation of *SYK*, a tyrosine kinase involved in transmitting signals from activated T- and B-cell receptors, leads to downstream NF-κB signalling and promotes the secretion of IL-23 (11). In contrast, an attenuation of cytokine expression was observed in SFCs of PsA compared to OA patients, which was also evident through hierarchal clustering. These results were not completely surprising. Although studies have reported increased IL-17-producing cells in circulation and within synovial fluid of PsA patients (12), this is not reflected in the expression of proteins such as IL-17 and IL-23 in serum and synovial fluid (13). Also, a reduction in IL-17-producing cells and IL-17 related gene expression has been shown in PsA synovium as compared to skin (8, 12); suggesting that IL-17 may be more involved in skin as compared to joint pathology in psoriatic disease.

Early diagnosis and treatment of PsA is especially important as the extent of joint disease at presentation can predict the progression of joint destruction and radiological damage (14). The identification of genetic components which distinguish those with PsA from the general population and patients with PsC may help develop tools for physicians to better diagnose PsA. Since peripheral blood is easily obtained, a clinical test could be developed to measure these biomarkers to aid physicians. We identified nine genes which had fold changes that were concordant between SFCs and PBCs of PsA compared to PsC patients. Two of these genes (*IL17C* and *CXCL5*) also had concordant fold changes with PsA as compared to control PBCs. These genes have the potential use as transcriptomic biomarkers of PsA, possibly in combination with other genetic markers or polymorphisms that have previously been identified in PsA. *CXCL5* has been identified as a possible transcriptomic biomarker in other diseases such as sepsis (15).

Limitations to the present study stem primarily from cellular heterogeneity in synovial fluid and peripheral blood, dilution of gene expression levels in PBCs, and the possibility of different proportions of cell subtypes between samples. Together, these factors may have resulted in small effect sizes and fold changes prone to reverse direction in PBCs. This might explain why some genes did not significantly differ in PBCs, or were significant but showed an opposite directionality of change as SFCs. Additionally, use of medications could not be completely excluded in these patients. A significant effect on expression levels was not observed in PsA patients receiving biologics or disease-modifying anti-rheumatic drugs in SFCs (5/14 patients) or in PBCs (14/19 patients, data not shown).

This study also had several strengths. Previous gene expression studies in PBCs compared PsA patients to healthy controls or patients with other inflammatory conditions such as systemic lupus erythematosus or rheumatoid arthritis (16, 17). With well-phenotyped cohorts of PsA and PsC patients, we could determine if gene expression differences identified in PsA SFCs were also detected as transcriptomic markers that are specific to joint inflammation in PsA PBCs. Also, the use of targeted arrays reinforced the involvement of the IL-17/IL-23 axis in PsA pathogenesis.

In conclusion, we identified a dysregulation of genes from the IL-17/IL-23 axis in PsA as compared to OA SFCs, with an elevation of signalling molecules and attenuation of cytokine expression. A subset of these genes was also differentially expressed in PBCs of PsA compared to both PsC and control subjects, potentiating their use as transcriptomic biomarkers of PsA. Further studies are necessary to elucidate how expression of Th17 genes is altered over the transition from psoriasis to PsA.

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