absence of fibrin deposition in $Fg^{-/-}$ mice was accompanied by significantly lower synovial inflammation, chondro-synovial adhesion and cartilage damage than in WT mice. Chondro-synovial adhesion correlated with cartilage damage in the WT and led to apparent mechanical stripping of the superficial cartilage, whilst this phenomenon was not observed in the $Fg^{-/-}$ mice. *In vitro*, autologous RA synoviocytes adhered to cartilage explants exclusively in the presence of fibrin deposition. Fibrinogen chains were not expressed by primary chondrocytes, indicating passive deposition from synovial fluid or tissue. In human RA cartilage explants, we found colocalization and a significant positive correlation between fibrin and calcific deposits. Fibrinogen caused exacerbated calcification in CPP-treated primary murine chondrocytes and induction of genes involved in chondrocyte calcification (*Pc1*, *Pit1*). Cartilage-oligomeric matrix protein (*Comp*) gene was also highly induced suggesting a pro-catabolic role of fibrinogen.

Conclusion: Fibrin deposition is an active trigger of cartilage degeneration in RA via induction of chondro-synovial adhesion (mechanical aspect) and induction of calcification (catabolic aspect). Newer therapeutic approaches may not merely focus on fibrinolysis but protect cartilage from fibrin-induced adhesion or calcification e.g. by fibrin-targeted immunotherapy.

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AB0093

PORPHYROMONAS GINGIVALIS - PERIODONTAL PATHOGEN WITH POTENTIAL ROLL IN RHEUMATOID ARTHRITIS

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Background: In recent literature, a strong association between periodontal disease (PD) and rheumatoid arthritis (RA) has been reported. PD is a common, progressive inflammatory disease, initiated by a bacterial infection that engages the supporting structures of the teeth and leads to tooth loss. A number of common features have been identified between PD and RA. One of the most important associations is the process of citrullination, which is caused by the production of PG specific enzyme so called Porphyromonas Gingivalis Peptidyl Deminase (PPAD)

Objectives: The aim of the study was to show the incidence of PG in RA patients, and to compare it with patients with osteoarthritis (OA) and healthy controls (HC) and to evaluate the possible correlation between the presence of PG in patients with RA and the positivity of anti CCP and anti MCV antibodies in RA patients.

Methods: The study included 30 patients with RA which fulfilled RA classification criteria from 2010, 26 patients with osteoarthritis (OA) and 24 healthy controls. All participants were genetically analyzed for the presence of PG by Chelex@100 method and polymerase chain reaction (PCR), by isolating amplified sequences of DNA in a sub gingival biofilm taken from the deep periodontal pockets. The presence of anti CCP and anti MCV autoantibodies was detected in the sera of RA patients with ELISA test.

Results: The average ages of the patients in the 3 groups were as follows -51 years for RA, 52 for OA and 58 years for HC. Seventy two percent of RA patients were females.

Significantly higher levels of PG were found in the periodontal pockets of RA patients.

Eighty percent of RA patients (80% or 24 RA patients) were PG positive in comparison with 35% of OA patients and 2% healthy controls.

Of the PG-positive RA patients, 83% had positive and 17% had anti-CCP negative test, while of the PG-negative patients, a positive anti-CCP test was present in 33% and a negative anti-CCP test was present in 67%. Accordingly, in PG-positive RA patients positive anti-MCV test was present in 79% and negative anti-MCV test was present in 21%, and in PG-negative RA patients anti-MCV test was positive in 17% and negative in 83% patients.

Table 1.

Patient's groups	RA	OA	HC	Significance
Porphyromonas gingivalis positive	24 (80%)	9 (35%)	7 (29%)	RA→OA (p < 0.0001)
Porphyromonas gingivalis negative	6 (24%)	17 (65%)	17(71%)	RA→HC (p < 0.0001) OA→HC (p 0,65)
Total	30	26	24	

Conclusion: The results of our study indicate that PG is found more frequently in periodontal pockets of patients with rheumatoid arthritis, which implies the important role of oral microbioma in RA pathogenesis, treatment and prevention.

