was used to measure the expression of 12 interferon signature genes in the extracted RNA. The study was approved by the University Research Ethics Committee.

Results: 92.4% of the cohort studied were female. 58.7% were receiving vitamin D3 supplementation at a mean dose of 1031IU daily. 27.2% had vitamin D insufficiency (25-hydroxyvitamin D 21-29ng/ml) and 15.2% were vitamin D deficient (25-hydroxyvitamin D <20ng/ml). Mean serum 25-hydroxyvitamin D was 30.75ng/ml (standard deviation 9.53ng/ml). Median SLEDAI-2K was 4 (range 0-12). Serum 25-hydroxyvitamin D had a significant negative correlation with body mass index (BMI) ($R=-0.258$, $p=0.006$) but there was no significant negative correlation with SLEDAI-2K or with the expression of the interferon signature genes. The expression of most interferon signature genes measured (IFI35, OAS1, MX1, IFITM1, STAT2, IFIT3, IFIT1, STAT1, SOCS1) had a significant positive correlation with SLEDAI-2K.

Conclusion: This study did not show a significant relationship between serum vitamin D level and disease activity. In keeping with this, there was no significant negative correlation between serum 25-hydroxyvitamin D and interferon signature gene expression. Further prospective studies and randomised controlled trials are required to study this relationship in greater depth.

References:

- [1] Kamen DL, Cooper GS, Bouali H, Shaftman SR, Hollis BW, Gilkeson GS. Vitamin D deficiency in systemic lupus erythematosus. *Autoimmun Rev*. 2006; 5: 114-7.
- [2] Sahebari M, Nabavi N, Salehi M. Correlation between serum 25(OH)D values and lupus disease activity: an original article and a systematic review with meta-analysis focusing on serum VitD confounders. *Lupus* 2014; 23: 1164-77.
- [3] Arasappan D, Tong W, Mummaneni P, Fang H, Amur S. Meta-analysis of microarray data using a pathway-based approach identifies a 37-gene expression signature for systemic lupus erythematosus in human peripheral blood mononuclear cells. *BMC Med*. 2011; 9: 65.

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THU0236

EFFICACY AND SAFETY OF NON-MITOGENIC ANTICD3 ANTIBODY ADMINISTRATION IN THE TREATMENT OF LUPUS-PRONE MICE

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Background: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by the production of autoantibody and systemic tissue damages including glomerulonephritis. Immune responses mediated by autoreactive T-cells, as well as by autoantibody, is involved in the development and progression of end-organ damages¹. Biologic agents which manipulate T-cell function such as CTLA4-Ig and anti-CD40L have been revisited and tried to treat human SLE, however, both of them failed to demonstrate efficacy.

A mouse specific anti-CD3ε mAb, clone 145-2C11 (2C11) is known to be immunosuppressive by down-modulation of TCR and depletion of T-cells². Administration of Fc-deleted 145-2C11 F(ab')₂ to lupus-prone mice was reported to reduce lymphadenopathy and prolong survival, but had no significant effect on anti-DNA antibody titer³. The mechanisms by which 2C11 ameliorates lupus are still unclear.

In this study, we used non-mitogenic Fc-modified silent 145-2c11 (2C11S), which disables interaction between target cells and Fc receptor-bearing cells, abolishes antibody directed cytotoxicity, and has longer half-life than F(ab')₂. 2C11S is expected to exert its effect in safe and stable as compared with functional parent-2C11 (2C11P) antibody.

Objectives: The purpose of our study is to clarify the difference between 2C11S and 2C11P and to examine their therapeutic effects against murine lupus-prone (NZB/W F1) mice.

Methods: 20 µg of 2C11P (absolute antibody), 2C11S (absolute antibody), or isotype control immunoglobulin G1 κ (IC)(BioLegend) were administered intraperitoneally to C57BL/6 mice. The difference of their action on T-cells were evaluated in a time series from peripheral blood. Plasma cytokine levels were measured within 24 hours after antibody administration.

In NZB/W F1 mice from weeks 10 or 20, 2C11P, 2C11S, and IC were administered (100 µg / week, 4 times, intraperitoneally). Plasma anti-dsDNA antibody titer, spleen and kidney blood cell subpopulation, and histology of renal tissue were evaluated before and/or after treatment.

Results: Duration of reduced TCR expression in 2C11S group was approximately twice as long as that in 2C11P group, and the levels of plasma TNF-α was not increased in 2C11S group while significant increase was observed in 2C11P group (IC; mean 48.3 ± SD 16.7 pg/ml, 2C11S; 57.9 ± 6.12, 2C11P; 168 ± 50.6, IC VS 2C11S; $p>0.99$, IC VS 2C11P; $p=0.03$, ANOVA).

