SAT0038

SEMAPHORINS: FROM ANGIOGENESIS TO INFLAMMATION IN RHEUMATOID ARTHRITISJérôme Avouac^{1,2}, Sonia Pezet², Eloise Vandebeuque¹, Yannick Allanore^{1,2}.¹Paris Descartes University, Cochin Hospital, Rheumatology, Paris, France;²INSERM U1016, Paris, France

Background: Synovial neoangiogenesis is an early and crucial event to promote the development of the hyperplastic proliferative pathologic synovium in rheumatoid arthritis (RA). A recent microarray analysis of Endothelial cells (ECs) issued from patients with RA and matched controls has revealed a differential expression of semaphorin family in RA ECs, which is known to contribute to angiogenesis and autoimmunity/inflammation.

Objectives: Our aim was to study the potential implication of semaphorins in RA pathogenesis.

Methods: mRNA levels of class 3 and 4 Semaphorins (SEMA) SEMA3A, SEMA3E, SEMA4A and SEMA4E, as well as their receptors PlexinD1 (PLXND1) and Neuropilin-1 (NRP1), were measured by real-time quantitative PCR in RA (n = 15) and control (n = 15) ECs. Protein expression of these 4 semaphorins and their receptors was evaluated in ECs of 10 RA patients and controls by western blot, and in the synovial tissue of 10 RA patients and 5 controls by immunohistochemistry and immunofluorescence. Serum concentrations of these 4 semaphorins were measured by sandwich ELISA in a cohort of 130 patients with RA (85% women, mean age: 58 ± 12 years and mean disease duration: 11 ± 12 years) and 30 age- (56 ± 10 years) and sex- (82% women) matched controls.

Results: SEMA4A, SEMA3E and their receptors PLXND1 and NRP1 mRNA (Real time quantitative PCR) and protein (western blot analyses) levels were found to be markedly increased in RA ECs compared to control ECs. The expression of SEMA4A, SEMA3A, SEMA3E, PLXND1 and NRP1 was strikingly increased in the synovial tissue of patients with RA. Confocal microscopy with double labeling for CD31 confirmed the prominent endothelial expression of these class 3 and 4 semaphorins and their receptors. SEMA3A serum levels were significantly lower in RA compared to controls (13.93±5.69 vs. 18.29±6.69 ng/mL, $P<0.001$). Levels of SEMA3E (1.21±0.884 vs. 0.78±0.80 ng/mL, $P=0.041$), SEMA4A (65922 ±27109 vs. 53189±25808 pg/ml, $P=0.010$) and SEMA4D (3.59±3.39 vs. 1.90±3.95 ng/mL, $P=0.012$) were significantly increased in patients with RA compared to controls. In patients with RA, SEMA3A, SEMA4A and SEMA 4D correlated with CRP levels ($r=-0.36$, $P<0.001$, $r=0.39$, $P<0.001$ and $r=-0.31$, $P=0.001$, respectively). SEMA4A and 4D correlated with the Disease Activity Score (DAS)-28, ($r=0.27$, $P=0.006$ and $r=-0.23$, $P=0.020$, respectively). SEMA3A, SEMA4A and SEMA4D also correlated with the Global OMERACT-EULAR Synovitis Score, reflecting the intensity of synovial vascularization measured by power Doppler ultrasound ($r=-0.22$, $P=0.023$, $r=0.21$, $P=0.030$ and $r=-0.242$, $P=0.013$, respectively). In addition SEMA4A and SEMA3E correlated with serum levels of the angiogenic markers Tie-2, angiotensin, Interleukin-8 and soluble Vascular Cell Adhesion Molecule.

Conclusion: Gene expression profiling of ECs identified semaphorins as potential biomarkers and therapeutic candidates in RA. Class 3 and 4 semaphorins are overexpressed in ECs, synovial vessels and the serum of patients with RA. They also correlated with validated markers of inflammation and angiogenesis. Thus, semaphorins might be novel and appealing EC-derived inflammatory and proangiogenic targets in RA.

