THU0015

VARIATION IN MACROPHAGES DIFFERENTIATION AND SREBF1 EXPRESSION BETWEEN INFRAPATELLAR FAT PAD AND SUBCUTANEOUS TISSUES FROM RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS PATIENTS

Shuhe Ma[1], Kosaku Murakami[1], Motomu Hashimoto[2], Masao Tanaka[2], Koichi Murata[2], Kohei Nishitani[2], Hiromu Ito[3], Tsuneyo Mimori[1]. [1]*Kyoto University Graduate School of Medicine, Dept. of Rheumatology and Clinical and Immunology, Kyoto, Japan*; [2]*Kyoto University Graduate School of Medicine, Advanced Medicine of Rheumatic Disease, Kyoto, Japan*; [3]*Kyoto University Graduate School of Medicine, Dept. of Orthopedic Surgery, Kyoto, Japan*

Background: Sterol regulatory element-binding protein 1 (SREBP1) has been known to upregulate the expression levels of regulators of ω-3 fatty acids in the resolution phase of macrophages, and this would in turn repress the production of pro-inflammatory cytokines.[1, 2] In OA joints, adipocytes might participate in inflammatory process.[3] We are interested in the proportion of M1 and M2 macrophages in stromal vascular fraction (SVF) from both tissues in both diseases. And to investigate how *srebf1* works in infrapatellar fat pad (IFP, or Hoffa) or subcutaneous (SC) tissue, we studied the expression levels of *srebf1*, pro-inflammatory cytokines and regulators of fatty acids.

Objectives: To investigate the proportion of CD14 positive cells as well as M1 and M2 macrophages in SVF of Hoffa and SC; and to explore contribution of SREBP1 to rheumatic disease pathological processes of RA and OA.

Methods: After treated with collagenase, macrophages (CD14 positive cells) in SVF were counted by flow cytometry. Then they were divided, half for calculating the ratio between CD80 positive cells (M1 macrophages) and CD163 positive cells (M2 macrophages), and half for performing qRT-PCR.

Results: Characteristics of the patients

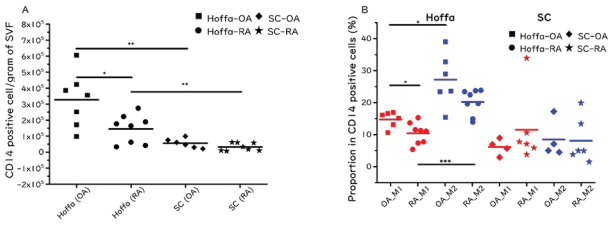
Table 1. Characteristics of the patients

		OA (n=7)	RA (n=8)	*p* value
Age (years)		72 (62-79)	63 (49-76)	0.055
Female patients (n)		6	7	
Male patients (n)		1	1	
Height (cm)		155 (151-164)	157 (147-161)	0.341
Body weight (kg)		62.7 (48.7-74.1)	58.7 (48.8-72.2)	0.386
pre-ope CRP (mg/L)		0.75 (0-2)	12.8 (0-17)	0.064
BMI	Underweight	0%	0%	
	Normal	42.9%	50.0%	0.234
	Overweight	42.9%	37.5%	0.05
	Obese	14.3%	12.5%	

OA: osteoarthritis; RA: rheumatoid arthritis; CRP: C-reactive protein; BMI: body mass index; BMI: <18,5 underweight; BMI: 18.5–24.99 normal; BMI: 25.0–29.99 overweight; BMI >30.0 obesity[4]

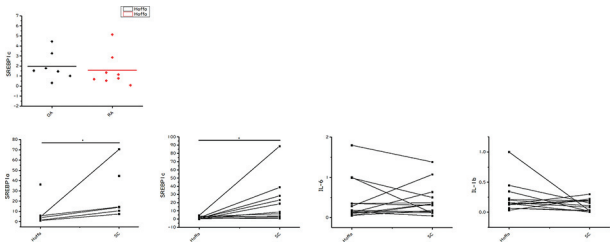
More CD14 positive cells exist in Hoffa comparing to SC, and M2 macrophages show higher proportion.

