POS0368 CITRULLINATION INDUCES EPIGENETIC MEMORY OF THE INNATE IMMUNE SYSTEM

K. Laskari[1], S. Sabu[1], O. Distler[1], M. Neidhart[1], E. Karouzakis[1,2]. [1]*University Hospital Zurich, University of Zurich, Department of Rheumatology, Center of Experimental Rheumatology, Zurich, Switzerland*; [2]*Galapagos, Translational Medicine, Basel, Switzerland*

Background: During trained immunity, monocytes and macrophages undergo a functional and transcriptional reprogramming toward activation, which is induced by a priming stimulus and results in enhanced responsiveness to subsequent triggers. Monocytes from patients with rheumatoid arthritis (RA) display features consistent with a trained immunity phenotype. Citrullinated proteins as citrullinated vimentin (c-vimentin), which function as damage-associated patterns in RA, may be implicated in the process of trained immunity.
Objectives: We aimed to investigate if c-vimentin induces trained immunity in vitro in healthy individuals.
Methods: Monocytes were isolated from the peripheral blood (EDTA blood, n=22; buffy coats, n=6) from healthy donors by Ficoll-paque centrifugation and negative selection using CD3/CD19/CD56 magnetic beads. The cells were stimulated with c-vimentin (0.1 µg/ml) for 24h and re-stimulated 5 days later with the lipopolysaccharide of *E.coli* (LPS) (10 ng/ml). Protein as well as lactate release were estimated in cell culture supernatants at day 6 by ELISA. RT-PCR and/or Western Blotting were applied to measure mRNA and/or protein expression. The Ligand-receptor glycocapture technology LRC-TRiCEPS was used to identify candidate cell surface targets of c-vimentin. The methylation of histone H3 at lysine 4 (H3K4) was examined by chromatin immunoprecipitation.
Results: Priming with citrullinated vimentin induced training in human monocytes, as suggested by the significantly increased levels of secreted interleukin-6 (IL-6), upon restimulation with LPS (1.29-fold increase, n=22, p<0.001). Likewise, the release of chemokines CXCL1 and CCL20/Macrophage Inflammatory Protein 3a was significantly increased (1.81-fold and 2.32-fold increase, respectively, n=14, both p<0.001). LRC-TRiCEPS enabled the identification of STING cell surface receptor for the ligand c-vimentin. Indeed, c-vimentin induced activation of TBK1, which is implicated in the STING signaling pathway, by phosphorylation, while STING inhibition with the covalent small molecule H151 (2µM) abolished this effect. Besides, H151 inhibited trained immunity by decreasing IL-6 release and expression (1.61-fold and 1.93-fold decrease, respectively, n=5). Trained monocytes also displayed high lactate production (primed vs. unprimed cells, n=9, p=0.004), reflecting a shift in metabolism with an increase in glycolysis. By inhibiting the metabolic pathway of glycolysis by 2-deoxyglucose (11mM), the induction of trained immunity could be counteracted (5.32-fold decrease in IL-6 release, n=7, p=0.016). Finally, c-vimentin induced H3K4 methylation with increased levels of this mark in the promoter of the IL-6 gene. By modulating the function of epigenetic enzymes with methylthioadenosine (1mM), which specifically inhibits histone methyltransferase, trained immunity was reversed (8.43-fold decrease in IL-6 release, n=6, p=0.031).
Conclusion: Citrullinated vimentin induces epigenetic modifications and metabolic changes in monocytes, probably through a STING and TBK1-dependent activation, resulting in enhanced cytokine and chemokine production upon restimulation. Inhibition of the STING signaling pathway may be a novel therapeutic target for myeloid activation in RA.
Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2021-eular.3302

POS0369 ELEVATED EXPRESSION OF TIM-3 ON NEUTROPHILS CORRELATES WITH DISEASE ACTIVITY AND SEVERITY OF ANKYLOSING SPONDYLITIS

X. Huang[1], T. W. Li[1], J. Chen[1], Z. Huang[1], S. Chen[1], X. Guo[1]. [1]*Guangdong Second Provincial General Hospital, Department of Rheumatology and Immunology, Guangzhou, China*

