(spp.), *L. reuteri, L. oris, L. johnsonii, L. gasseri* and *L. rhamnosus*, attenuated lupus-liked clinical signs, including splenomegaly and lymphadenopathy. However, our understanding of the mechanism was limited. In this study, we used the lupus-prone MRL/*lpr* mouse model to delineate the mechanisms through which *Lactobacillus* spp. modulate lupus pathogenesis. We first investigated the effects of individual species. Surprisingly, none of the species individually recapitulated the benefits of the mix. Instead, *Lactobacillus* spp. acted synergistically to attenuate splenomegaly and renal lymphadenopathy through secreted factors and a CX_3CR1-dependent mechanism. Interestingly, oral administration of MRS broth exerted the same benefits likely through increasing the relative abundance of endogenous *Lactobacillus* spp. Mechanistically, we found increased percentages of FOXP3-negative type 1 regulatory T cells with administration of the mix in both spleen and mesenteric lymph nodes. In addition, oral gavage of *Lactobacillus* spp. decreased the percentage of central memory T cells while increasing that of effector memory T cells in the lymphoid organs. Furthermore, a decreased percentage of double negative T cells was observed in the spleen with the mix. These results suggest that *Lactobacillus* spp. might act on T cells to attenuate splenomegaly and lymphadenopathy. Together, this study advances our understanding of how *Lactobacillus* spp. attenuate lupus in MRL/*lpr* mice. The synergistic action of these bacteria suggests that multiple probiotic bacteria in combination may dampen systemic autoimmunity and benefit lupus patients.

Microbiome

1302 THE THERAPEUTIC EFFECT OF GLYCOLYSIS INHIBITION IN LUPUS-PRONE MICE IS TRANSFERABLE THROUGH THE FECAL MICROBIOME

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Gut microbial dysbiosis has been reported in patients with lupus. Results obtained with mouse models suggest that dysbiosis contributes to lupus pathogenesis through the pathobionts that induce inflammation by translocating out of the gut and/or producing proinflammatory metabolites. We and others have shown that fecal microbiota transfers (FMT) from lupus-prone mice induced autoantibodies and immune activation in non-autoimmune mice. On the other hand, we have shown that the production of autoantibodies and associated expansion of follicular T (Tfh) cells and germinal center (GC) B cells can be eliminated by treating lupus-prone mice with 2-deoxy-D-glucose (2DG), a glycolysis inhibitor. Here, we investigated the effect of 2DG on the fecal microbiome in two models of lupus with different etiologies, the (NZB × NZW) F1 and (NZW x BXSB)F1 mice.

Anti-dsDNA IgG-positive (NZB × NZW)F1 and (NZW x BXSB)F1 mice were treated with 2DG. The composition of their fecal microbiome was determined by 16S rDNA sequencing and their metabolome by LC-MS analysis, and compared to age-matched controls. Fecal samples from these mice were used for FMT 3 times per week in pre-autoimmune lupus-prone mice of the same strain that were pre-treated with antibiotics for 2 weeks. FMT lasted for 26 weeks in (NZB × NZW)F1 mice and 9 weeks in (NZW x BXSB)F1 mice. Age-matched controls were gavaged with PBS. Autoantibodies were measured by ELISA and indirect immunofluorescence. Immunophenotypes were assessed by flow cytometry in the spleen and mesenteric lymph nodes. Renal pathology was evaluated by light microscopy on PAS-stained sections and immunofluorescence on frozen sections with antibodies to complement C3, IgG2a, F4/80 and CD3.

We showed that a 2DG treatment started in reduced the changes in bacterial populations that occurred as disease developed in control mice in both models. 2DG also altered the distribution of fecal metabolites in these treated mice. Next, we investigated the effect of serial FMT from 2DG-treated or control mice into pre-autoimmune lupus-prone mice of the same strain that were pre-treated with antibiotics. In both models, FMT from 2DG-treated mice was protective, with a reduction of anti-dsDNA IgG production, immune cell activation, and renal pathology as compared to FMT from control mice.