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SAT0039

ZAP-70 CONTROLS AUTOIMMUNE ARTHRITIS VIA REGULATION OF APOPTOSIS THROUGH BCL-2, BIM AND CBL IN T CELLSReka Kugyelka¹, Lilla Prenek¹, Zoltan Kohli¹, Tibor Glant², Peter Nemeth¹, Timea Berki¹, Ferenc Boldizsar¹. ¹University of Pecs, Department of Immunology and Biotechnology, Pecs, Hungary; ²Rush University Medical Center, Department of Molecular Medicine, Chicago, United States of America

Background: The recombinant human G1(rhG1)-induced mouse arthritis (GIA) model resembles human rheumatoid arthritis in many aspects¹. Similarly, to RA, GIA is also a T cell dependent disease: T cell activation and apoptosis has been shown to regulate arthritis severity². ZAP-70 kinase is a key molecule of T cell receptor signaling, in its absence T cell differentiation and activation is seriously hindered³. In preliminary

experiments, we found that partial ZAP-70 deficiency (in ZAP-70^{+/-} heterozygous mice) ameliorated autoimmune arthritis in the GIA model, supported by both *in vivo* (decreased severity and milder arthritis) and *in vitro* tests (decreased T cell proliferation, IL-4 and IL-6 production).

Objectives: In the present work we wanted to test the hypothesis that the observed changes in GIA in partial ZAP-70 deficient mice are due to complex alterations in T cell apoptosis.

Methods: T cells were isolated from healthy and arthritic BALB/c and ZAP-70 heterozygous (ZAP-70^{+/-}) knockout mice, then stimulated *in vitro* for 72 hours with anti-CD3/anti-CD28-coated beads. After stimulation, cells were lysed and the homogenates were subjected to Western-blotting using antibodies against activated Caspases-3,-8, and -9, respectively, and other apoptotic markers (cytochromeC), and some important regulator molecules (Bcl-2, Bim and Cbl).

Results: Overall, as expected, we have seen decreased tyrosine phosphorylation in the T cells of arthritic ZAP-70^{+/-} mice compared to the controls. The levels of cleaved (activated) Caspase-8 and -9 were comparable in ZAP-70^{+/-} samples to the controls, however, we detected elevated levels in case of Caspase-3. There was slightly lower Cytochrome C release and Bcl-2 and Bim levels in the activated T cells of ZAP-70^{+/-} mice. Importantly, there was markedly less Cbl-b in the T cells of arthritic ZAP-70^{+/-} mice.

Conclusion: Our results show the complex changes in the apoptotic pathways of activated T cells caused by the partial deficiency of ZAP-70. Impaired T cell activation through the T cell receptor complex, as shown by decreased phosphorylation, accompanied by slightly increased apoptosis sensitivity of T cells (stronger Caspase-3 signal) might explain the less severe arthritis seen in ZAP-70^{+/-} mice. Furthermore, based on these results Cbl-b (a negative regulator of T cell activation) might serve as an important early molecule interacting with ZAP-70 and thus fine regulate T cell activation and apoptosis in autoimmune arthritis.

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SAT0040

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA COACTIVATOR-1B FACILITATES PSEUDOPODIA FORMATION OF FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS VIA ACTIVATING RHO GTPASESMA Jianda¹, Jun Jing¹, Tao Yan², Junwei Wang¹, Lie Dai¹. ¹Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Department of Rheumatology, Guangzhou, China; ²Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, China

Background: Fibroblast-like synoviocytes (FLS) play critical roles on joint inflammation and destruction of cartilage and bone in rheumatoid arthritis (RA) due to their aggressive behavior including increased migration. The Rho family of small GTPases are the master regulators of actin cytoskeleton remodeling which leads to pseudopodia formation and migration. Peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) β is a transcriptional regulator which plays important roles on regulating multiple signaling pathways. Our previous study revealed that elevated PGC-1 β in RA-FLS promoted their migration and invasion. However, the underlying mechanism remains elusive.

Objectives: To investigate the role of PGC-1 β on regulating pseudopodia formation of RA-FLS and its underlying mechanism.

