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.

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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.

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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, monoclonization 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

murine colonization testing for immune modulatory properties of individual strains.

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THU0240

SELECTIVE DEPLETION OF AUTOREACTIVE B CELLS USING A PEPTIDE VACCINATION APPROACH

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Background: B cell depletion is currently approved or being tested in clinical trials for the treatment of many autoimmune diseases including rheumatoid arthritis, myositis, systemic sclerosis, and lupus. We previously demonstrated that the heavy chain variable regions (V_H) of anti-DNA antibodies contain epitopes that can bind MHC class I molecules.¹ Vaccination of autoimmune-prone NZB/NZW F1 mice with plasmid DNA vectors carrying minigenes that encode such epitopes induced CD8⁺ cytotoxic T lymphocytes (CTL) that killed anti-DNA antibody-producing B cells, reduced serum anti-DNA antibody levels, retarded the development of nephritis, and improved survival.¹ Treatment with peptides alone did not induce such CD8⁺ T-cells, as we and others have reported impaired CD8⁺ regulatory and CTL responses in mice and humans.^{2,3} Here, we asked if we could overcome such impairment using synthetic oligodeoxynucleotides containing unmethylated cytidine-phosphate-guanosine dinucleotides (CpG-ODN) that can enhance innate and adaptive immunity.

Objectives: To determine if peptides representing MHC class I binding epitopes in the autoantibody V_H regions conjugated to CpG-ODN will elicit CD8⁺ CTLs that will ablate autoantibody-producing B cells, reduce serum autoantibody levels, and retard the development of autoimmune disease.

Methods: We first screened V_H regions of anti-dsDNA and other autoantibodies for MHC class I-binding epitopes using various bioinformatic approaches, and then verified the binding using cellular binding approaches. We immunized lupus-prone (NZB/NZW F1) mice that develop lupus that mimics human lupus with CpG-ODN or a control ODN, and assessed CTL responses. We then treated these mice with CpG-ODN conjugated to MHC class I-binding VH epitopes. We monitored lupus mice for proteinuria, serum anti-dsDNA antibodies, and survival.

Results: Immunization with CpG-ODN corrected the impairment in peptide-specific CTL responses in lupus-prone mice. The CpG-ODN conjugated with MHC class I-binding, anti-dsDNA antibody V_H -derived epitopes induced potent peptide-specific CTL responses against autoreactive B cells that expressed the respective epitopes, and against B cells from diseased lupus-prone mice. The animals treated with CpG-ODN-peptide conjugates had reduced serum anti-DNA antibody levels and proteinuria, significantly delayed development of nephritis, and improved survival as compared to animals injected with a control ODN.

Conclusion: Selective ablation of autoreactive B cells by CTLs induced by peptide vaccines conjugated to CpG-ODN represents a novel approach to treat autoantibody-mediated diseases.

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THU0241

ALTERATIONS OF THE FECAL MICROBIOTA ASSOCIATED WITH EXACERBATION OF LUPUS ACTIVITY BY HCMV PP65 422-439 IMMUNIZATION IN NZB/W F1 MICE

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Background: Systemic lupus erythematosus (SLE) is a multifactorial disease. Gut microbiota is an important environmental factor for SLE¹. The perturbation of gut microbiota is often observed at onset or during the disease course. The fragment of HCMV phosphoprotein 65⁴²²⁻⁴³⁹ (HCMVpp65⁴²²⁻⁴³⁹) containing B cell epitopes has been reported to elicit humoral immunity and accelerate the autoimmune response in murine lupus^{2,3}. However, little is there to know about the interplay between viral trigger for SLE and the change of gut microbiota during lupus progression.

Objectives: By using a murine lupus model with NZB/W F1, we investigated the differential alteration in gut microbiota associated with the progression of lupus disease in HCMVpp65⁴²²⁻⁴³⁹ immunized mice and control mice.

Methods: Ten weeks-old NZB/W F1 mice were given or not given an intraperitoneal injection of 100-µg HCMVpp65⁴²²⁻⁴³⁹ peptide biweekly for four times. Fecal samples, urine and blood of mice were collected once every two weeks followed by 16S rRNA genes sequencing and ELISA tests. The pathological investigation of renal tissue from sacrificed mice was conducted at 24 weeks of mice age. Statistical analysis for dynamics and alteration of the gut microbiota as well as functional prediction of bacterial communities related to the progression of lupus-like activity was performed.

Results: HCMVpp65⁴²²⁻⁴³⁹ immunization results in the onset of lupus-like activities in NZB/W F1 mice with a higher titer of anti-dsDNA antibody, creatinine and proteinuria, and severe glomerular damage (figure 1). Also, higher diversity and increased family abundance of several bacterial species were observed in HCMVpp65⁴²²⁻⁴³⁹ immunized mice (Table 1 and Figure 2a). The predicted metagenomic taxonomic profile in NZB/W F1 mice showed statistically significant enrichment of flagellar assembly, bacterial motility, and chemotaxis (Figure 2b). Spearman's correlation analysis revealed that a significant association between the increased relative family abundance for *Saccharimonadaceae*, *Marinifilaceae*, *Desulfovibrionaceae*, and *Rikenellaceae* and HCMVpp65⁴²²⁻⁴³⁹ induced lupus-like activity in NZB/W F1 mice (Figure 2c).

Table 1. Significant test of microbial community structure between two groups

Groups	MRPP		Adonis		Anosim	
	E-Δ	P	R ²	P	R	P
Pre-disease vs. Control	0.43	0.1	0.77	0.1	1	0.112
Pre-disease vs. HCMVpp65 ⁴²²⁻⁴³⁹	0.52	0.007	0.55	0.009	0.97	0.01
Control vs. HCMVpp65 ⁴²²⁻⁴³⁹	0.45	0.013	0.35	0.014	0.58	0.019

Conclusion: Our results demonstrated that HCMVpp65⁴²²⁻⁴³⁹ immunization induced the change in gut microbiota composition and suggested the association of gut microbiota alteration with lupus-like activity in NZB/W F1 mice.

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Figure 1

HCMVpp65422-439 immunization accelerates the SLE disease progression in NZB/W F1 mice. ELISA analysis for the levels of (A) anti-dsDNA antibody, (B) serum creatinine, and (C) proteinuria in control mice and HCMVpp65422-439 immunized mice. (D) The representative photograph and diagram shows the size of spleen collected from both groups. (E) Hematoxylin and eosin staining of the glomerular from control mice and HCMVpp65422-439 immunized mice. (F) The glomerulonephritis score of renal lesions from control and immunized mice.