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AB0094

FUNCTIONAL TREG CELLS MAY BE CONVERTED INTO T EFFECTOR PHENOTYPE ON EXPOSURE TO INFLAMMATORY MILIEU IN RHEUMATOID ARTHRITIS (RA) SYNOVIAL FLUID: IN-VITRO STUDY

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Background: The debate on functional versus numerical difference in T regulatory cell population among patients with Rheumatoid arthritis (RA) is not clear. Tregs expressing Inflammatory subset phenotype markers, such as Th1(Cxcr3, Tbet) and Th17(ccr6,Ror γ) are reported. (1) Though the reported numbers of synovial fluid tregs are higher in RA, (2) the fate of Tregs on entering the synovial inflammatory milieu from peripheral blood (PB) has not yet been investigated.

Objectives: To compare Treg frequencies in PB and synovial fluid between osteoarthritis (OA) and RA

To compare cytokine levels in PB and SF between OA and RA

To study the effect of autologous synovial fluid on RA and OA Treg isolated from peripheral blood

Methods: The Peripheral Blood (PB) and synovial fluid (SF) of RA (n=80) and OA (n=30) patients were analyzed for CD4+T-cell subset frequencies and phenotypes by flow cytometry. Cytokine concentrations in plasma and SF were measured by cytometric bead array. Tregs from 5 RA-PB and 5 OA-PB were isolated and cultured in autologous synovial fluid for 24 hrs. Phenotypic expression of Th1 and Th17 chemokines on the cell surface were analyzed by flow cytometry and expression levels of T-bet, ROR γ and FOXP3 in those Treg cells were measured with quantitative real-time PCR (RT-qPCR).

Results: The PB and SF frequencies of Th1, Th17 and Tregs are shown in Table 1. The pro-inflammatory cytokines were high in the plasma and SF of RA but the anti-inflammatory cytokines were similar (Fig 1A&B). Treg cells were isolated from RA and OA PB and cultured in autologous SF for 24 hrs. RA Treg showed increased cell surface expression of CXCR3+ and CCR6+ (Fig 1C) and there was no difference in OA Treg. Gene expression studies showed an increased expression of T-bet, ROR γ and decreased expression of Foxp3 in RA Tregs while there was no difference in OA Tregs before and after in-vitro culture (Fig 1D).

Table 1. Tcell subsets in Rheumatoid Arthritis and Osteoarthritis.

CD4 subtype	RA		OA	
	PB	SF	PB	SF
	N=80	N=30	N=30	N=30
Th1	26.65 ± 5.59	34.99 ± 1.30	25.97 ± 7.24	26.45 ± 1.87*
Th2	5.19 ± 1.9	16.36 ± 1.73	5.24 ± 2.15	1.44 ± 0.10*
Th17	14.05 ± 3.29	21.18 ± 2.04	10.81 ± 2.78*	2.45 ± 0.23*
Treg	10.68 ± 2.47	12.53 ± 2.10	11.162.95	13.04 ± 2.57

*P<0.05

Conclusion: Tregs in RA may be converted to Th1 and Th17 phenotype on exposure to inflammatory cytokine in the synovial fluid, thus losing their regulatory functions. Understanding factors influencing stability of Treg cells may help improve future therapeutics.

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Fig1: A. Comparison of plasma cytokine levels between RA and OA patients; B. Comparison of synovial fluid cytokine levels between RA and OA patients; C. Surface characterization of in-vitro cultured Peripheral blood Treg cells of RA patients. C1 & C2 CXCR3(Th1) and CXCR6(Th17) chemokines expressing Treg cells isolated from peripheral compartment of RA patients; C3 & C4 CXCR3(Th1) and CXCR6(Th17) chemokines expressing Treg cells cultured in autologous synovial fluid. D. Comparison of expression of transcription factors in peripheral blood Treg cells of RA and OA patients: cultured with autologous synovial fluid. Expression of (D1) T-bet, (D2) ROR-g, (D3,D4) Foxp3. Mann-Whitney U test was used to analyze cytokine data, unpaired student t-test was used to analyze Treg frequency data, Paired t-test was used to analyze gene expression data; * P Value < 0.05 considered significant

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AB0095

PRECLINICAL CHARACTERIZATION OF CJ-15314, A HIGHLY SELECTIVE JAK1 INHIBITOR, FOR THE TREATMENT OF AUTOIMMUNE DISEASES

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Background: Janus kinases (JAK1, JAK2, JAK3, and TYK2) play critical roles in mediating various cytokine signaling, and has been developed as a target for autoimmune diseases such as RA. Tofacitinib, oral Pan-JAK inhibitor, demonstrated efficacy in RA patients, but its widespread use is limited by safety issues. Baricitinib, JAK1/2 inhibitor, is also known to interfere with the hematopoiesis system, such as anemia and thrombocytopenia associated with suppression of JAK2 signals. Therefore, it is necessary to develop a new potent compound that selectively inhibits JAK1 over JAK2, 3

Objectives: To identify the pharmacological characteristic based on efficacy of CJ-15314 as potent and selective JAK1 inhibitor for treatment of autoimmune disease.