Background: Ankylosing spondylitis (AS) is a type of common, chronic inflammatory disease that compromises the axial skeleton and sacroiliac joints, causing inflammatory low back pain and progressive spinal stiffness, over time some patients develop spinal immobility and ankylosis which can lead to a decrease in quality of life. The last few decades, evidence has clearly indicated that neutrophil also plays key roles in the progression of AS. However, the immunomodulatory roles and mechanisms of neutrophils in AS are poorly understood. T-cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) has been reported as an important regulatory molecule, expressed and regulated on different innate immune cells, plays a pivotal role in several autoimmunity diseases. Recent study indicates that Tim3 is also expressed on neutrophils. However, the frequency and roles of Tim3-expressing neutrophils in AS was not clear.
Objectives: In this study, we investigated the expression of Tim3 on neutrophils in AS patients and explored the correlation between the level of Tim3-expressing neutrophils and the disease activity and severity of AS.
Methods: Patients with AS were recruited from Guangdong Second Provincial General Hospital (n=62). Age/sex-matched volunteers as Healthy controls (HC) (n=39). The medical history, clinical manifestations, physical examination, laboratory measurements were recorded. The expression of costimulatory molecules including programmed death 1 (PD-1), Tim-3 on neutrophils were determined by flow cytometry. The mRNA expression of PD-1 and Tim-3 was determined by real-time PCR. The levels of Tim3-expressing neutrophils in AS patients were further analyzed for their correlation with the markers of inflammation such as ESR,CRP,WBC and neutrophil count(NE), as well as disease activity and severity of AS. The expression of Tim3 on neutrophils was monitored during the course of treatment (4 weeks).
Results: The expression of Tim3 on neutrophils in patients with AS was increased compared to the HC (Figure 1A). However, significant difference was observed in the frequency of PD-1-expressing neutrophils between AS patients and HC (Figure 1B). The expression analysis of Tim-3 mRNA, but not PD-1, confirmed the results obtained from flow cytometry (Figure 1C). The level of Tim3-expressing neutrophils in patients with AS showed an positive correlation with ESR, CRP and ASAS-endorsed disease activity score (ASDAS) (Figure 1D). Moreover, the frequency of Tim3-expressing neutrophils in active patients(ASDAS≥1.3) was increased as compare with the inactive patients (ASDAS<1.3) (Figure 1E). As shown in Figure 1F, the frequency of Tim3-expressing neutrophils decreased after the treatment.

