AB0134

SERUM LL-37, GALECTIN-3, AND TOLL-LIKE RECEPTORS-3 LEVELS DECREASE IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythematosus (SLE) is a systemic inflammatory disease characterized by heterogeneous clinical manifestations (1). Although there are significant developments with its pathogenesis, it is still not fully known. In recent years, pathways such as NETosis and plasmacytoid dendritic cell (pDC) activation have been emphasized in the pathogenesis of SLE (2, 3).

Objectives: In our study, we aimed to investigate serum LL-37, Galectin-3, and Toll-like receptors-3 (TLR)-3 levels, which are thought to be related to pathogenetic pathways in SLE patients.

Methods: 17 SLE patients and 33 healthy controls were included in the study. The clinical and laboratory features of the patients were determined. Serum LL-37, Galectin-3, and TLR-3 levels were determined by ELISA (enzyme-linked immunosorbent assay) method using the appropriate commercial kit, and the results were evaluated according to the manufacturer's instructions.

Results: The clinical and laboratory features of the groups are described in Table 1. In our study, serum LL-37, Galectin-3, and TLR-3 levels were decreased statistically significantly in SLE patients compared to healthy control ($p = 0.007$, $p = 0.002$, and $p = 0.008$, respectively).

Table 1. Clinical and laboratory features of the groups in the study

	Healthy control (n=33)	SLE (n=17)	p
Age (years)	34.1 ± 3.7	40 ± 11.4	0.05
Sex (n; female/male)	33/0	17/0	
LL-37 (ng/ml)	78.6 ± 92.6	14.2 ± 19.3	0.007
Galectin-3 (ng/ml)	25.5 ± 23.2	6.7 ± 7.6	0.002
Toll-like receptors-3 (pg/ml)	7893.4 ± 1041.3	916.2 ± 469.7	0.008

Conclusion: It is suggested that LL-37, galectin-3, and TLR-3 levels have various effects on NETosis and pDC activation pathways in SLE pathogenesis. In our study, low levels of serum LL-37, galectin-3, and TLR-3 in SLE patients suggest that they are associated with SLE pathogenesis.

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5782

AB0135

THE MITOCHONDRIAL-RETICULAR NETWORK (MRN) OF NEUTROPHILIC LEUKOCYTES OF SYNOVIAL FLUID (SF) OF PATIENTS WITH SLE AND RA

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Background:

Objectives: It has been established that in cells, in particular in neutrophilic leukocytes of SF, mitochondria form a mitochondrial-reticular dynamic spatial network (MRN). MRN is the epicenter of apoptosis, reflecting structural and functional changes in the immuno-complex pathology in SLE and RA.

Methods: SF was analyzed in patients: 10 SLE (43 ± 2.3 years), 13 RA (45 ± 1.6 years) and 8 donors (42 ± 3.7 years, postmortem). Neutrophilic leukocytes from the SF were isolated by standard methods and resuspended in a composition medium: 70 mM NaCl; 140 mM sucrose; 5.6 mM KCl; 10 mM pyruvate; 8 mM MOPS; pH = 7.4. The cell suspension was centrifuged for 5 min at 800 g. MRN was isolated by centrifuging the resulting supernatant for 15 min at 12 000 g. The resulting MRN fragments were resuspended in citrate-phosphate buffer (pH = 7.4) and used in experiments. The activity of adenosine monophosphate-activated protein kinase (AMPK) was evaluated by Western blotting. Quantitative determination of cytochrome C (Cyt C) was carried out by enzyme immunoassay method using the Human Cytochrome

c Platinum ELISA kit (eBioscience, USA). Active forms of oxygen free radicals (AFRF) were registered by EPR. The swelling rate of MRN fragments was determined spectrophotometrically at 540 nm. The electrophoretic mobility (EM) of MRN fragments was determined by the automatic microscope "Parmoquant-2".

Results: MRN of neutrophilic leukocytes of the SF undergoes significant adaptive rearrangements during the development of SLE and RA (tab.1). On average, the expression of biochemical indicators of autophagy (AMPK), apoptosis (Cyt C), necrosis (level of oxygen free radicals, low-amplitude swelling rate) increases by 2-3 times compared with the conventional norm. Particular attention should be paid to pathological changes in the electrokinetic potential of MRN, which determines the functional state of the SF as a whole as a colloidal system. Obviously, in SLE and RA, depletion of the energy of MRN (a sharp increase in the activity of AMPK), activation of free radical processes, disruption of intracellular ion homeostasis due to an increase in the rate of swelling of MRN as a manifestation of a compensatory-adaptive reaction. It ultimately leads to a decrease in electrokinetic properties of MRN. Thus EM is an integral indicator of physico-chemical properties and architectonics of MRN pointing to the development of autoimmune pathology.

