AB0169

NEURONAL SURFACE P ANTIGEN (NSPA), THE CROSS-REACTING TARGET OF LUPUS ANTI-RIBOSOMAL P AUTOANTIBODIES, IS A POTENTIAL UBIQUITIN-LIGASE THAT REGULATES NMDAR FUNCTION

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Background: Anti-ribosomal P protein autoantibodies (anti-P) are found in 10%–15% of all patients with lupus¹ and their pathogenic role is supported by clinical studies showing association with psychosis² and cognitive deficit,³ as well as by experiments in rodents showing depression like behaviour,⁴ memory impairment⁵ and electrophysiological alterations.^{6,7} The Neuronal Surface P Antigen (NSPA) identified as an anti-P cross-reacting protein⁸ very likely mediates anti-P-driven brain diffuse dysfunctions through AMPAR and NMDAR synaptic transmission and plasticity.⁶

Objectives: To analyse molecular link between NSPA and NMDAR function involved in synaptic transmission and plasticity associated with memory, as a new approach to understand the neuropathogenic mechanism of anti-P antibodies.

Methods: Comparative studies in wild-type and NSPA knock-out mice on water maze performance (spatial memory), electrophysiology of CA3-CA1 glutamatergic transmission by field excitatory postsynaptic potential in hippocampal slices, biochemical analysis of synaptosomes and post-synaptic densities, including the mass and post-translational modifications (tyrosine phosphorylation and ubiquitylation) of relevant proteins.

Results: NSPA KO mice displayed the following alterations: 1) Impaired hippocampal-mediated spatial memory; 2) Decreased NMDAR-mediated glutamatergic transmission and long-term potentiation deficit, reflecting synaptic plasticity defects; 3) Selective lowered mass of GluN2A and GluN2B subunits of NMDAR in hippocampal synaptosomes and synaptic densities, accompanied by an enhanced mass and decreased ubiquitylation of the tyrosine phosphatase PTPN4 that interacts with NSPA and dephosphorylates the Tyr1472 of GluN2B of NMDAR. In addition, NSPA contains an APC-10 domain belonging to E3 ubiquitin ligases and a cotransfection assay in HEK293 cells demonstrated NSPA ubiquitylation as expected for these kinds of enzymes.

Conclusions: All together, these results suggest that NSPA is an ubiquitin ligase that having PTPN4 as a substrate regulates the tyrosine phosphorylation status and consequently the function of NMDAR at the synaptic region.

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AB0170

CCR9+ PATHOGENIC T HELPER CELLS FROM PRIMARY SJÖGREN'S SYNDROME PATIENTS ARE CHARACTERISED BY DEREGULATED PATHWAYS ASSOCIATED WITH EFFECTOR T CELL FUNCTION

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Background: CCR9 +Th cells produce large amounts of IFN-γ and IL-10, lack CXCR5 expression but have features similar to Tfh cells and the recently

described pathogenic PD1 +CXCR5 cells, including expression of ICOS, PD-1, IL-21 and Bcl6, but no CXCR5 expression. CCR9 Th cells and their ligand CCL25 are elevated in salivary glands of primary Sjögren's syndrome (pSS) patients. Since CCR9 Th cells strongly induce antibody production and robustly respond to IL-7, which is indicated to play an essential role in pSS pathogenesis and in formation of ectopic lymphoid structures, these cells may play an important role in pSS immunopathology.

Objectives: The goal of this study was to identify the molecular dysregulation of CCR9 Th cells in pSS patients.

Methods: CCR9+, CXCR5 + and CCR9-CXCR5- Th cells were sorted from peripheral blood of 7 healthy individuals and 7 pSS patients and RNA sequencing of these sorted cell subsets was performed. Computational analysis identified differentially expressed genes (DEG) and gene expression networks. Pathway enrichment analysis was performed in order to assess differentially regulated pathways. Target genes are technically validated and knockdown experiments will assess the functional role of identified targets.

Results: The sorted Th subsets could robustly be distinguished based on their transcriptomes. In the CCR9 +Th cell subset 2777 DEGs (1249 up and 1528 down) were identified between healthy controls and pSS patients, and 1416 and 1077 in the CXCR5 + and CCR9-CXCR5- subsets, respectively. Using network analysis 15 modules were constructed, consisting of genes showing coherent expression patterns. Four modules of interest were selected based on pathway enrichment analysis, revealing pathways involved in e.g. cytokine and chemokine production, proliferation and migration. DEGs of interest within these networks were selected, including upregulated expression of integrin αE, integrin α1 and downregulation of regulatory T cell associated genes FoxP3 and IL2RA. Expression of these markers is being validated using flow cytometry. In addition, knock-down of predicted key transcription factors is studied to reveal their role in the pathogenic potential of CCR9 +Th cells.