Overall, our results demonstrated for the first time that the therapeutic effect of glucose inhibition in lupus is transferable through the gut microbiota. These results suggest that the enhanced glucose metabolism in lupus-prone mice promotes the expansion of pathogenic gut bacteria either directly or indirectly through the immune system that normalized by glucose inhibition.

Lay summary High glucose metabolism sustains the activation of the immune system in lupus. Inhibition of glucose metabolism with a drug called 2DG reverses the production of pathogenic autoantibodies in mice. Here we showed that 2DG also changes the gut microbiome in lupus- prone mice. Further, transfers of fecal bacteria from 2DG-treated lupus mice protected younger mice to develop lupus. The results showed that the gut microbiome contributes significantly to the pathogenic effects of glucose metabolism is lupus, and suggest that the beneficial effect of reducing glucose metabolism includes the restoration of a healthy gut microbiome.

1303 BACTERIAL DNA INDUCES REGULATORY B CELLS AND ATTENUATES LUPUS THROUGH A B CELL-EXTRINSIC, TLR9-DEPENDENT MECHANISM

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Our recent study has demonstrated induction of regulatory B cells (Bregs) by bacterial DNA in MRL/*lpr* mice leading to attenuation of SLE. This suggests a regulatory role for the gut bacteria in SLE development. However, the mechanism by which bacterial DNA induces Breg cell differentiation remains to be elucidated. Bacterial DNA contains unmethylated CpG motifs which are recognized by the innate immune molecule, toll-like receptor 9 (TLR9). We therefore hypothesize that gut bacteria-derived DNA induces Bregs in a TLR9-dependent manner, which in turn protects against lupus initiation. To test our hypothesis, TLR9 global knockout ($Tlr9^{-/-}$) MRL/*lpr* mice and their heterozygous ($Tlr9^{+/-}$) littermates were randomized into 2 groups per genotype. Beginning at 4 weeks of age, one group in each genotype received 80 µg *E. coli* double- stranded DNA (dsDNA) once a week by oral gavage for 4 consecutive weeks, whereas the other group received phosphate buffered saline (PBS) as control. Urine samples were collected before euthanasia whereas blood, spleen, lymph nodes, and kidneys were collected and processed following euthanasia at 15 weeks of age. *In vitro* culture treatment with bacterial DNA was used to investigate a TLR9-dependent, B cell-intrinsic or -extrinsic mechanism in Breg cell induction. Our data showed a trend in decrease of proteinuria, as well as sizes of spleen and lymph nodes, with bacterial DNA treatment in $Tlr9^{+/-}$ mice. Interestingly, serum levels of anti-dsDNA IgM, which has been shown to be protective against lupus, were significantly increased in bacterial DNA-treated $Tlr9^{+/-}$ mice, although the pathogenic anti-dsDNA IgG remained unchanged. Additionally, flow cytometry analysis of splenocytes showed an increasing trend in the percentage of interleukin-10 positive (IL-10^+) Bregs in bacterial DNA-treated $Tlr9^{+/-}$ mice and a concomitant decrease in double negative (DN, or $CD4^-CD8^-$) T cells. In the kidney, bacterial DNA treatment also decreased the percentage of $CD45^+$ cells in $Tlr9^{+/-}$ mice. Importantly, all bacterial DNA- induced changes observed in $Tlr9^{+/-}$ MRL/*lpr* mice, including those of proteinuria, organ weight, anti-dsDNA IgM, splenic Bregs, splenic DN T cells and renal $CD45^+$ cells, were abrogated in $Tlr9^{-/-}$ MRL/*lpr* mice. Bacterial DNA treatment of cocultures of B and non-B cells from $Tlr9^{+/+}$ and $Tlr9^{-/-}$ MRL/*lpr* mice revealed a TLR9-dependent, but B cell-extrinsic, induction of Bregs mediated by IL-6 produced most likely from myeloid cells. Altogether, our study suggests possible attenuation of SLE by gut bacteria-derived DNA through a B cell-extrinsic, TLR9-dependent mechanism that involves the induction of IL-10^+ Bregs and secretion of potentially protective anti- dsDNA IgM.

SLE Genetics

1401 A GENOME WIDE ASSOCIATION SCAN OF SLE GENETIC RISK IN A COHORT OF AFRICAN-AMERICAN PERSONS

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