Table 1. EXPRESSION OF INDUCERS OF AUTOPHAGY, APOPTOSIS, NECROSIS AND ELECTROPHORETIC MOBILITY OF MRN FRAGMENTS OF NEUTROPHILIC LEUKOCYTES OF SF IN SLE AND RA

Experience Terms	AMPK, cond.unit/mg protein	Cyt C, ng/ml	AFRF, unit/mg protein	Swelling rate of MRN, /min · mg protein	EM, m/v · sec
Donor (8)	0,51±0,05	23,7±5,4	7,3±2,4	0,177± 0,004	1,58± 0,07
SLE (10)	1,73±0,04**	49,3±6,5*	21,3±5,1**	0,435±0,005***	0,35±0,05***
RA (13)	1,25±0,07**	47,8±4,8*	15,7±4,3*	0,410±0,007***	0,41±0,07***

Notes: differences with the control norm: * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Conclusion: Endoplasmic stress occurs in SF cells during the development of SLE and RA, blocking of autophagy and apoptosis leads to a breakdown of neutrophilic leukocyte MRN, accumulation of high molecular products of tissue decay - phlogogens in the intercellular space, among which the expression in the context is characterized by proteins - chaperones Hsp 60-100. These processes are accompanied by a shift in the bioelectric homeostasis of MRN neutrophilic leukocytes, an increase in their swelling rate and a significant decrease in their electrokinetic potential. The described MRN reactions of neutrophilic leukocytes of the SF should be taken into account when developing pharmacologically induced apoptosis as a new approach in the treatment of autoimmune diseases

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.1258

AB0136

THE SUSTAINED POSITIVITY FOR ANTI-DSDNA ANTIBODIES FOSTERS THE ESTABLISHMENT OF AN ATHEROTHROMBOTIC STATUS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background:

Objectives: 1. This study, developed within the Innovative Medicines Initiative Joint Undertaking project PRECISESADS framework, aimed to identify specific molecular profiles involved in the enhanced CV-risk present in SLE patients and to analyze the relevance of the sustained positivity for anti-dsDNA on the establishment of their atherothrombotic status.

Methods: One hundred and twenty-four SLE consecutive patients (not including patients with associated antiphospholipid syndrome), belonging to the PRECISESADS project, were evaluated for the presence of CVD and its association with positivity for anti-dsDNA antibodies. A second cohort of 62 SLE patients was included, of which endothelial dysfunction, lipid profile, the presence of atheroma plaques (identified by a pathologic increase in the carotid intima media thickness -CIMT-), and the frequencies of anti-dsDNA positivity for 7 years, were evaluated. Serum inflammatory and oxidative stress biomolecules, and

NETosis-derived bioproducts were further evaluated by multiplex assay and specific commercial kits, respectively. Besides, miRNomes were identified using next-generation sequencing. Clinical significance of the biomolecules analyzed was explored by correlation/association studies with immunological and CV-risk features.

Results: A significant relationship among the incidence of CVD (i.e. thrombosis or cardiac involvement) and the positivity for anti-dsDNA antibodies was recognized in the first SLE cohort. Accordingly, in the second SLE cohort, significantly impaired micro-vascular endothelial function (identified by reduction of hyperemia post-occlusion area), increased atherogenic index and pathologic increase in the CIMT were assessed in patients positive for anti-dsDNA in relation to anti-dsDNA negative patients. Around a 65% of SLE patients displayed a sustained positivity for anti-dsDNA antibodies for more than 7 years. These patients showed a distinctive and specific molecular profile compared with patients that had remained negative for anti-dsDNA, including increased inflammatory profile (IL1B, IL2, IL6, IL17, EOTAXIN, FGF, GM-CSF, IFN γ , IP10, RANTES, TNF), enhanced oxidative status (lipoperoxides), and higher NETosis (nucleosomes, elastase). Levels of those biomolecules were closely interconnected and associated to their regulatory miRNAs, which accordingly exhibited differential expression in SLE anti-dsDNA(+) vs anti-dsDNA(-) patients. Finally, the frequency for positivity of anti-dsDNA significantly correlated both with markers of endothelial dysfunction and with the presence of atheroma plaques in SLE patients, pointing at the direct involvement of anti-dsDNA-Abs in the development of these processes.