Conclusions: Transcriptomic analysis of CCR9 +Th cells from pSS patients revealed multiple dysregulated pathways previously shown to be involved in increased effector T cell function. Upregulation of genes associated with pathogenicity and downregulation of regulatory T cell associated genes were found in pSS patients. Targeting predicted key molecules might reveal (novel) therapeutic targets to halt pathogenic processes induced by CCR9 +Th cells.

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AB0171

B CELL SUBPOPULATIONS IN LUPUS NEPHRITIS PATIENTS: CORRELATIONS WITH DISEASE ONSET AND OUTCOMES

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Background: the relationship between B cells subsets distribution, clinical and laboratory parameters, therapeutic response and prognosis in lupus nephritis (LN) is still underestimated.

Objectives: The aim of our study is to establish the value of B cells subsets as biomarkers in patients with active LN than controls, in patients at the onset of renal manifestation than patients with renal flare, and finally in nephritic patients in relation to their clinical and laboratory characteristics at the baseline and during the course of the disease.

Methods: 50 patients with a diagnosis of LN have been evaluated every three months. Laboratory, immunological and disease activity data were collected at the baseline and at 6 (T6), 12 (T12), 24 (T24), 36 (T36) months and at the last follow-up (FU). Number of renal flares, time to renal remission and persistent proteinuria at the last FU were evaluated. B cell subsets were obtained through cytofluorimetry and classified using C27/IgD classification. The characterisation of B cells subsets was realised in 50 LN patients and 37 healthy controls.

Results: LN patients had a lower percentage of CD19 + cells than controls (9.2% vs 10.6%; p=0.01) as well as a lower percentage of memory unswitched cells CD27 + IgD+ (10.7% vs 15.3%; p<0.001) while patients had a higher percentage of plasmablasts and double negative memory cells CD27-IgD- (respectively 5.9% vs 1%; p<0.001% and 10.9% vs 4.1%; p=0.01).

No significant differences regardless B cells subsets were found between early LN patients and long ones as well as between LN patients at the onset and LN patients during renal flare. We found a correlation between an higher disease activity (assessed with SLEDAI 2K) and lower percentage of memory B cells IgD-CD27+ (p=0.02). Double negative B cells CD27-IgD- tended to be correlated with an higher disease activity. Of interest the correlation between persistent proteinuria detected during the follow-up and a lower percentage of plasmablasts at the baseline (p=0.01).

Conclusions: The alteration of B cells subsets is an early event in LN without differences regardless the period of renal involvement (nephritic onset or later LN development). The association between persistent proteinuria and a lower percentage of plasmablasts at the baseline could be a negative prognostic factor considering the correlation between persistent proteinuria and worse renal outcome.

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AB0172 THE RELATIONSHIP BETWEEN THE DIFFERENT TYPES OF CELL DEATH IN SYSTEMIC CONNECTIVE TISSUE DISEASES

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Objectives: To study the basic mechanism of cell death (autophagy, apoptosis and necrosis) typical of patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSD). During systemic connective tissue diseases and especially in SLE, a close relationship between various types of cellular death is observed.

Methods: 7 SLE-, 10 RA-, 10 SSD patients' and 5 donors' sera were studied. The level of Ca ions was registered by the method of atomic absorption spectrometry. Adenosine monophosphate-activated protein kinase (AMPK) was estimated by the Western blotting method. The activity of ATP-ase was measured spectrophotometrically. In order to find out the level of p53 protein, the immune-enzyme "Human p53 Platinum ELISA" method was employed. The quantity of hemoprotein (Cyt c) and the level of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) was measured by employing the immune-enzyme method.