A comparison of proportion of CD14 positive cells between Hoffa and SC showed significance both from OA and from RA. To M2 macrophages proportion, higher percentages of M2 macrophages (OA: 26.3±5.5%, RA: 22.5±2.4%) exist in Hoffa from both OA and RA patients, but no significance was found between the diseases. However, the proportion of M1 macrophages (OA: 15.6±1.7%, RA: 11.3±2.1%) indicated significance.



Abstract THU0015 Figure 1. (A) Proportion of CD14 positive cells per gram of SVF from OA and RA patients. (B) Proportion of M1 and M2 macrophages in CD14 positive cells from OA and RA patients. (*p<0.05, **p<0.01, ***p<0.001, as determined by One-Way ANOVA.)

Srebf1 expressed less in Hoffa than in SC



Abstract THU0015 Figure 2. qRT-PCR analysis of cytokine and *srebf1* expression levels displayed among total patients. (*p<0.05, as determined by One-Way ANOVA.)

Results show that in Hoffa, *srebf1c* expressed less in RA patients than that in OA patients, but no significance was indicated. In the comparison of expression levels between Hoffa and SC, both *srebf1a* and *srebf1c* showed significance, and more *IL-6* and *IL-1*β expressed in Hoffa than in SC.

Conclusion: In RA patients, more M2 macrophages exist in Hoffa than in SC. Lower expression levels of *srebf1c* in Hoffa from RA patients suggests that macrophages differentiation can be reprogrammed by fatty acid metabolism.

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Disclosure of Interests: Shuhe Ma: None declared, Kosaku Murakami: None declared, Motomu Hashimoto Grant/research support from: Astellas, Brystol-Meyers, Eisai, Employee of: M. H. is affiliated with the department (Department of Advanced Medicine for Rheumatic Diseases, Kyoto University), which is financially supported by four pharmaceutical companies (Tanabe-Mitsubishi, Chugai, Ayumi, UCB Japan), Speakers bureau: Tanabe Mitsubishi, Brystol-Meyers, Masao Tanaka: None declared, Koichi Murata: None declared, Kohei Nishitani: None declared, Hiromu Ito: None declared, Tsuneyo Mimori: None declared
DOI: 10.1136/annrheumdis-2019-eular.460

THU0016

ALTERED EXPRESSION AND FUNCTION OF P2X7 RECEPTOR IN PATIENTSAFFECTED BY SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Anna Lisa Giuliani[1], Federica Furini[2], Alessandra Bortoluzzi[2], Marcello Govoni[2], Francesco DI Virgilio[1]. [1]*University of Ferrara, Morphology, Surgery and Experimental Medicine, Ferrara, Italy*; [2]*University of Ferrara, Medical Science, Ferrara, Italy*

Background: Extracellular ATP (eATP) is one of the most diffuse danger associated molecular patterns (DAMPs) released actively through specific mechanisms from intact cells, or passively from damaged or dying cells[1]. An implication for eATP has been found in SLE[2]. Among receptors for eATP, P2X7R is deeply involved in inflammatory and immune processes and its activation drives different intracellular pathways such as NLRP3-inflammasome activation, IL-1β maturation and release, IL-6 and TNF-α production, regulation of lymphocyte proliferation and cell apoptosis[3]. Previous studies pointed out a possible relationship between P2X7R signaling pathways and SLE pathogenesis[4] [5]. A marked inflammatory condition characterize serositis, that are among the most common manifestations of SLE, and chloroquine is one of the main drugs employed.

Objectives: The aim of this study was to investigate P2X7R expression and activity in SLE.

Methods: 48 SLE patients, and 20 healthy control (HC) subjects were enrolled. Among SLE patients, 16 (SLE-S) presented, and 32 (SLE-NS) did not present history of serositis. All subjects gave written informed consent to peripheral venous blood withdrawal after approval by the local ethic committee. Plasma samples were used to measure IL-1β, IL-6 and TNF-α levels by ELISA. Mononuclear cells were isolated from blood samples by Ficoll gradient sedimentation and employed as follow: i) assessment of IL-1β, IL-6 and TNF-α release after stimulation with lipopolysaccharide (LPS) and/or Benzoyl ATP (BzATP); ii) evaluation of P2X7R mRNA expression by RT-PCR; iii) measurement of P2X7R activity as BzATP-induced increase of intracellular Ca^{2+} concentration using the Fura2/AM probe.

