Dynamics of Gut Microbiota in Autoimmune Lupus

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Gut microbiota has been recognized as an important environmental factor in health, as well as in metabolic and immunological diseases, in which perturbation of the host gut microbiota is often observed in the diseased state. However, little is known on the role of gut microbiota in systemic lupus erythematosus. We investigated the effects of host genetics, sex, age, and dietary intervention on the gut microbiome in a murine lupus model. In young, female lupus-prone mice resembling women at childbearing age, a population with the highest risk for lupus, we found marked depletion of lactobacilli, and increases in *Lachnospiraceae* and overall diversity compared to age-matched healthy controls. The predicted metagenomic profile in lupus-prone mice showed a significant enrichment of bacterial motility- and sporation-related pathways. Retinoic acid as a dietary intervention restored lactobacilli that were downregulated in lupus-prone mice, and this correlated with improved symptoms. The predicted metagenomes also showed that retinoic acid reversed many lupus-associated changes in microbial functions that deviated from the control. In addition, gut microbiota of lupus-prone mice were different between sexes, and an overrepresentation of *Clostridaceae* and *Lachnospiraceae*, both harboring butyrate-producing genera, were more abundant in the gut of lupus-prone mice at specific time points during lupus progression. Together, our results demonstrate the dynamics of gut microbiota in murine lupus and provide evidence to suggest the use of probiotic lactobacilli and retinoic acid as dietary supplements to relieve inflammatory flares in lupus patients.

The mammalian gut harbors trillions of microorganisms known as the microbiota (1). Increasing evidence in recent years suggests that the host microbiota and immune system interact to maintain tissue homeostasis in healthy individuals (2–6). The importance of microbiota for the host is highlighted by altered immune responses in the absence of commensal bacteria, where both systemic and gut-specific lymphoid tissues are generally smaller and less developed (7). Higher susceptibility to infectious pathogens (8) and, in some cases, attenuated symptoms in autoimmune disorders (2), have been observed in mice raised under germfree conditions. Indeed, perturbation of the host microbiota, especially that in the gut, is shown to be associated with many diseases. These include inflammatory bowel disease (9, 10), metabolic syndrome (e.g., obesity [11–15] and type 2 diabetes [16], and autoimmune diseases, e.g., type 1 diabetes [17–20], rheumatoid arthritis [5, 21–23], and multiple sclerosis [24, 25]).

However, whether gut microbiota differ in systemic lupus erythematosus (lupus) versus healthy individuals has not been reported. Lupus is an autoimmune disorder characterized by the generation of autoantibodies, recruitment of autoreactive or inflammatory T cells, and abnormal production of proinflammatory cytokines (26, 27). The hallmark of the disease is severe and persistent inflammation that leads to tissue damage in multiple organs, including kidneys, lungs, joints, heart, and brain (26). According to the Lupus Foundation of America, about 2 million Americans currently live with the disease. The prevalence ranges from 20 to 200 cases per 100,000 persons, with higher prevalence for people of African, Hispanic, or Asian ancestry. Although the disease affects both males and females, women of childbearing age are diagnosed nine times more often than men. African-American women suffer from more severe symptoms and a higher mortality rate. The cause of lupus is unclear and there is currently no cure. To date, treatment and prevention of disease flares have relied on long-term use of immunosuppressants where side effects, such as susceptibility to infections, are of particular concern (28). There is a need for better understanding of the disease and for better approaches in lupus treatment and management.

Here, we report the dynamics of gut microbiota in a murine lupus model. Disease-, sex-, and time-dependent differences were identified. Using vitamin A as a potential intervention to reduce lupus nephritis (29–32), we also showed that specific changes of the gut microbiota strongly correlated with disease attenuation. Predicted metagenomes associated with murine lupus and vitamin A treatments were also described.

MATERIALS AND METHODS

Mice. MRL/Mp (MRL), MRL/Mp-Faslpr (MRL/lpr, stock number 009485), C57BL/6 (B6), and B6.MRL-Faslpr (B6/lpr) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred and maintained in a specific-pathogen-free facility according to the requirements of Institutional Animal Care and Use Committee at Virginia Polytechnic Institute and State University. All four strains had been housed in the same room under the same condition for at least 12 months prior to the initiation of the study. The breeding rotations were 3 months for MRL and MRL/lpr due to the short life span of MRL/lpr and 6 months for mice of B6 background. All-trans-retinoic acid (RA) and all-trans-retinol palmitate...
were

were from known bacterial genomes. The normalized OTU were used for amplified

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protein (5 to 20 mg/dl), and 30, 100, and 300 mg/dl total protein, re-

total protein, re-

lates within strains (TukeyHSD test, P < 0.005, Fig. 1B), indicating an overall phylogenetic difference. The gut microbiota of lupus-prone MRL/lpr mice were characterized by a significant reduction in the family Lactobacillaceae (under “Firmicutes_ Bacilli”) and a concurrent increase in the family Lachnospiraceae (under “Firmicutes_Clostridia”), which together accounted for more than 65% of all bacteria in the distal gut (Fig. 1C and D). A total of 105 operational taxonomic units (OTU) representing all Lactobacillaceae sequences were further classified to genus Lacto-