Conclusion: 1. Positivity for anti-dsDNA antibodies confers a specific molecular profile linked to an enhanced CV-risk in SLE patients. 2. Moreover, the sustained positivity for anti-dsDNA antibodies fosters the establishment of an atherothrombotic status in these autoimmune patients.

Acknowledgments: Supported by the EU/EFPIA-IMI-JU PRECISESADS (n° 115565) and ISCIII (PI18/0837 and RIER RD16/0012/0015), Co-funded with FEDER.

Disclosure of Interests: Alejandra M. Patiño-Trives: None declared, Maria A Aguirre: None declared, Carlos Pérez Sánchez: None declared, Pérez Sánchez Laura: None declared, María Luque-Tévar: None declared, Iván Arias de la Rosa: None declared, Rafaela Ortega Castro: None declared, María del Carmen Abalos-Aguilera: None declared, Mario Espinosa: None declared, Pedro Seguí Azpilcueta: None declared, Jacques-Olivier Pers: None declared, Nuria Barbarroja Puerto Grant/research support from: ROCHE and Pfizer., Speakers bureau: ROCHE and Celgene., Marta Alarcon-Riquelme: None declared, Eduardo Collantes Estevez Grant/research support from: ROCHE and Pfizer, Speakers bureau: ROCHE, Lilly, Bristol and Celgene, Chary Lopez-Pedreria Grant/research support from: ROCHE and Pfizer.

DOI: 10.1136/annrheumdis-2020-eular.5216

AB0137 DIVERSITY ANALYSIS OF INTESTINAL FLORA IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythematosus (SLE) is a multiple systemic autoimmune disease and its pathogenesis is still not fully understanding. In recent years, there has been reports on the relationship between SLE and intestinal flora.

Objectives: To study the diversity and the intestinal flora intestinal microbes in patients with SLE and further provide new ideas for clinical treatment.

Methods: The stool samples of 28 patients with SLE and 125 normal healthy adults were collected. The 16S rRNA in the specimen was sequenced using the Roche/45 high-throughput sequencing platform, and the differences between the two groups were compared at the level of the phylum and genus.

Results: In SLE patients, as the picture show, the levels of fusobacteria, proteobacteria and TM7 were significantly higher ($P<0.05$) and the number of firmicutes was significantly decreased ($P<0.05$) than that of healthy controls at the phylum level. The percentage of bifidobacterium, collinsella, enterococcus, leuconostoc, streptococcus, bilophila were significantly higher ($P<0.05$) and the number of lachnospira, roseburia, gemmiger, devosia, desulfovibrio were significantly decreased ($P<0.05$) than that of healthy participants at the genus level.

Figure 1. the differences between patients with SLE and normal healthy adults were compared at the level of the phylum

Figure 2. the differences between patients with SLE and normal healthy adults were compared at the level of the genus

Conclusion: The diversity of intestinal flora in patients with SLE altered from that of normal population. The differences are likely to be one of the pathogenesis of lupus, which might provide theoretical foundation for the regulation of intestinal flora to treat autoimmune diseases such as lupus.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5943

AB0138 INCREASED CD38 EXPRESSION LEVELS ON IMMUNE CELL SUBSETS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Plasma Cells (PCs) are implicated in the pathogenesis of Systemic Lupus erythematosus (SLE) and their targeting proved a promising treatment modality. As there is a monoclonal therapeutic antibody targeting CD38 licensed for clinical use in multiple myeloma, plasma cell depletion via CD38 seems to represent a promising path in SLE treatment. While CD38 is highly expressed on plasmacells, it is present on the surface of subsets of T and B lymphocytes as well as myeloid cells.

Objectives: Here we aim to identify the differential expression of CD38 on peripheral blood leukocytes in SLE compared to healthy controls (HC) investigate the function of CD38+ T lymphocytes

Methods: We performed flow cytometry to investigate the expression of CD38 on peripheral blood mononuclear cells of SLE patients (n=36) and HCs (n=20). We additionally analyzed the expression of T lymphocytes within the urine of patients with lupus nephritis as well as the skin of SLE patients. We investigated the