Methods: In vitro, cell-based, kinase panel, Kd value and human whole blood assay were performed to determine the inhibition potency and selectivity for JAK subfamily kinases. In vivo therapeutic potential was evaluated by RA model including rat Adjuvant-Induced Arthritis (AIA) and collagen-induced arthritic (CIA). To confirm the possibility of further expansion into the autoimmune disease, BioMAP® Diversity PLUS® Panel was performed by discoverX.

Results: In vitro assay, CJ-15314 inhibited JAK kinase family in a concentration-dependent manner with IC50 values of 3.8 nM against JAK1, Selectivity for

JAK1 over JAK2, 3 was approximately 18, 83 fold greater for CJ-15314. In 1mM ATP condition, CJ-15314 has been confirmed to have the highest JAK1 selectivity over competing drugs, under 1mM ATP condition that reflects the physiological environment in the body. Similarly, Kd values has also confirmed the selectivity of JAK1, which is 10 fold higher than JAK2, 3. Accordingly, in human whole blood assays, CJ-15314 is 11 fold more potent against IL-6 induced pSTAT1 inhibition through JAK1 (IC50 value: 70 nM) than GM-CSF-induced pSTAT5 inhibition (JAK2) whereas baricitinib and filgotinib exhibited only 2 fold and 7 fold respectively.

In vivo efficacy model, CJ-15314 inhibited disease severity scores in a dose dependent manner. In the rat AIA model, CJ-15314 at 30mg/kg dose showed 95.3% decrease in arthritis activity score, 51.2% in filgotinib at 30 mg/kg, 97.7% showed baricitinib at 10 mg/kg. CJ-15314 showed superior anti-arthritis efficacy than filgotinib. CJ-15314 also minimally affected anemia-related parameters but not baricitinib end of the 2-week treatment. In the rat CIA model, like 10 mg/kg of baricitinib, 30 mg/kg of CJ-15314 also has a similar effect, with a significant reduction in histopathological scores.

In biomap diversity panel, CJ-15314 inhibited the expression of genes such as MCP-1, VCAM-1, IP-10, IL-8, IL-1, sTNF-α and HLA-DR confirming the possibility of expansion into other diseases beyond arthritis.

Conclusion: CJ-15314 is a highly selective JAK1 inhibitor, demonstrates robust efficacy in RA animal model and is good candidate for further development for inflammatory diseases.

* CJ-15314 is currently conducting a phase I trial in south Korea.

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AB0096

FCER1G GENE METHYLATION AND MIR-106/MIR-17 AS A NEW POTENTIAL EPIGENETIC MARKERS IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease that leads to joints destruction. One of the most important cytokine responsible for this process is interleukin 6 (IL-6). Fc receptor gamma chain (FcRγ), encoded by *FCER1G* gene, is responsible for neutrophils activation, phagocytosis, cell surface signaling pathway as well as IL-4, IL-6, IL-10 and tumor necrosis factor production. Epigenetic factors, including DNA methylation and micro-RNAs (miRs) expression regulate the genes expression on transcriptional and post-transcriptional mechanisms. There are miRs responsible for cytokines production, for example GU rich miRs, miR-106b and miR-155 were reported as associated with IL-6 overproduction.

Objectives: The aim of our study was to evaluate *FCER1G* gene methylation and miR-17 family members as epigenetic markers associated with RA, disease activity and IL-6 expression.

Methods: Bioinformatics analysis were applied to select the miRs with a possible target sites in a promoter region of *FCER1G* gene. The MiR-17 family members, including miR-17, miR-93 and miR-106b were selected for investigation. A total of 74 individuals, 50 RA patients, 84% female, aged 53.7±12.3 years (mean±SD) and 24 healthy controls (HC), 87.5% female, aged 53±8.49 years were enrolled. RA patients were selected based on DAS-28 scoring. RA patients with high disease activity (DAS28 >5.1; 58%) and remission (≤2.6; 42%) were included in the analysis. DNA was extracted from a whole blood and miRs were extracted from plasma. Quantitative real-time PCR was used for analyze both methylation and expression levels. In a randomly selected samples (16 from high