TNF-ALPHA INDUCES NECROPTOSIS-LIKE DEATH OF MACROPHAGES AND PROMOTES EXTRACELLULAR RELEASE OF 14-3-3-JETA

Guizhan Trimova1, Kaoru Yamagata1, Shigeru Iwata1, Tong Zhang1, Fumi Uemura1, Minoru Satoh1, Michelle Zaharik1, Norma Bihn2, Shintaro Hirata3, Shingo Nakayamada4, Yoshiya Tanaka1.

1University of Occupational and Environmental Health, Japan, The First Department of Internal Medicine, Kitakyushu, Japan; 2University of Occupational and Environmental Health, Japan, Department of Clinical Nursing, School of Health Sciences, Kitakyushu, Japan; 3Augures Life Sciences Corp, North Vancouver, BC, Canada; 4Hiroshima University Hospital, Hiroshima, Japan

Background: 14-3-3 is an intracellular protein detected in the serum and synovial fluid of patients with rheumatoid arthritis (RA) [1]. While presence of 14-3-3 is both diagnostic for early and established RA [2], and prognostic for radiographic progression [3], the mechanism of 14-3-3 externalization in RA remains unclear.

Objectives: To clarify the mechanism of externalization of 14-3-3 into the extracellular space using human PBMC-derived macrophages (Mϕ).

Methods: Distribution of 14-3-3 in synovial tissue of patients with RA or osteoarthritis (OA) was examined by immunohistochemistry; cellular morphology was studied by confocal microscopy and electron microscopy (EM). Mϕ were stimulated with TNF-α, Diamide (induces ligand-independent TNFR signaling) or IL-6/sIL-6R. Western blotting was used to detect S-358-phosphorylated MLKL (a mediator of necroptosis) and presence of 14-3-3 in Mϕ culture supernatants.

Results: Dense and ring-shaped staining of 14-3-3 was detected in Mϕ, but not OA, synovial tissues. 14-3-3 and p32 were endogenously detected in RA synovial tissue; 14-3-3 was not detected in CD68+ cells from RA lung tissue or CD4+ T cells from RA synovium. The outer space around the nucleus of healthy control Mϕ treated with TNF-α or Diamide, but not IL-6/sIL-6R, demonstrated abnormal actin distribution by phallidin staining and presence of cellular and organelle swelling by EM. Further, magnified images showed partial destruction of the cell membrane in TNF-α and Diamide-treated Mϕ. Phosphorylation of MLKL was observed between 20 min and 24 h after stimulation of healthy control Mϕ with TNF-α, with maximum signal at 8 h post-stimulation, but was not observed upon IL-6/sIL-6R stimulation at any time point. After 8 h of stimulation no 14-3-3 was detected in the culture supernatant of healthy control Mϕ endogenously expressing 14-3-3 or Mϕ stimulated with IL-6/sIL-6R, while a high concentration of 14-3-3 was detected in culture supernatants in Mϕ treated with TNF-α or Diamide.

Conclusion: 14-3-3 protein was abundant in RA, but not OA, synovial tissues and co-localized with PAD4 in CD68+ cells; this close proximity to PAD4 may promote citrullination of 14-3-3 in vivo. Treatment of healthy control Mϕ with TNF-α induced phosphorylation of MLKL and cell swelling and disintegration of the plasma membrane characteristic of necroptosis, correlated with the release of intracellular 14-3-3 protein into cellular supernatants. Our results shed light on mechanism of externalization of 14-3-3 and how it achieves elevated levels in RA synovial fluid.

REFERENCES:

Competing Interests: M. Zaharik and N. Bihn are employees of Augurex, Y. Tanaka, has received speaking fees and/or honoraria from Daiichi-Sankyo, Astellas, Eli Lilly, Chugai, Sanofi, Abbvie, Pfizer, YL Biologics, Bristol-Myers, Glaxo-Smithkline, UCB, Mitsubishi-Tanabe, Novartis, Eisai, Takeda, Janssen, Asahi-kasei and has received research grants from Mitsubishi-Tanabe, Novartis, Pfizer Japan Inc, Sanofi, Takeda, UCB, YL Biologics. Fumi Uemura: None declared, Minoru Satoh Grant/research support from: Mitsubishi-Tanabe, Novartis, Pfizer Japan Inc, Sanofi, Takeda. THU0057B

