murine colonization testing for immune modulatory properties of individual strains.

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Disclosure of Interests: : Gregg Silverman Consultant of: Work with industry is unrelated to the topic in this abstract, Doua Azzouz: None declared, Ze Chen: None declared, Jing Deng: None declared, Zhi Li: None declared, David Fenyo: None declared, Alexander Alekseyenko: None declared
DOI: 10.1136/annrheumdis-2020-eular.5091

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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Disclosure of Interests: : None declared

DOI: 10.1136/annrheumdis-2020-eular.2678

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.