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Background: Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease that is potentially fatal. There is an unmet need to improve current therapies. In patients with SLE, we observed that serum CXCL5 levels were significantly lower than healthy control subjects and negatively correlated with disease activity⁽¹⁻⁹⁾.

Objectives: The aim of this study is to elucidate the effect of supplemental serum CXCL5 in abrogating the pathological processes of SLE.

Methods: Ten doses of exogenous CXCL5 (3µg/kg) was administered to 16-week-old Fas^{br} mice weekly by intravenous injection. Mice were monitored for 10 weeks. Splenic immune profile was measured by flow cytometry. Circulating cytokine and immunoglobulin profile were detected by Luminex technology. Renal function was evaluated by urinary spot albumin creatinine ratio. In situ renal immune cell infiltration and complement 3 deposition were detected by Haematoxylin and Eosin (H&E) and immunohistochemistry staining. The molecular pathways involved were examined by RNA sequencing.

Results: In Fas^{br} mice, intravenous administration of exogenous CXCL5 significantly improved mouse survival with concomitant reduction of autoantibody secretion, proteinuria, complement 3 deposition, neutrophil infiltration and lupus nephritis classes. Through evaluating the changes of immune profile, cytokine profile and molecular pathways, we found that intravenous CXCL5 reduced inflammation via an orchestral effect of regulating neutrophil trafficking and modulating helper T cell-mediated immune response. Pharmacokinetic and real-time Polymerase Chain Reaction studies further demonstrated that this orchestration was triggered by a cascade reaction - restoring vascular under-expressed CXCL5 by an exogenous stimulation, re-establishing the normal serum levels of endogenous CXCL5 and reverting the CXCL5 chemokine gradient between inflamed tissues and blood circulation.

Conclusion: Managing the dysregulation of CXCL5 by exogenous supplement may provide a new option for SLE therapy.

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POS0418

SPLenic EXTRAMEDULLARY HEMATOPOIESIS IS OMNIPRESENT AND CORRELATES WITH DISEASE SEVERITY IN THE LUPUS NZB/W F1 MURINE MODEL

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Background: Extramedullary hematopoiesis (EMH) is increasingly recognized as an integral component of systemic inflammatory diseases; compared to their bone marrow counterparts, hematopoietic progenitors of EMH have an enhanced role in target organ damage^{1,2}. We have found that β-glucan -a non-specific inducer of reprogramming of innate immunity- results in dramatic EMH with marked increase in Long-Term (LT)-HSCs, massive splenomegaly and worsening of nephritis in the NZB/W F1 lupus murine model (unpublished data).

Objectives: To investigate EMH's time course and contribution to inflammatory target-organ damage (kidney) in the NZB/W F1 lupus murine model.

Methods: Spleens and kidneys were isolated from female NZB/W F1, at pre-nephritic stage (3-month-old) and nephritic stage (>6-month-old), and age/sex matched C57BL/6 (WT controls). Single-cell suspensions of spleens were analyzed by flow cytometry for Hematopoietic Stem and progenitor cells (HSPCs) phenotyping. Formalin-fixed and paraffin-embedded sections of spleens and kidneys were stained with conventional histological stains (H&E, Silver, Trichrome Masson). Spleens were histologically assessed for the presence of EMH and kidneys were assessed for activity and chronicity through the NIH Lupus nephritis scoring system.

Results: Histological analysis revealed that NZB/W F1 mice at the nephritic stage display massive splenomegaly with concomitant expansion of the red pulp, increased presence of megakaryocytes and disorganized splenic architecture. This is further corroborated by the flow cytometry analysis which demonstrated a significant increase of all HSPCs subsets (Long-term/Short-term Hematopoietic Stem Cells and Multipotent progenitors) compared to the C57BL/6 WT controls at nephritic stage. The degree of HSPC expansion and splenic architecture disorganization correlates strongly with the activity of lupus nephritis as quantified by the NIH scoring system. Of note, evidence of splenic EMH were present even in 3-month-old animals before overt nephritis ensues.

Conclusion: Extramedullary hematopoiesis is present before overt nephritis and is dramatically expanded at the nephritic stage of the NZB/W F1 mouse model. The degree of EMH positively correlates with the severity of lupus nephritis. These data support a pathogenic role of EMH, and spleen derived HSPCs, in driving lupus nephritis.

