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POS1414

TYPE I INTERFERONS INDUCE TIE2-MEDIATED ENDOTHELIAL CELL DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

Keywords: Systemic lupus erythematosus, Cytokines and chemokines, Cardiovascular disease

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Background: Endothelial cell (EC) dysfunction is a hallmark of Systemic Lupus Erythematosus (SLE) and has been generally accepted to be one of the important factors contributing to the higher risk of thrombosis and atherosclerotic events observed in SLE patients. Although the presence of traditional factors (smoking, diabetes, increased age, obesity) and the presence of autoantibodies are associated with atherosclerosis and thrombotic events, they do not completely explain the higher risk of these events in SLE, suggesting the existence of other mechanisms/factors. TIE2 is a tyrosine kinase receptor essential for vascular development and blood vessel remodeling through its interaction with its ligands angiopoietin-1 (Ang-1) and Ang-2. In homeostatic conditions, both Ang-1 and Ang-2 activate TIE2 signaling and induce vascular stabilization in a TIE1-dependent manner. However, inflammatory processes induce TIE1 cleavage, leading to the inhibition of Ang-1-induced TIE2 activation, and to the increase of Ang-2 now acting as a TIE2 antagonist, culminating in vascular dysfunction and EC activation[1,2]. Importantly, this process has been implicated in both atherosclerosis and thrombosis.

Objectives: As type I interferons (IFN-α and IFN-β) are key cytokines in the pathogenesis of SLE, the aim of this study is to determine whether these cytokines induce TIE2 signaling-mediated endothelial cell dysfunction.

Methods: Serum levels of Ang-1, Ang-2 and sTIE1 in SLE patients (n=48) and healthy control (HC, n=29) were measured by ELISA. Human Umbilical Vein EC (HUVEC) were stimulated with SLE serum (20%), IFN-α and IFN-β (1000 IU) for 5, 15, 30 min and 1, 2, 4, 6, 8, 12, 24, 48 and 72 hours and mRNA and protein expression of Ang-1, Ang-2, TIE1 and TIE2 were determined by quantitative PCR (qPCR) and ELISA, respectively. The phosphorylation of TIE2 was determined by Western Blot and HUVEC viability by calcein assay. The angiogenic capacity was measured by tube formation assay. Silencing assays were performed with siRNAs addressed to IFNAR1 and TIE1 receptors.

Results: Type I IFNs, mainly IFN-β, significantly reduced TIE1 and TIE2 levels. IFN-β stimulation significantly increased the secretion of the Tie1 ectodomain (sTie1). Both IFNs significantly reduced protein secretion of Ang-1 and Ang-2 at early time points (<4h). Furthermore, IFN-α and IFN-β stimulation reduced TIE2 activation (Figure 1). Both type I IFNs significantly reduced the viability of HUVEC. Serum increased Ang-2 and sTIE1 secretion levels in HUVEC at early time points (<1h). We found reduced levels of Ang-1 and elevated Ang-2 and sTIE1 in SLE patients compared to HC. Also, IFN-β induced tubule formation at short times (4h) and decreased at 24h. Remarkably, this effect was reversed by silencing the receptors Tie1 and IFNAR1.

Conclusion: Our results demonstrate that type I IFNs play a relevant role in the stability of endothelial cells by inhibiting Tie2 signaling, suggesting that these processes may be implicated in the cardiovascular events observed in SLE patients.

REFERENCES:

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Disclosure of Interests: None Declared.

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POS1415

ENDOTHELIAL INFLAMMATING AND MICHONDRIAL DYSFUNCTION IN THE ANTIPHOSPHOLIPID SYNDROME

Keywords: Anti-phospholipid syndrome, Cardiovascular disease

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Background: Inflammageing, vascular ageing and mitochondrial dysfunction are important contributors to cardiovascular risk in the rheumatic diseases. In antiphospholipid syndrome (APS), endothelial dysfunction is underpinned by a thrombo-inflammatory state mediated by antiphospholipid antibodies (aPL). The contribution of endothelial senescence and metabolic disturbances is not known.

Objectives: To assess senescence, inflammation and metabolic function in ex vivo blood derived endothelial colony forming cells (ECFC), a novel source of patient endothelium.