Results: In SLE patients respect to HC, plasma IL-1β levels were unmodified whereas IL-6 was higher, resulting significantly increased in SLE-S. Monocytes isolated from SLE patients released lower quantities of IL-1β after stimulation with BzATP, whereas the release of both IL-6 and TNF-α was significantly augmented in SLE-NS respect to both HC and SLE-S subjects after all types of stimulation. RT-PCR showed reduced P2X7R and augmented NLRP3 mRNA expression in SLE patients. Accordingly, P2X7R activity was significantly reduced in all SLE patients and did not appear to be influenced by a chloroquine pre-treatment.
Conclusion: In SLE patients, compared to HC subjects, we found reduced P2X7R mRNA expression, increased NLRP3 mRNA, as a possible compensating mechanism, and correspondingly, significantly lower BzATP-induced intracellular Ca^{2+} increase, without an apparent influence by chloroquine, one of the drugs most diffusely used for SLE treatment. The in vitro IL-1β release was reduced, whereas plasma IL-1β was unaltered, indicating an alternative source, other than monocytes, of this cytokine. Conversely, IL-6 and TNF-α levels were increased in vitro, and IL-6 was present in plasma at higher levels. The possible consequences of reduced P2X7R, mainly on cytokines network deregulation and lymphocyte proliferation, will be further investigated as well as the role of IL-6 and TNF-α as possible therapeutic targets.

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Disclosure of Interests: Anna Lisa Giuliani: None declared, Federica Furini: None declared, Alessandra Bortoluzzi: None declared, Marcello Govoni: None declared, Francesco Di Virgilio Consultant for: FDV is a member of the Scientific Advisory Board of Biosceptre Ltd, a UK-based biotech Company involved in the development of P2X7R-targeted therapeutics.
DOI: 10.1136/annrheumdis-2019-eular.1736

THU0017 IN VITRO MECHANISTIC STUDIES DEMONSTRATE FILGOTINIB ACTIVITY THAT HAS POTENTIAL IMPLICATIONS FOR DIFFERENTIATION AMONG JAK INHIBITORS

Pei Han, Amy Meng, Nevena Mollova, Yuanjiang Yu, Julie A. DI Paolo. *Gilead Sciences, Inc., Foster City, United States of America*

Background: Inhibition of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway has demonstrated efficacy in immune-mediated diseases and has been identified as a therapeutic target for the treatment of rheumatoid arthritis (RA). Differences in JAK inhibitor specificity for JAK1, JAK2, JAK3, and TYK2 may influence their safety profiles, but the mechanism is not known. Selective JAK1 inhibition by filgotinib (FIL) may modulate a subset of proinflammatory cytokines associated with RA pathogenesis and improve the risk-benefit profile by minimizing other non–JAK1-related adverse events. JAK2 inhibition is associated with cytopenias, while JAK3 inhibition has been associated with increased risk for opportunistic infections (eg, tuberculosis and herpes zoster) and chronic low-grade inflammation. In clinical trials, FIL did not negatively impact hemoglobin, LDL/HDL ratios, or natural killer (NK) cell counts.[1-3]
Objectives: To compare the in vitro profile of JAK inhibitors with different JAK selectivity profiles, for effects on erythroid progenitor cell expansion, NK cell proliferation, and liver X receptor (LXR) agonist-induced cholesteryl ester transfer protein (CETP) expression, an enzyme responsible for the conversion of HDL to LDL.
Methods: JAK inhibitors (FIL, FIL metabolite [GS-829845], baricitinib [BARI], tofacitinib [TOFA], and upadacitinib [UPA]) were evaluated in vitro in human cell-based assays: growth of erythroid progenitors from human cord blood $CD34^+$ cells using a HemaTox™ liquid expansion assay, IL-15–induced NK cell proliferation, and LXR agonist-induced CETP expression in the hepatic cell line (HepG2). Using IC_{50}s generated from these assays and the reported human plasma concentrations of the JAK inhibitors from clinical studies,[4-6] we calculated the target coverage for each compound at clinically relevant doses. The activity of FIL in humans was based on a PK-PD modeling algorithm[7] of FIL + GS-829845.
Results: In vitro assay results are described in the table. Based on these results, human exposure data, and modeled PK-PD relationships, FIL 100 mg and FIL 200 mg result in lower calculated cellular inhibition than the other JAK inhibitors at clinical exposures. Notably, FIL 100 mg and FIL 200 mg, but not the other inhibitors, are calculated to reduce CETP expression by 17% and 27%, respectively, while BARI, TOFA, and UPA are not expected to alter CETP levels.