In NZB/W F1 mice, the number of follicular helper T (Tfh) cells in spleen significantly decreased in 2C11S group (IC; median 9.0×10^4 [interquartile range 8.5×10^4], 2C11S; 1.8×10^4 [1.0×10^4], 2C11P; 1.0×10^5 [9.4×10^4], IC VS 2C11S; $p=0.03$, IC VS 2C11P; $p>0.99$, Kruskal-Wallis). The number of germinal center B (GCB) cells in spleen also decreased in 2C11S group (IC; 1.2×10^5 [1.7×10^5], 2C11S; 9.0×10^3 [2.3×10^4], 2C11P; 8.0×10^4 [2.3×10^5], IC VS 2C11S; $p=0.03$, IC VS 2C11P; $p>0.99$). The number of infiltrating CD4⁺T-cells in kidney significantly reduced in 2C11S group (IC; 3.4×10^3 [1.0×10^4], 2C11S; 6.4×10^2 [8.8×10^2], 2C11P; 1.2×10^3 [4.4×10^3], IC VS 2C11S; $p=0.048$, IC VS 2C11P; $p=0.23$). In addition, the rate of increase in anti-dsDNA IgG titers significantly decreased in 2C11S group (IC; 2.3 [1.3], 2C11S; 0.9 [1.0], 2C11P; 1.3 [1.4], IC VS 2C11S; $p=0.03$, IC VS 2C11P; $p=0.24$). Finally, glomerular hypercellularity was markedly alleviated only in 2C11S group (IC; 4.4×10 [8.4], 2C11S; 3.8×10 [1.1], 2C11P; 3.9×10 [8.2], IC VS 2C11S; $p=0.02$, IC VS 2C11P; $p=0.57$).

Conclusion: 2C11S did not induce cytokine release with maintaining longer effect on TCR down-modulation. 2C11S reduced autoantibody production by suppressing GCB differentiation, possibly through down-regulation of Tfh cell number. Consequently, 2C11S ameliorated lupus nephritis. On the other hand, 2C11P did not show therapeutic effect.

References:

- [1] George C Tsokos. et al. *Nat. Rev. Rheum* (2016) 12: 716-730.
- [2] Kuhn C. et al. *Immunotherapy* (2016) 8: 889-906.
- [3] Henrickson M. et al. *Arthritis Rheum* (1994) 37: 587-589.

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THU0237

UNCOVERING DIFFERENCES BETWEEN THE SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) AND HEALTHY IMMUNOMES USING MULTI-PARAMETRIC INTERROGATION

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Background: Systemic lupus erythematosus (SLE) is a complex, systemic autoimmune disease that interferes with the balance between regulation and immunity, resulting in immune system dysfunction. Disease course is unpredictable due to alternating remissions and flares¹.

Disease activity and treatment response are measured with composite scores such as the SLE Disease Activity Index (SLEDAI)². However, disease heterogeneity can impede reliable patient assessments. Mechanistic insights into SLE are required for better assessment.

Current studies are mainly descriptive, and a complex disease like SLE is best interrogated with multi-parametric, holistic approaches such as mass cytometry (CyTOF).

Objectives: (1)To characterise immune signatures of newly diagnosed SLE patients and in the process:

a.Study the roles of B and T cells in SLE

b.Gain a holistic understanding of the adaptive immune response

(2)To compare immunological profiles of newly diagnosed SLE patients with age-matched healthy controls

We hypothesise that significant differences exist between immunomes of newly diagnosed SLE and healthy subjects.

Methods: Peripheral blood mononuclear cells (PBMCs) of 5 SLE subjects (median age 125 months) were tested with CyTOF. Data was uploaded to an online analytical platform, the Extended Polydimensional Immunome Characterization (EPIC) discovery tool, for comparison with 51 age-matched controls in its database.

Subsequently, normalization and FlowSOM (Flow cytometry analysis by Self-Organising Maps) clustering to 50 nodes were performed with 37 functionally and phenotypically important immune markers. The Mann-Whitney U test identified significantly different cluster frequencies.

Results: Correspondence analysis comparing global differences in cluster frequencies showed segregation of SLE subjects away from healthy controls. Multiple significant differences were identified ($p < 0.05$). Notably, a memory CD4⁺CD152⁺PD1⁺ T cell subset (CD4⁺CD152⁺PD1⁺CD45RO⁺CD25⁺FoxP3⁺) was enriched in SLE (median: 2.17%, interquartile range: 1.66 to 7.74% of CD45⁺ PBMCs) versus control (1.34%, 1.06 - 1.58%; $p = 0.00267$). Expression

of these known checkpoint inhibitors (PD1, CD152) could be important for SLE immunopathogenesis.