Results: Assessing the functional activity of AMPK is an specific marker and a strategic biopower regulator of autophagy, as well as a specific indicator of red-ox cellular potential. In systemic connective tissue diseases, the oxidative stress is matched by urinary calcium and a decrease in the level of calcium in the blood. It reflects the level of seriousness of osteoporosis especially in case of RA. Molecular chaperones (HSP) play a key role in the changing of the way of cellular death. The family of chaperones HSP60 – HSP 100 shows ATPase activity which is most distinct during SLE and RA. The de-energisation of cells during systemic connective tissue diseases and the disappearing of the link between respiration and oxidative phosphorylation lead to proapoptotic protein – Cyt c is being released from mitochondria. High levels of Cyt c reflect cellular mitochondrial apoptosis and show the growing of hypoxia in SLE and RA. The level of protein p53 – a biological marker of apoptosis – is expressed when the DNA is destroyed. It reflects a higher level of the oxidative damage done to the DNA and the extent of the oxidative stress in SLE. This is also evidenced by the data collected by finding out the level of 8-OH-dG which is a biological marker of the free-radical damage of the DNA.

Comparison of markers of autophagy, apoptosis and necrosis in sera in SLE, RA and SSD: AMPK activity (units-mg of protein), the total level of ATP-ase active of HSP-60 – HSP-100 (nmol Pi/min-mg protein), (mM/l), quantity of (ng/ml): Cyt c, p53-protein, 8-OH-dG

Abstract AB0172 – Table 1

Groups	AMPK	ATP-ase	Cyt c	p53 protein	8-OH-dG
SLE	8,7 ±0,3	8,4±0,3 ±0,12	39,7 ±1,4	1,45±0,03	27,9±3,2
RA	5,7 ±0,2	7,9±0,2 ±0,24	26,8 ±2,3	1,27±0,04	24,2±2,3
SSD	2,8 ±0,4	5,1±0,2 ±0,09	14,9 ±2,6	0,98±0,03	18,6±3,4
Donor	1,4 ±0,3	4,0±0,7 ±0,32	11,7 ±0,3	0,73±0,07	12,56 ±2,4

Conclusions: Chaperone-mediated induction of the immune response by autophagy, an evolutionary enshrined only in mammals, perhaps, is the central link in the pathogenesis of systemic connective tissue diseases.

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AB0173 OVERWHELMING INFLAMMATION INCREASED SUSCEPTIBILITY OF SLE-PRONE MICE TO PULMONARY BACTERIAL INFECTION

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Background: Aside from the disease itself, infections represent the major cause of morbidity and mortality in systemic lupus erythematosus (SLE) patients. Inherent defects in immune system play an important role in increasing rates of infection. However, the underlying mechanism of this deficiency remains largely unknown.

Methods: Lupus-prone mice B6/lpr were anaesthetized and infected with 1×10⁸ CFU of *Haemophilus influenzae* (Hi) intranasally. Then bacterial clearance, body weight change and lung pathology were monitored. Apoptosis of lung cells was analysed by TUNEL assay. Both innate and adaptive immune response in the lung cells determined by flow cytometry. Cytokines in the bronchoalveolar lavage fluid (BALF) were measured by ELISA.

Results: Although both wild-type (WT) and B6/lpr mice survived after pulmonary Hi infection, a delay of bacterial clearance and inflammatory resolution was observed in B6/lpr mice. Tissue damage was more severe in the lungs of B6/lpr mice, as more apoptotic cells were detected on Day2 after infection. Cells from lupus-prone lungs produced more pro-inflammatory cytokines IL-6, MCP-1 and KC. TNF-α is comparable between the two groups. NK, γδ T and CD4 T cells are required for control bacterial infection. We that compared with WT controls, in response to infection fewer NK cells were detected in B6/lpr lungs. The numbers of γδ and CD4 T cells were not different, but their ability to secrete IFN-γ was significant lower in B6/lpr mice.

Abstract AB0173 – Figure 1

Conclusions: The increased susceptibility of SLE-prone mice to pulmonary *Haemophilus influenzae* infection may due to the elevated inflammatory responses and the deficient production of IFN-γ by immune cells.

Disclosure of Interest: None declared

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AB0174 TNF-A MODULATES MICROGLIA ACTIVATION VIA NF-KB ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS WITH DEPRESSION

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Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease accompanied by damage to a variety of tissue injuries, such as joints, kidney, and the peripheral and central nervous systems (CNS).¹ The diffuse CNS lupus manifests with a diverse array of neuropsychiatric symptoms that range from headaches, anxiety, depression, to cognitive impairment and, in rare cases, psychosis.² Additionally, up to 24% of SLE patients will display