Methods: ECFC were isolated from PBMC of 17 thrombotic APS and 11 healthy control (HC) donors, cultured in endothelial growth media containing 20% FBS. ECFC frequency, days from PBMC seeding to first colony appearance and days to initial passage (from P0 to P1) were similar between APS and HC. ECFC were phenotyped at P4 by flow cytometry (CD31+CD144+CD146+CD14-CD45-) and assayed at P4-P5. Proliferation was measured by fluorescent Edu incorporation. Senescence was determined by staining for senescence-associated β-galactosidase activity (SA-β-gal) and nuclear DAPI, expressed as % senescent cells against total cell number. Mitochondrial function and glycolysis were assessed in live cells using extracellular flux assays (Seahorse). Molecular analysis was informed by RNAseq (5 APS, 5 HC) and targets validated in the extended cohort at the mRNA and protein level. Data was analysed by non-parametric Mann-Whitney, ANOVA and Spearman’s correlation tests.

Results: Impaired proliferative capacity and a greater proportion of senescent cells were observed in APS vs HC ECFC (median % senescent, 13.1% vs 7.9%) (p=0.02). RNA-seq analysis revealed 266 differentially expressed genes in APS ECFC vs HC ECFC (FDR<0.05). Among these, upregulated genes included elevated inflammatory cytokines, chemokines, cell adhesion and fibrosis-remodelling molecules. Target validation at the mRNA level confirmed a hyproallergic inflammatory phenotype in APS; elevated genes included CXCL5, IL1B, PTX3, SERPINE1. Controls showed lower expression of these genes.

Conclusion: Our results suggest that ECFC may serve as a new model for studying the contribution of ECFC to APS. ECFC may be a new model for studying the contribution of ECFC to APS.

Disclosure of Interests: None Declared.

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This work is dedicated to Professor Justin Mason who passed away in 2022.

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cardiovascular risk. Ongoing work is assessing the impact of

treatment

published studies associating vascular ageing and mitochondrial damage with

phenotype in ECFC from patients with CVE compared to no-CVE, agrees with

metabolically perturbed phenotype. Evidence for a more severe dysfunctional

endothelial ageing, defined by a hypoproliferative-senescent, inflamed and

function in CVE, while greater loss of proliferative capacity was observed in

no-CVE. Inflammatory markers varied between the two patient groups e.g. PTX3

was higher in CVE and CXCL10 in no-CVE. Collectively, these observations sug-

gest different underlying biological processes in patients with severe thrombotic

complications compared to those without.

Conclusion: We propose APS as a paradigm disease for immune-mediated

endothelial ageing, defined by a hyperproliferative-senescent, inflamed and

metabolically perturbed phenotype. Evidence for a more severe dysfunctional

phenotype in ECFC from patients with CVE compared to no-CVE, agrees with

published studies associating vascular ageing and mitochondrial damage with

cardiovascular risk. Ongoing work is assessing the impact of ex vivo treatment

with IgG aPL, cytokines and relevant drugs capable of modulating senescence

and immunometabolic processes.

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**IL-6, IL-8, TNF-α ARE SIGNIFICANTLY INCREASED IN CEREBROSPINAL FLUID AND ASSOCIATED WITH ALTERATIONS OF EYE SIGN IN PATIENTS WITH NEUROPSYCHIATRIC LUPUS ERYTHEMATOSUS**

**Keywords:** Biomarkers, Systemic lupus erythematosus, Cytokines and chemokines

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Background: Several pro-inflammatory cytokines such as interleukin (IL)-6, IL-8 have been implicated in the pathogenesis of in patients with neuropsychiatric systemic lupus erythematosus (NPSLE) [1]. The alterations of eye sign has been reported and correlated with disease activity in NPSLE [2].

Objectives: To ascertain whether the intrathreal levels of cytokines/chemokines relative to serum levels is associated with microvascular changes of eye sign in the patients with neuropsychiatric systemic lupus erythematosus (NPSLE).

Methods: NPSLE Patients (>18 years) were consecutively enrolled, for whom the cerebrospinal fluid (CSF) and serum samples were collected at the same time, 6 kinds of cytokine/chemokine concentrations in the CSF and serum samples were measured by Chemiluminescent Immunoassay. The diagnosis of NPSLE was evaluated and based on the consensus from rheumatologist, neurologist, radiologist and psychiatrist. Bulbar conjunctival microvascular changes in eye sign were performed and scored for all NPSLE patients by rheumatologist using our criteria. NP assessments were evaluating in all NPSLE patients by psychiatrist, including the mini-mental state examination (MMSE), self-rating anxiety scale (SAS), self-rating depression scale (SDS). Demographic and clinical data were compared between two groups and to identify potential predictors for NPSLE by using multivariable logistic regression analysis.