Methods: Synovial tissues were obtained from six patients with active RA and FLS were isolated and cultured *in vitro*. RA-FLS were transfected with a lentivirus vector for PGC-1 β knockdown or over-expression, and transfected with same lentivirus vectors marked Lv-sh-GFP or Lv-GFP as negative controls. The change of cytoskeleton was stained with fluorescent phalloidin to visualize polymerized actin in lentivirus transfected RA-FLS. The mRNA expression of RhoA, Rac1, and Cdc42 was measured

by qPCR. Their activity was measured using a Rac1, RhoA or Cdc42 Pulldown & G-Lisa Activation Assay Kit.

Results: 1) PGC-1 β knockdown inhibited lamellipodia and filopodia formations of RA-FLS compared with Lv-sh-GFP transfection group (cells with lamellipodia: 24% $\pm$ 7% vs. 40% $\pm$ 5%, $P=0.035$; cells with filopodia: 34% $\pm$ 9% vs. 49% $\pm$ 4%, $P=0.041$), while PGC-1 β over-expression promoted lamellipodia and filopodia formations of RA-FLS compared with Lv-GFP transfection group (cells with lamellipodia: 50% $\pm$ 4% vs. 34% $\pm$ 6%, $P=0.040$; cells with filopodia: 67% $\pm$ 7% vs. 52% $\pm$ 6%, $P=0.045$, Figure 1A).

2) PGC-1 β knockdown or over-expression did not affect the mRNA expression of Rac1, RhoA or Cdc42 (all $P>0.05$, Figure 1B). However, PGC-1 β knockdown suppressed the activity of Rac1, RhoA and Cdc42 (Pulldown assays: 65%~78% reduction; G-LISA assays: 28%~53% reduction, all $P<0.05$), while PGC-1 β over-expression significantly increased their activity (Pulldown assays: 1.55~1.72 folds; G-LISA assays: 1.43~1.68 folds, all $P<0.05$, Figure 1C, D).

Conclusion: Our findings revealed that elevated PGC-1 β in RA-FLS promoted pseudopodia formation by activating Rho family proteins which imply a novel target on regulating RA-FLS migration.

Figure 1 Effects of PGC-1 β on pseudopodia formation and the activity of Rho GTPases in RA-FLS. RA-FLS were transfected with lentivirus for PGC-1 β knockdown or over-expression. (A) Comparison of lamellipodia and filopodia formations by F-actin staining in RA-FLS. (B) The mRNA expression of Rac1, RhoA and Cdc42 was measured by qPCR. (C) The activity of Rac1, RhoA and Cdc42 was measured by Pulldown assays. (D) The activity of Rac1, RhoA and Cdc42 was measured by G-LISA assays. Data were representative as means $\pm$ SD, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

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SAT0041

GENOTYPE OF THE RHEUMATOID ARTHRITIS SEVERITY LOCUS, RS26232, IS ASSOCIATED WITH INVASIVENESS OF RASFs IN VITRO

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Background: The single nucleotide variant rs26232 has been associated with both the susceptibility to, and severity of, rheumatoid arthritis (RA). There is an allele dose response between rs26232 and radiological damage, with the minor, T, allele being protective against disease severity. Rs26232 is situated in the first intron of C5orf30, which is a negative regulator of tissue damage and inflammation in RA.

Objectives: The objective of this study is to elucidate the mechanism by which rs26232 may mediate disease severity. We also aimed to determine the genotype-phenotype association of rs26232 in rheumatoid arthritis synovial fibroblasts (RASFs).

Methods: RASF were derived from knee biopsies of RA patients taken at arthroscopy (n=33). RASFs were used between passages 3-8. Matrigel-coated Boyden transwell chambers were used to measure invasion. Wound healing (scratch assays) were used to measure migration, whereas proliferation was measured via crystal violet staining of fixed RASF. Intracellular cytokine staining and cell surface markers were measured via flow cytometry. Secreted cytokines were measured in the supernatant of RASF using ELISA. Rs26232 genotype was determined by

PCR genotyping assay with allelic discrimination analysis. Anticitrullinated protein antibody (ACPA) status was measured for each participant. Quantitative real-time PCR was used to measure gene expression. Mann-Whitney U test was used to compare two independent groups, Kruskal-Wallis test was used to compare groups of greater than two.