bacillus, with the majority (92 of the 105 OTU) being uncultured species of this genus (see Table S1, highlighted rows, in the sup-

plemental material). The Lachnospiraceae family comprises a phy- logenetically diverse group of anaerobes defined as Clostridium cluster XIVa (48) that includes butyrate-producing genera such as Butyribrio and Roseburia (49). The predominant Lachno-

spiraceae-affiliated OTU were uncultured (see Table S2, high-

lighted rows, in the supplemental material). Other lupus-enriched families included Ruminococcaceae (uncultured) and Rikenel-

laceae (genus Alistipes) (Fig. 1D). Rare OTU (defined as <0.1% of total bacteria from all samples) accounted for <3.5% of individual microbiota (see Fig. S1 in the supplemental material). Among them, the RF9 group (“Tenericutes_Mollicutes”), Clostridiales family XIII, and the Streptococcaceae were significantly enriched in lupus-prone mice (see Fig. S1 in the supplemental material).
Overall, gut microbiota in lupus-prone mice had a higher diversity measured as Faith’s phylogenetic diversity and Shannon’s entropy (Fig. 1E). These results show that lupus-prone mice have distinct bacterial composition and diversity compared to healthy controls, suggesting that lupus could alter gut microbiota.

Microbiota dynamics associated with sex and age in murine lupus. Lupus is a sex-specific disease affecting more women than men (26). In the MRL/lpr model, while lupus develops spontaneously in both females and males, female mice exhibit accelerated disease (50). We thus analyzed the gut microbiota separately in female and male mice (Fig. 2A and see Fig. S2A in the supplemental material). To try to minimize the cage effect as a confounding factor, we randomly selected one mouse of each sex from four different litters for each mouse strain (MRL or MRL/lpr, n/strain). In 14-week-old females, comparing lupus-prone MRL/lpr mice to MRL controls, significantly lower levels of Lactobacillaceae and higher levels of Lachnospiraceae were noted (Fig. 2A), a finding consistent with our observations in 5-week-old females (Fig. 1D). In males, however, the two strains of mice were not significantly different from each other in their gut microbial composition (Fig. 2A). Since sex and disease status appeared to interact, we also compared female and male mice within each mouse strain. In MRL mice, females had significantly higher Lactobacillaceae and Streptococcaceae levels and lower Lachnospiraceae and Clostridiaceae levels than did male mice (Fig. 2A and see Fig. S2B in the supplemental material). In contrast, in the lupus-prone MRL/lpr group, female mice had higher levels of Lachnospiraceae and Bacteroidetes S24-7, and lower levels of Bifidobacterium and Erysipelotrichaceae, whereas the levels of Lactobacillaceae were comparable between female and male mice (Fig. 2A). Principal coordinate analysis showed distinct microbiota between sexes, albeit the difference depended on mouse strains (see Fig. S2C in the supplemental material). Although no sex-dependent difference in bacterial diversity was observed in control mice, female lupus-prone mice had significantly higher diversity than their male counterparts (see Fig. S2D in the supplemental material). These results suggest that the gut microbiota of lupus-prone mice are different between sexes and that female-specific changes in certain bacterial phylotypes (such as the higher abundance of Lachnospiraceae) may be associated with an earlier onset of and/or more severe lupus symptoms in female MRL/lpr mice.

We next determined time-dependent changes of gut microbiota in murine lupus. Because MRL/lpr mice have a short life span of approximately 18 weeks (47), we chose to investigate the time course in another lupus-prone model with the same Fas<sup>br</sup> mutation. B6.MRL-Fas<sup>br</sup> (B6/lpr) mice, which are of C57BL/6 (B6) background, have a life span of at least 7 months. Both MRL/lpr and B6/lpr mice start to develop lupus-like symptoms at about 2 months of age, but disease progression is slower in B6/lpr mice (51–53). We found that the gut microbiota of female B6/lpr mice was distinct from healthy B6 mice, as shown by principal coordinate analysis (Fig. 2B, n = 3 per strain). In this pair of mouse strains we observed dynamic changes in microbiota composition over a 5-month time period (Fig. 2C). Notably, B6/lpr mice had
higher levels of Clostridiaceae than did B6 mice from 2 to 5 months \((P < 0.05\), unpaired \(t\) test), a time frame wherein B6/lpr mice have just started to show lupus-like symptoms. The family Clostridiaceae includes the butyrate producers in Clostridium cluster I (48). In addition, the abundance of Lactobacillaceae oscillated in the guts of both B6/lpr and B6 mice, whereas Lachnospiraceae appeared to be more abundant in B6/lpr mice during the last month \((P = 0.003\), Fig. 2C). These results suggest that the composition of gut microbiota change over time in both healthy and lupus-prone mice. Certain bacterial phylotypes, such as Clostridiaceae and Lachnospiraceae (both butyrate producers), are more abundant in the guts of B6/lpr mice at specific time points during lupus progression.