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POS0419

ABERRANT SPLICEOSOME AND ALTERED EXPRESSION OF IFN-RESPONSE RELATED GENES ARE HALLMARKS OF MONOCYTES FROM LUPUS PATIENTS WITH RENAL DISEASE

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Background: To date, novel mechanisms such as the involvement of splicing machinery components in lupus nephropathy and its interplay with the transcriptome in innate immune cells have not been evaluated.

Objectives: 1- To identify altered transcriptomic signatures associated with the immune response of monocytes from SLE patients and its association with clinical features. 2- To evaluate the role of the spliceosome linked to the transcriptomic profile of SLE monocytes. 3- To analyze mechanistically the impact of

anti-dsDNA antibodies (Ab) and the modulation of the spliceosome in the SLE monocytes activity.

Methods: Sixty SLE patients and forty healthy donors (HD) were included in the study. Infiltration rate of myeloid cells and its association with clinical features were analyzed in kidney biopsies by Immunohistochemistry. In parallel, circulating monocytes were purified from peripheral blood by immune-magnetic selection. The expression of a set of 770 genes related to autoimmune/inflammatory diseases was evaluated using NanoString Technologies. The levels of the main 45 components of the splicing machinery were further analyzed in these samples using a microfluidic qPCR array (Fluidigm). An extensive clinical/serological evaluation was also performed, comprising disease activity, renal involvement parameters, autoAb profile, and the systemic inflammatory status (27-plex Assay). Finally, *in vitro* studies involving anti-dsDNA-IgG Ab treatment and over/down-expression of splicing machinery components were carried out to analyze their effects in the monocyte activity.

Results: Infiltration of CD68 expressing cells was confirmed in kidney biopsies and associated with parameters of kidney failure (C3/C4, chronic index), highlighting the key role of the myeloid compartment in lupus nephropathy. Gene expression profiling recognized 156 genes differentially expressed in SLE monocytes compared with HDs, including 87 genes up-regulated and 69 down-regulated. Functional analysis showed that most dysregulated genes were associated with the IFN response (i.e. IFIT1, IFI44, IFI44L, RSAD2). In parallel, the altered expression of 27 spliceosome components was demonstrated in SLE monocytes compared with HD, including 3 up-regulated and 24 down-regulated. Correlation studies demonstrated that the aberrant expression of splicing machinery components was linked to the altered interferon signature and the plasma inflammatory profile. This aberrant profile at molecular level was associated with the disease activity status, anti-dsDNA positivity and C3/C4 levels. Interestingly, SLE patients with renal disease displayed a simultaneous alteration of both, the IFN and the spliceosome signatures in monocytes, along with an enlarged pro-inflammatory profile in plasma. Logistic regression models that integrated the concomitant alteration of some splicing machinery components and IFNs genes identified lupus nephritis patients with high accuracy. Mechanistic studies showed that *in vitro* treatment of monocytes from HDs with anti-dsDNA promoted a concomitant deregulation of the IFN signature and the expression of several spliceosome components (i.e. PTB, RBM17, RNU6ATAC). Finally, the over/down-expression of selected spliceosome components (PTB and RBM17) in monocytes from SLE patients reduced the active release of inflammatory cytokines and their adhesion capacity.

Conclusion: 1) Monocytes from SLE patients with renal involvement exhibit a remarkable alteration of genes associated with the IFN response, further linked with the aberrant expression of several splicing machinery components. 2) Anti-dsDNA promoted the dysregulation in monocytes of both the IFN and spliceosome signatures, along with an active release of pro-inflammatory mediators. 3) The modulation of key splicing components in monocytes from SLE patients reduce their pro-inflammatory status and migration capacity. Ongoing studies may provide novel biomarkers and therapeutic tools to treat lupus nephropathy.

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POS0420 DYSREGULATED CD38 EXPRESSION ON PERIPHERAL BLOOD IMMUNE CELL SUBSETS IN SLE

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by pathogenic antinuclear autoantibodies, which are secreted by autoreactive plasma cells. Among novel plasma cell-depleting strategies, CD38 has been identified as promising target. The monoclonal anti-CD38 antibody daratumumab is approved for treatment of multiple myeloma and provided a therapeutically relevant depletion of plasma cells in patients with SLE¹.

Objectives: Beyond plasma cells, CD38 is widely expressed across innate and adaptive immune cells and additional cellular targets of anti-CD38 treatment, especially in patients with SLE, are largely unknown. Therefore, this study aimed to systematically characterize the expression of CD38 in peripheral blood leukocytes to identify potential target cells of CD38-directed therapies that may contribute to or limit therapeutic benefits in SLE.