Abstract THU0017 Table 1. IC_{50} ± SD in in vitro assays (nM, unless otherwise noted).

Assay	FIL	GS-829845	BARI	TOFA	UPA
Early erythroid progenitors	1960 ±137	19300 ±1730	38.6±2.9	210 ±15.2	42 ±2.9
Mature erythroid progenitors	1140 ±112	10600 ±1270	25.7±2.9	110 ±10	24.5 ±2.75
NK cell proliferation	314.8 ±53	9697 ±8100	6.6±1.9	12.2 ±2.1	4.1 ±1.7
Inhibition of LXR agonist-induced CETP expression	15.3 ±7.1 μM	19.4 ±4.2 μM	>1 μM weak induction[a]	No effect	

[a] Weak stimulation of LXR agonist-induced CETP expression.
Conclusion: JAK1 selectivity of FIL and GS-829845 resulted in less inhibition of erythroid progenitor expansion and NK cell proliferation compared with BARI, TOFA, and UPA. FIL also reduced LXR agonist-induced CETP expression, while the other inhibitors did not alter these levels. These results provide a potential mechanistic link to the observed reduction of CETP concentration and activity following FIL treatment, and the observed reduction in LDL:HDL in RA patients.[8]

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Disclosure of Interests: Pei Han Shareholder of: Gilead Sciences, Inc., Grant/research support from: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc., Amy Meng Shareholder of: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc., Nevena Mollova Shareholder of: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc., Yuanjiang Yu Grant/research support from: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc., Julie A. Di Paolo Shareholder of: Gilead Sciences, Inc., Employee of: Gilead Sciences, Inc.
DOI: 10.1136/annrheumdis-2019-eular.3361

THU0018 ANTI-GALECTIN-9 ANTIBODY AS A NOVEL TREATMENT OPTION IN RHEUMATOID ARTHRITIS TARGETING PATHOGENIC FIBROBLAST-LIKE SYNOVIOCYTES

Ditte Køster[1], Malene Hvid[1,2], Aida Solhøj Hansen[1], Tue Wenzel Kragstrup[1,3], Pierre Busson[4,5,6], Bent Deleuran[1,3], Morten Aagaard Nielsen[1]. *[1]Aarhus University, Biomedicine, Aarhus, Denmark; [2]Aarhus University, Clinical Medicine, Aarhus, Denmark; [3]Aarhus University Hospital, Rheumatology, Aarhus, Denmark; [4]UMR 8126, CNRS, Villejuif, France; [5]Universite Paris-Saclay, Gustave Roussy, Villejuif, France; [6]Universite Paris-Saclay, Univ Paris Sud, Le Kremlin-Bicêtre, France*

Background: Fibroblasts-like synoviocytes (FLS) present in the stromal environment are key effector cells in the persistence of synovial inflammation and joint damage in rheumatoid arthritis (RA). A particular subset of FLS characterized as $Thy\text{-}1^+Podoplanin^+CD34^-$ is significantly expanded in RA patients and has been described to be disease-associated[1]. Currently, no treatment targeting the stromal environment in RA is available[2].
Objectives: To identify a novel treatment option targeting the stromal environment in RA to modify the disease-associated subset of fibroblasts.
Methods: An *in vitro* model was established with FLS derived from synovial fluid mononuclear cells (SFMC) from RA and osteoarthritis (OA) patients (n=6). FLS between passage 2-5 were used for further analyses. Untreated FLS in cultures were analyzed by flow cytometry for expression