Results: 120 SLE patients were recruited (30 [24-41] years) including 30 NPSLE and 90 non-NPSLE. In multivariable logistic analysis, total score of eye sign in model 1 and ramified loops, microangioma and wound spot of eye sign in model 2 were predictors for NPSLE patients and were also increased in NPSLE [2]. This allowed the categorization of patients at the time of clinical assessment into four distinct groups: “type 1”: patients displaying only type 1 symptoms; “type 2”: patients displaying only type 2 symptoms; “mixed”: patients displaying both type 1 and type 2 symptoms; “minimal”: patients with no symptoms. This classification was built upon the SLEDAI score (type 1 symptoms), which is widely available in NPSLE studies, and on American College of Rheumatology (ACR) criteria for each (type 2 symptoms), which are less commonly used in SLE and are rarely available.

Objectives: Our aim was to develop a type 2 score derived from the Short-Form health survey (SF-36) to categorize SLE patients and to compare immunological and transcriptomic signatures between groups.

Methods: Seventeen items from the SF-36 were selected to build a type 2 score for 50 SLE patients (100 visits; LUPUCE cohort) and the SLEDAI was used to define type 1 symptoms. Patients were categorized in four groups: minimal (no symptoms), type 1, type 2 and mixed (both type 1 and type 2 symptoms).

Table 1. Multivariable logistic regression analysis of predictors for developing NPSLE.

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Model 2</th>
</tr>
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<tbody>
<tr>
<td>OR 95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>ACP</td>
<td>3.94 (0.83-18.89)</td>
</tr>
<tr>
<td>aPL</td>
<td>10.43 (0.65-166.61)</td>
</tr>
<tr>
<td>Rp</td>
<td>3.83 (0.47-314.1)</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>1.53 (0.98-2.38)</td>
</tr>
<tr>
<td>ESRI</td>
<td>0.97 (0.89-1.08)</td>
</tr>
<tr>
<td>MMSE</td>
<td>1.27 (0.79-2.03)</td>
</tr>
<tr>
<td>SAS</td>
<td>0.95 (0.87-1.04)</td>
</tr>
<tr>
<td>SDS</td>
<td>1.01 (0.92-1.10)</td>
</tr>
</tbody>
</table>

*Statistically significant at p < 0.05.ACCI: age adjusted Charlson Comorbidity Index; aPL: Antiphospholipid antibody; Rp: Raynauds’ phenomenon; SLEDAI: Systemic lupus erythematosus disease activity index; ESR: Erythrocyte sedimentation rate.

Figure 1. The association between IL-6, IL-8, TNF-α in CSF and predictors of eye sign for NPSLE.

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**Disclosure of Interests:** None Declared.

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**CATEGORIZATION OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS USING DISEASE ACTIVITY, PATIENT-REPORTED OUTCOMES AND TRANSCRIPTOMIC SIGNATURES**

**Keywords:** Systemic lupus erythematosus

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Background: Patients with systemic lupus erythematosus (SLE) display symptoms that are not always related to disease activity and may distort clinical trial results. Recently, a clinical categorization based on the presence of type 1 (inflammatory manifestations) and/or type 2 (widespread pain, fatigue, depression) symptoms has been proposed in SLE [1] and tested in a cohort of 212 patients [2]. This allowed the categorization of patients at the time of clinical assessment into four distinct groups: “type 1”: patients displaying only type 1 symptoms; “type 2”: patients displaying only type 2 symptoms; “mixed”: patients displaying both type 1 and type 2 symptoms; “minimal”: patients with no symptoms. This classification was built upon the SLEDAI score (type 1 symptoms), which is widely available in SLE studies, and on American College of Rheumatology (ACR) criteria for each (type 2 symptoms), which are less commonly used in SLE and are rarely available.

Objectives: Our aim was to develop a type 2 score derived from the Short-Form health survey (SF-36) to categorize SLE patients and to compare immunological and transcriptomic signatures between groups.

Methods: Seventeen items from the SF-36 were selected to build a type 2 score for 50 SLE patients (100 visits; LUPUCE cohort) and the SLEDAI was used to define type 1 symptoms. Patients were categorized in four groups: minimal (no symptoms), type 1, type 2 and mixed (both type 1 and type 2 symptoms).

Table 1. Multivariable logistic regression analysis of predictors for developing NPSLE.