Secondly, the innate lymphoid cell 2 (ILC2) subset (Lin⁺CD7⁺CD25⁺CD127⁺GATA3⁺) was markedly depressed in SLE (0.11%, 0.1 - 0.255%) versus control (0.41%, 0.25 - 0.55%; $p = 0.0293$). ILC2s protect epithelial integrity; a reduction suggests impaired protective roles in SLE.

Supervised cell frequencies from bivariate analysis correlate strongly with unsupervised cell frequencies, validating these results (Pearson's correlation coefficient $r = 0.9926$, $p < 0.001$ (CD4⁺CD152⁺PD1⁺CD45RO⁺CD25⁺FoxP3⁺); $r = 0.8863$, $p < 0.05$ (ILC2)).

Conclusion: With a multi-parametric, unbiased approach comparing SLE subjects to a large database of age-matched healthy controls, we identified two immune subsets of potential immunopathogenic importance. With this information, the CyTOF panel can be redesigned to probe more specifically into the SLE immunome, facilitating disease-specific interrogation.

References:

- [1] Tsokos, G.C. (2011). Systemic lupus erythematosus. *N Engl J Med* 365(22), 2110-2121.
- [2] Bombardier, C., Gladman, D.D., Urowitz, M.B., Caron, D., Chang, C.H. (1992). Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 35(6), 630-640.

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THU0238

ASSOCIATION OF SYSTEMIC DISEASES OF CONNECTIVE TISSUE (SDCT) AND GERONTOLOGICAL PROCESSES

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Background: The approach from the point of view of the evolutionary perspective of finding targets for therapeutic effects among the components of the cellular destruction system is critical both in the treatment of rheumatological diseases and in gerontology.

Objectives: To identify the relationships between the pathways of cell death in synovial fluid (SF) of people of different age groups with SDCT.

Methods: : SF was analyzed in patients of two age groups. Group N 1 of patients: 10 SLE (43±2.3 years), 13 RA (45±1.6 years), 7 SSD (35±1.8 years) and 8 donors (42±2.7 years, postmortem). Group N 2 (age) of patients: 9 SLE (69±1.8 years), 14 RA (65±1.6 years), 5 SSD (65±0.7 years) and 9 donors (66±2.3 years, postmortem). SF treated with 0.1% Triton-x-100, resuspended in 0.1% citrate buffer (pH = 7.4) and centrifuged at 25000 g for 30 minutes. The supernatant has been used in the experiment. The activity of adenosine monophosphate-activated protein kinase (AMPK) was evaluated by Western blotting. The level of p53 protein was analyzed by the enzyme immunoassay method using ELISA kit (eBioscience, USA). The content of 8-hydroxy-2-deoxyguanosine (8-OH-dG) was evaluated using the EIA Kit (USA). Quantitative determination of cytochrome C (Cyt c) was carried using the ELISA kit. Active forms of oxygen free radicals (AFRF) were registered by EPR.

Results: : An interaction was established between the pathways of cell death in SDCT (mostly distinctive in SLE), age-related changes and clinical manifestations of the autoimmune process were established. The severity of cell death types depends on the nosological form of SDCT (tab.1).

TAB.1. SOME MARKERS OF AUTOPHAGY, APOPTOSIS AND NECROSIS IN SF OF DONORS AND PATIENTS WITH SDCT (M±m): AMPK (cond.unit/mg protein), ATP-ase (nM Pi/min·mg protein), AFRF (unit/mg protein); Cyt c, p 53, 8-OH-dG - (ng/ml)

Group N1	AMPK	ATP-ase	AFRF	Cyt c	p 53	8-OH-dG
Donor (8)	1,3±0,2	3,7±0,3	9,7±2,3	10,4±0,3	0,7±0,1	9,5±0,8
SLE (10)	9,4±0,3***	10,3±0,3*	44,9±3,6**	40,1±2,9*	1,7±0,1*	28,9±1,4**
RA (13)	7,3±0,2***	9,7±0,5*	32,8±4,6**	29,3±3,7*	1,5±0,06*	23,8±0,9*
SSD (7)	4,3±0,2*	5,9±0,4	17,8±4,3*	18,9±3,7*	0,9±0,1	23,7±0,8*
Group N2	AMPK	ATP-ase	AFRF	Cyt c	p 53	8-OH-dG
Donor (9)	3,7±0,4	0,9±0,2	24,8±5,1	15,2±3,4	1,3±0,2	18,3±0,2
SLE (9)	18,2±0,2***	4,2±0,3**	64,6±3,1**	52,8±3,9*	3,4±0,1*	44,8±0,7*
RA (10)	17,0±0,4***	4,0±0,2**	54,9±1,6*	44,3±3,3*	3,2±0,3*	42,2±0,4*
SSD (5)	9,8±0,3**	2,8±0,2*	38,8±4,4*	38,6±2,9*	2,9±0,1*	30,7±0,3*