Results: 49% (n=16) of our RASF cohort were homozygous for the major allele CC, 42% (n=14) were heterozygous CT, 9% (n=3) were TT. Rs26232 is associated with invasion of RASFs with the CC genotype being more invasive than the CT genotype ($p=0.021$). RASFs with the CC genotype had increased ICAM1 and VEGF cell surface expression compared to CT genotype ($p=0.001$, $p=0.05$ respectively). MCP1/CCL2 was decreased in CC compared to CT ($p=0.013$). No association was found between rs26232 genotype and migration, proliferation, or expression of MMP3, TIMP3, IP10 or MIP1a.

rs26232 is located in the first intron of C5orf30. There was no differential expression of total C5orf30 or with any of the 3 individual transcript variants in rs26232 genotype groups. In silico analysis of the region in which rs26232 is located on chromosome 5 identified a DNase Hypersensitivity cluster. Furthermore, mining of the Genotype-Tissue Expression (GTEx) expression quantitative trait loci (eQTL) database revealed a cluster of genes located upstream on chromosome 5 (102,850,000-103,150,000) associated with rs26232. This region upstream from rs26232 contains 4 genes (EIF3KF1, PPI5K2, PAM and GIN1), of which three are eQTLs of rs26232. In silico analysis revealed that the 3 eQTLs (EIF3KF1, PPI5K2 and PAM) also contain the active enhancer mark H3K27Ac, whereas GIN1 does not.

Conclusion: The CC genotype of rs26232 is associated with both increased invasiveness of RASFs and increased adhesion markers compared to CT genotype. Rs26232 does not mediate its affect via its nearest gene, C5orf30. Rather, in silico analysis predicts rs26232 may function as a distal regulator of EIF3KF1, PPI5K2 and PAM. Future work will test the hypothesis that rs26232 genotype phenotype association is mediated by EIF3KF1, PPI5K2 and PAM.

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SAT0042

TWO POPULATIONS OF PD-1^{hi}CD4⁺ T CELLS WITH DISTINCT B CELL HELPING CAPACITY, ARE ELEVATED IN THE PERIPHERAL BLOOD OF PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

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Background: A novel population of B helper cells, phenotypically CD4⁺CXCR5⁺PD-1^{hi}, has been described in the synovial tissues and peripheral blood of seropositive RA patients with an established disease, and termed 'peripheral helper' (Tph) cells. Contrary to CD4⁺CXCR5⁺PD-1^{hi} follicular helper T (Tfh) cells, Tph cells are not located in lymphoid organs but accumulate in inflamed tissues. The frequency and kinetics of circulating Tph cells have not been examined in patients with early, untreated RA.

Objectives: To study the frequency of circulating Tph (cTph) cells, and also of circulating Tfh cell counterparts (cTfh), in patients with early RA (eRA).

Methods: Peripheral blood was drawn from DMARD-naïve early RA patients (2010 ACR criteria) with a disease duration < 24 weeks (n=48). For each patient, an age and gender-matched healthy control was also studied (HC, n=48). After isolation by Ficoll-Hypaque gradient, freshly isolated PBMCs were stained with antibodies to CD3, CD4, CXCR5, ICOS, PD-1 and CCR2, and examined by flow cytometry. Autologous cocultures of naïve or memory B cells were established with CXCR5⁺ (+) or (-) memory CD4⁺ T cells.

Results: Seropositive (RF+ and/or ACPA+, n=31) but not seronegative eRA patients (n=17), demonstrated increased frequencies and absolute numbers of cTph and cTfh cells. cTph but not cTfh cells expressed CCR2. Those eRA patients who experienced a significant clinical improvement at 12 months, demonstrated a marked reduction of their cTph whereas their cTfh cell numbers remained unchanged; at the same time, rheumatoid factor titres decreased significantly but ACPA (anti-citrullinated peptide antibodies) titres did not vary. Both CXCR5⁺ and CXCR5⁺CD4⁺ T cells were able to induce maturation of memory B cells, whereas only CXCR5⁺CD4⁺ T cells could differentiate naïve B cells.

Conclusion: Two populations of PD-1^{hi}CD4⁺ T cells with distinct phenotype and B cell helping capacity, are increased in the peripheral blood of