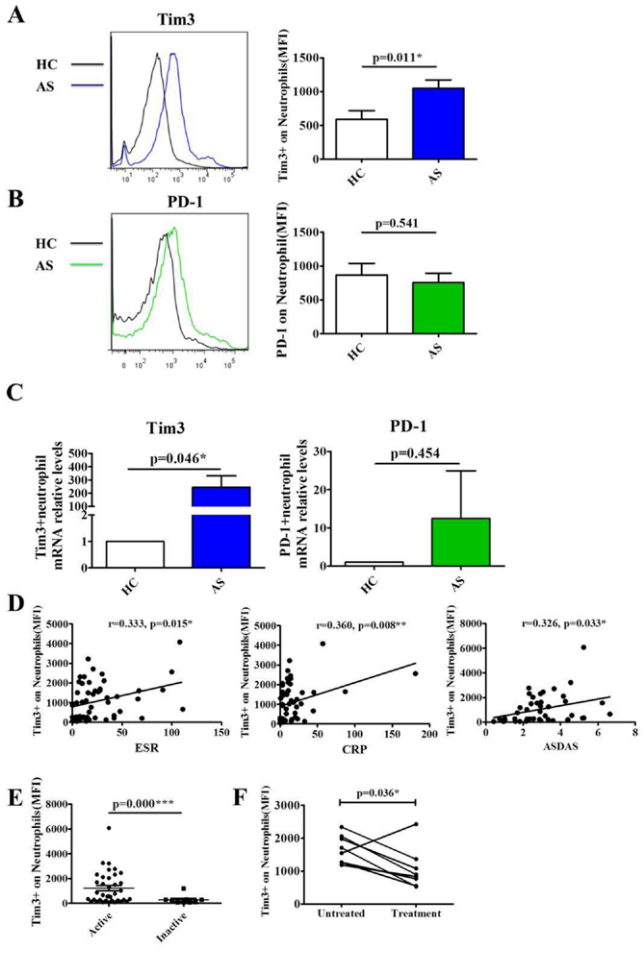

Figure 1. (A,B) The expression of Tim3 and PD-1 on neutrophils in AS and HC were determined by flow cytometry.**(C)** The expression of Tim3 and PD-1 on neutrophils in AS and HC were determined by RT-PCR.**(D)**The correction between Tim3-expressing neutrophils and ESR,CRP,ASDAS.**(E)** The expression of Tim3 on neutrophils in active and inactive patients.**(F)** Influence of treatment on the frequency of Tim3-expressing neutrophils.

Conclusion: Increased Tim-3 expression on neutrophils may be a novel indicator to assess disease activity and severity in AS, which may serves as a negative feedback mechanism preventing potential tissue damage caused by excessive inflammatory responses in AS patients.

REFERENCES:

[1] Han, G., Chen, G., Shen, B. & Li, Y., Tim-3: an activation marker and activation limiter of innate immune cells. FRONT IMMUNOL 4 449 (2013).

[2] Vega-Carrascal, I. et al., Galectin-9 signaling through TIM-3 is involved in neutrophil-mediated Gram-negative bacterial killing: an effect abrogated within the cystic fibrosis lung. J IMMUNOL 192 2418 (2014).

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.3516

POS0370

TYPE I INTERFERON PATHWAY ASSAYS IN PATIENTS WITH RHEUMATIC AND MUSCULOSKELETAL DISEASES - SYSTEMATIC LITERATURE REVIEW (SLR) AND DEVELOPMENT OF CONSENSUS TERMINOLOGY FROM A EULAR TASKFORCE

A. Burska[1], J. Rodriguez Carrio[2], P. G. Conaghan[1], W. A. Dik[3], R. Biesen[4], M. L. Eloranta[5], G. Cavalli[6], M. Visser[7], D. Boumpas[8], G. Bertsias[9], M. Wahren-Herlenius[10], J. Rehwinkel[11], M. L. Frémond[12], M. K. Crow[13], L. Ronnblom[5], E. Vital[1], M. Versnel[14]. [1]*University of Leeds, Leeds Institute of Rheumatic and Musculoskeletal Medicine, Leeds, United Kingdom*; [2]*University of Oviedo, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain*; [3]*Erasmus MC, Department of Medical Immunology, Rotterdam, Netherlands*; [4]*Charité University Medicine Berlin, Department of Rheumatology, Berlin, Germany*; [5]*Uppsala University, Department of Medical Sciences, Rheumatology, Uppsala, Sweden*; [6]*Vita-Salute San Raffaele University, Unit of Immunology, Rheumatology, Allergy and Rare Diseases, Milan, Italy*; [7]*EULAR, PARE Patient Research Partners, Amsterdam, Netherlands*; [8]*University of Crete, Medical School, Department of Internal Medicine, Heraklion, Greece*; [9]*University of Crete, Medical School, Department of Internal Medicine, Heraklion, Greece*; [10]*Karolinska Institutet, Division of Rheumatology, Stockholm, Sweden*; [11]*University of Oxford, MRC Human Immunology Unit, Oxford, United Kingdom*; [12]*Hôpital Necker-Enfants Malades, Immuno-Hématologie et Rhumatologie pédiatriques, Paris, France*; [13]*Hospital for Special Surgery, Weill Cornell Medical College, Mary Kirkland Center for Lupus Research, New York, United States of America*; [14]*Erasmus MC, Department of Immunology, Rotterdam, Netherlands*

Background: The interferon (IFN) pathway is a complex system with multiple proteins and diverse downstream effects on gene and protein expression. IFNs have been implicated in multiple RMDs. Despite significant potential, IFN assays have not progressed into clinical practice.

Objectives: To perform a SLR on IFN assays in RMDs and propose a consensus terminology.

Methods: OvidMedline, Embase and Web of Science were searched for reports of IFN and RMDs up to October 2019. Information about the properties of assays measuring type I IFN and measures of truth were extracted and summarised. Terminology was agreed through an interactive consensus process with reference to the existing evidence.