THU0058

B CELL SYNOVITIS AND CLINICAL PHENOTYPES IN RHEUMATOID ARTHRITIS AT DIFFERENT DISEASE STAGES

Felice Rivellese1, Frances Hurby1, Serena Bugatti2, Liliane Fossati-Jimack2, Hasan Rizvi3, Davide Lucchesi1, Gloria Litoa Riberia1, Alessandra Nervianni1, Gulzhan Trimova1, Kaoru Yamagata1, Shigeru Iwata1, Tong Zhang1, Costantina Pitzalis3, 1Queen Mary University of London, Centre for Experimental Medicine and Rheumatology, London, United Kingdom; 3University of Pavia, IRCCS Policlinico San Matteo Foundation, Pavia, Italy; 2Barts Health NHS Trust, Department of Pathology, London, United Kingdom

Background: The role of B cells in the pathogenesis of Rheumatoid Arthritis (RA) is well recognised and has been reinforced by the established efficacy of B cell depleting treatments. However, B cell infiltration in synovia is highly variable and their association with clinical disease activity has been inconsistently reported, with conflicting results possibly linked to the lack of standardization in quantitative and qualitative assessment of B cell synovitis. In particular, the presence of B cells in synovia has never been systematically assessed in large cohorts.

Objectives: To evaluate B cells and their association with clinical phenotypes in the synovia of patients with RA at various disease stages.

Methods: A total of 432 synovial biopsies from the following cohorts of RA patients were analysed: i. early (<1 year) treatment-naive RA (n=165), ii. Synthetic Disease-modifying Anti-Rheumatic Drugs inadequate responders (sDMARDs-ir) (n=103), iii. TNF-inhibitors inadequate responders (TNFi-ir) (n=164). Haematoxylin and eosin staining was used for the assessment of synovitis according to a previously validated score (Krenn). Upon immunohistochemical staining for CD20, semi-quantitative (Sq) scoring (0-4) was used to classify patients into B cell rich (≥ 2) and poor (< 2) and automated digital image analysis (DIA) to calculate the B cell area fraction. B cell expression markers, including CD20 mRNA counts and a composite B cell module, were obtained by RNA-sequencing from early RA synovial biopsies (n=91).

Results: Semi-quantitative synovial B cell scores positively correlated with the B cell area fraction obtained by DIA (Spearman r 0.93 in early RA and 0.88 in TNFi-ir, p<0.0001). Accordingly, B cell rich patients (Sq score ≥ 2) had a significantly higher B cell area fraction (p<0.0001). RNA-sequencing from 91 patients with early RA showed a positive correlation between the Sq B cell scores and CD20 mRNA counts and the B cell module (Spearman r=0.6 and 0.69, respectively, p<0.0001). Similarly, a positive correlation was found between the B cell area fraction obtained by DIA and CD20 mRNA counts and B cell module (r=0.67 and 0.69, respectively, p<0.0001) when comparing B cell presence in the three cohorts, B cell-rich synovitis was present in 35% of early RA, 36% of sDMARDs-ir and 47.1% of TNFi-ir (p=0.025 comparing early RA vs late-stage TNFi-ir patients). Finally, while B cell-rich patients showed significantly higher synovial inflammatory scores across all cohorts, higher disease activity (number of swollen joints, DAS28), and higher prevalence of autoantibody positivity (ACPA and RF) in B cell-rich patients were observed exclusively in the early RA cohort.

Conclusion: We here describe a robust and validated synovial B cell score that can potentially contribute to patient stratification in RA, as it helps identifying an enrichment of B cell synovitis in established disease, uncoupled from clinical disease activity. 

Acknowledgement: We would like to thank all the investigators and recruitment centers from PEAC (http://www.peac-mrc.mds.qmul.ac.uk/centres.php), STRAP (http://www.matura-mrc.wiki.qmul.ac.uk/strap_recruiting_centers.php) and R4RA (http://www.r4ra-nihr.wiki.qmul.ac.uk/recruiting_centers.php) and the EMMI clinical trial team at Queen Mary (http://www.4ra.qmul.ac.uk/doc/Authors/4ra_b_item.pdf).

Disclosure of Interests: Felice Rivellese: None declared, Frances Hurby: None declared, Serena Bugatti Speakers bureau: Bristol-Myers Squibb, Celgene, Lilly, Novartis, Sanofi, Janssen, Liliane Fossati-Jimack: None declared, Hasan Rizvi: None declared, Davide Lucchesi: None declared,