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

Autophagy (especially in the case of SLE, as well as RA) is directly involved in the formation of the immune response and the inflammatory process. During aging, as well as during SDCT, there is a sharp increase in the activity of AMPK (intracellular energy sensor), expression of apoptosis inducers

increases and also the acid-base balance shifts, the number of active forms of oxygen radicals increases, ox-red changes, the components of cellular destruction are activated, the activity of the cytokine system of the organism is disturbed (cytokines - regulators of apoptosis), the expression of chaperones decreases and immuno-oxygenase homeostasis shifts. Inhibition of the genetically determined process of cell death, apoptosis, underlies the development of autoimmune diseases, immunoproliferative pathology (carcinogenesis) and gerontological changes.

Conclusion: The chaperone-mediated induction of the immune response as a signaling mechanism of autophagy, supposedly, is a common central link and a molecular switch that causes both the development of autoimmune diseases of connective tissue and gerontological processes.

References:

- [1] Shishkin V. I. et al. *Ann Rheum Dis* 2018; 77: sup.2 – A1274

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THU0239

DYNAMIC TEMPORAL CHANGES IN CLINICAL DISEASE ACTIVITY AND GUT MICROBIOTA REPRESENTATION OF A PATHOBIONT LINKED TO LUPUS NEPHRITIS

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Background: From a cross-sectional cohort, we have identified a candidate human gut anaerobic pathobiont, *Ruminococcus gnavus* (RG) of the family *Lachnospiraceae* that was linked to active Lupus nephritis (LN)(1). Based on 16S rRNA amplicon analysis, LN patients displayed increased fecal RG abundance, concordant with serum IgG anti-RG antibody responses that appeared intertwined with anti-dsDNA responses implicated in renal pathogenesis. Indeed, monocolonization of germ-free mice is reported to result in generalized inflammation and expansions of Th17 cells. However, RG at low levels are also prevalent in healthy adults, and the temporal dynamics of RG representation within Lupus microbiota ecosystems have not been investigated. Also, genomic sequences of few RG strains have been reported, and these vary greatly in genome structure, gene representation and sequence, which may have broad implications for adaptation to a host with systemic inflammation and/or factors that contribute to immune activation in a susceptible host.

Objectives: To investigate the relationships between *in vivo* RG expansions and disease activity that often wax and wane overtime, we initiated longitudinal studies in Lupus patients and controls. As representation of RG strains alone might alter pathogenic potential, we also sought to characterize RG strains from active LN patients.

Methods: From our cohort, patients were characterized for demographics, clinical disease activity, and serologies including standard autoantibody and complement levels, and anti-bacterial responses of interest. High throughput 16S rRNA amplicon libraries from fecal samples were analyzed using QIIME 2 and DADA2 (1). Also, individual RG colonies were isolated and subjected to whole genome sequencing. Species and strains were then assigned in part based on multi-locus sequence typing and reference guided genomic assemblies.

Results: 16S rRNA analysis of 34 samples, at 2-4 timepoints from 14 SLE patients, documented highly conserved patterns of gut community representation overtime in 10/14 patients, based in part on unsupervised hierarchical cluster analysis. Notably, independent of vaccinations in clinical disease activity of up to 8 SLEDAI points, conserved microbiome phylogenetic abundance/composition was documented at a family level, and the level of amplicon sequence variants that approximate identification of individual species. In pilot studies, from two active lupus nephritis patients hundreds of fecal bacterial colonies were isolated, with initial assignments by 16S rRNA sequence. From highly redundant whole genome sequence analysis, these Lupus-patient fecal colonies were found to distribute into only four distinct RG strains, which differed from reported strains.

Conclusion: Our findings suggest that many Lupus patients have little or no detectable perturbations in representation of the *Lachnospiraceae* family or abundance of RG species overtime. Moreover, this seeming microbiota stability was documented even in patients with dramatic changes in disease activity. However, these approaches are inadequate to detect shifts between RG strains. In pilot studies we have isolated and characterized the genomes of four unique RG strains from active LN patients, which include variations in gene content and sequence that may have implications for the host-commensal relationship and immune activation. Broadening of these studies to larger number of SLE patients and healthy subjects, with metagenomic surveys of strain representation in genomic shotgun libraries are currently in progress, in coordination with