Results: 10037 abstracts were identified. 275 fulfilled eligibility criteria, and were used for data extraction. Some used more than one technique to measure IFN-I pathway activation. Hence, 275 papers generated data on 393 methods. There was great heterogeneity in the methods used and presentation of results. IFN-I pathway activation was measured using: qPCR (n=121), immunoassays (n=101), microarray (n=69), reporter cell assay (n=38), DNA methylation (n=14), flow cytometry (n=14), cytopathic effect assay (n=11), RNA sequencing (n=9), Plaque reduction assay (n=8), Nanostring (n=5), bisulphite sequencing (n=3). All papers fulfilled Face Validity. Due to lack of gold standard for IFN-I pathway activation, evidence of criterion validity was variable. Concurrent validity was presented for n=150 assays. The terminology used to describe aspects of type I IFN pathway activation was not consistent, so a consensus terminology for IFN research (Table 1) was proposed by the taskforce.

Table 1. Consensus terminology

Term	Abbreviation	Definition
Interferon	IFN	Proteins with anti-viral activity; IFNs are mediators of an anti-viral response. They belong to the Type I, Type II and Type III IFN families.
Type I interferon	IFN-I	The IFNs alpha, beta, omega, kappa, epsilon, secreted by any nucleated cell, and binding to the IFNAR, which is expressed on any nucleated cell.
Type II interferon	IFN-II	IFN gamma, mostly secreted by T cells, binding to the IFNGR, which is expressed on most leucocytes.
Type III interferon	IFN-III	IFN lambda, which are structurally more similar to IL-10 but share downstream signalling and gene expression with IFN-I.
Interferon-stimulated genes	ISGs	Genes whose expression is known to be upregulated by any kind of IFN. Individual ISGs may not exclusively represent Type I IFN pathway activation.
Type I Interferon pathway activation		Any evidence for function of the components of the Type I IFN pathway. This includes: secretion of a Type I IFN protein, binding to the IFNAR, initiation of JAK/STAT signalling pathways, expression of IFN-stimulated genes, expression of IFN-stimulated proteins.
Type I interferon pathway assay		An assay measuring one or more components of the Type I IFN pathway at a molecular or functional level.
Interferon stimulated gene expression signature		A qualitative description of coordinated expression of a set of ISGs that is indicative of Type I IFN pathway activation.
Interferon stimulated gene expression score		A quantitative variable derived from expression of a defined set of ISGs that is indicative of Type I IFN pathway activation.
Interferon stimulated protein score		A variable derived from expression of a defined set of soluble biomarkers known to be upregulated by IFN, although not specific for Type I IFN.
Interferonopathy		Monogenic diseases in which there is constitutive Type I IFN pathway activation with a causal role in pathology. The clinical picture may resemble rheumatic musculoskeletal diseases. However, most diseases with IFN pathway activation are not Interferonopathies.

Conclusion: Diverse methods have been reported as IFN assays and these differ in what elements of type IFN-I pathway activation they measure. The taskforce consensus terminology on type I IFN reporting should be considered for research and clinical applications.

Disclosure of Interests: Agata Burska: None declared, Javier Rodriguez Carrio: None declared, Philip G Conaghan: None declared, Willem A Dik: None declared, Robert Biesen: None declared, Maija-leena Eloranta: None declared, Giulio Cavalli: None declared, Marianne Visser: None declared, Dimitrios Boumpas: None declared, George Bertsias: None declared, Marie Wahren-Herlenius: None declared, Jan Rehwinkel: None declared, Marie-Louise Frémond: None declared, Mary K. Crow Consultant of: AstraZeneca, Bristol Meyers Squibb, Lilly, Shannon Pharmaceuticals, Grant/research support from: Gilead, Lars Ronnblom Consultant of: AstraZeneca, Edward Vital Speakers bureau: GSK, Consultant of: AURINIA, SANDOZ, GSK, AstraZeneca, Roche, Modus, Grant/research support from: AstraZeneca, Marjan Versnel: None declared

DOI: 10.1136/annrheumdis-2021-eular.3788