Methods: We analyzed the expression of CD38 on peripheral blood leukocytes in two different cohorts, comprising a total of 56 SLE patients and 39 healthy controls, by flow and mass cytometry. CD38 expression levels across major immune cells were analyzed for changes between controls and SLE, as well as for correlation across immune cell lineages, and with clinical and serological disease parameters.

Results: We detected increased CD38 expression levels on circulating NK cells, plasmacytoid dendritic cells, CD4⁺ and CD8⁺ memory T cells, as well as IgD⁺CD27⁺ and marginal zone-like B cells in SLE compared to healthy controls. In myeloid and NK cells, CD38 expression was associated with an activated cellular phenotype, reflected by co-expression of molecules such as HLA-DR, CD11c or Syk. In the B cell compartment, IgA⁺ plasmablasts and plasma cells expressed more CD38 than their IgA⁺ counterparts. Also, HLA-DR^{high} plasmablasts showed higher CD38 expression compared to HLA-DR^{low} plasma cells. The strongest differences in CD38 expression between controls and SLE were found in CD8⁺ central and effector memory T cells. Additionally, we detected an expansion in CD38^{high} and CD38^{int} cells in the T cell memory compartment, with some patients showing distinctly increased expression values. We observed a high intra-individual correlation of CD38 expression across immune cell lineages, yet without correlation of CD38 expression levels with clinical activity (SLEDAI-2K), serological markers of SLE or the type I interferon surrogate marker CD169 (SIGLEC-1).

Conclusion: Our data indicate that not only pathogenic plasma cells are potential target cells of CD38-targeting antibodies. The highly dysregulated CD38 expression across innate and adaptive immune cells in SLE could be of pathophysiological importance with respect to the potential efficacy and side effects of such therapies. Since CD38 expression did not correlate with disease activity, it may be assumed that it is not a response protein solely induced and modulated by type I interferons. Nevertheless, our comprehensive characterization of CD38 expression in the immune system might have important implications for personalized approaches with emerging CD38-directed therapeutics.

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POS0421 COMBINED ANALYSIS OF METABOLIC AND TRANSCRIPTOMIC KIDNEY PROFILES OF NZW/B-F1 MURINE LUPUS UNCOVERS BIOLOGICAL MECHANISMS PRECEDING THE ONSET OF NEPHRITIS

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Background: Metabolic pathways are important regulators of immune differentiation and activation in kidneys. Kidneys directly impact systemic metabolism, circulating metabolite levels, and express intrinsic metabolic activity. The integration of renal metabolomic and transcriptomic profiles may unravel unique gene-metabolite pairs of biological significance in lupus nephritis (LN).

Objectives: To decipher gene-metabolite signatures at both pre-nephritic and nephritic stages of lupus.

Methods: Kidneys were isolated and snap-frozen after perfusion from female NZB/NZW-F1 lupus mice at the pre-nephritic (3-month-old) and nephritic (6-month-old exhibiting ≥ 100 ng/dL of urine protein) stage of lupus (n=6/group). Age-matched female C57BL/6 mice were used as healthy controls. Sample extracts were used for RNA sequencing and ¹H-NMR spectroscopy metabolic profiling. DESeq2 was used to identify differentially expressed genes. Univariate analysis was used to reveal metabolic differences characteristic for nephritis.

Results: Comparative transcriptomic analyses uncovered multiple transcripts related to metabolic pathways: In pre-nephritic kidneys, lipid metabolism, cellular respiration, TCA cycle, amino acid metabolism processes were overrepresented in the upregulated genes while in nephritic kidneys, amino acid metabolism processes were overrepresented among the downregulated genes (Figure 1). ¹H-NMR analysis revealed a total of 49 metabolites. Comparison of the metabolic levels of nephritic and pre-nephritic animals revealed that ADP, ATP, NAD⁺, Taurine and Myo-inositol decreased, while Thr increased significantly. The comparison to corresponding control animals, demonstrated that only myo-inositol increased significantly. Integration of kidney metabolomics and transcriptomics indicated the involvement of processes related to glutathione metabolism, leukocyte trans-endothelial migration and antigen presentation during the established renal disease stage.

Conclusion: The combined transcriptomics and metabolomics analysis revealed metabolic derangements in lupus-affected kidneys both during subclinical and overt LN. Deregulated tissue-levels of taurine and myo-inositol at the subclinical stage of the disease suggest aberrant renal biochemistry preceding the development of overt LN that may directly impact systemic metabolism and circulating metabolite levels.