**Restoration of lactobacilli and improvement of lupus symptoms with RA.** Vitamin A has been shown to reduce lupus nephritis (29–32). However, while orally administered vitamin A may come directly in contact with commensal bacteria in the intestinal tract during absorption, little is known about whether and how this nutrient affects gut microbiota. We thus investigated the effects of vitamin A (retinol) and a major physiological active metabolite of vitamin A, retinoic acid (RA), on gut microbiota in the MRL/lpr mouse model. We treated female MRL/lpr mice, orally and daily, with RA at 6 mg/kg of body weight (suspended in canola oil as the vehicle) from 6 to 14 weeks of age. RA of comparable doses and with similar treatment time frames was previously reported to reduce spleen and lymph node sizes and improve renal pathology in murine lupus models (29, 30). To mimic natural vitamin A supplementation, where retinol (retinyl palmitate) is the primary ingredient, we also treated female MRL/lpr mice daily with 11.2 mg retinyl palmitate/kg of body weight (equivalent to 6 mg retinol/kg) mixed with 0.6 mg RA/kg of body weight (10% of the amount of retinol) from 6 to 14 weeks of age. The small amount of RA in this formulation, which we refer to as VARA, has been shown to enhance the storage of retinol in the body (54). Because the vehicle itself (canola oil, which contains polyunsaturated fatty acids) may affect the gut microbiota (55–57), MRL/lpr mice treated with canola oil were used as the diseased control, whereas MRL mice treated with canola oil were used as the healthy control. To try to minimize the cage effect as a confounding factor, we randomly selected female MRL/lpr mice from four different litters and housed them in four cages. Each cage had one mouse randomly assigned to vehicle treatment, one with RA, and a third with VARA. The MRL mice were from two different litters.
Colonic contents were collected after treatment and the microbiota were analyzed.

Gut microbiota after vitamin A treatments were not significantly different from the vehicle control among MRL/lpr groups based on principal coordinate analysis (see Fig. S3A in the supplemental material, n = 4 per group). However, we noted a number of significant differences in the taxonomy breakdown at the family level for abundant bacterial OTU. Strikingly, we found that RA restored Lactobacillaceae (Fig. 3A and B) that was downregulated in MRL/lpr mice (Fig. 1D, Fig. 2A, and Fig. 3B). VARA, on the other hand, did not have such an effect. Rather, it significantly increased the levels of Lachnospiraceae and Rikenellaceae (Fig. 3B), two bacterial families that were already higher in MRL/lpr than MRL mice, at least for 5-week-old mice (Fig. 1D). Importantly, we observed strong correlations between the abundance of two bacterial families, Lactobacillaceae and Lachnospiraceae, and lupus disease indices (Fig. 3C and see Table S3 in the supplemental material).

Lupus disease parameters, lymphadenopathy (increased spleen and mesenteric lymph node weights) and glomerulonephritis (impaired renal function indicated by increasing scores), were associated with lupus, we used PICRUSt to infer putative metabolic pathways and functions from the 16S rRNA gene profiles of 14-week-old female MRL and MRL/lpr mice (41). Comparing the predicted gut microbiome of lupus-prone mice to that of the control, categories representing genes important for genetic information processing (e.g., DNA replication and repair, protein synthesis) and nucleotide metabolism were significantly lower in MRL/lpr microbiome (Fig. 4A), indicating reduced turnover of bacterial DNA, RNA, and protein synthesis (58). This was accompanied by a significant increase in cell motility genes (e.g., bacterial chemotaxis and flagellar assembly) in the gut microbiome of lupus-prone mice.

Both RA and VARA also decreased the levels of Erysipelotrichaceae compared to the vehicle control in MRL/lpr mice (Fig. 3B). In addition, lupus-prone mice had significantly lower levels of Bifidobacterium compared to the healthy control, and neither RA nor retinol restored the abundance of this bacterial family (Fig. 3B). Lower representation of Erysipelotrichaceae and Bifidobacterium could potentially make available more substrates for other commensal bacteria, such as Lactobacillaceae in the case of RA treatments and Lachnospiraceae in the case of VARA treatments (Fig. 3B). Consistent with prior observations (Fig. 1E), vehicle-treated MRL/lpr microbiota had higher diversity than vehicle-treated MRL microbiota based on Shannon’s entropy analysis, whereas neither RA nor VARA treatments changed this phenomenon (see Fig. S3B in the supplemental material) despite their effects on lupus symptoms, suggesting that microbial diversity in the gut may depend on genetic predisposition of the mouse strains but not their phenotype.

Changes of microbiome metabolic functions with lupus and vitamin A treatments. To investigate microbiome functions associated with lupus, we used PICTRUST to infer putative metabolic pathways and functions from the 16S rRNA gene profiles of 14-week-old female MRL and MRL/lpr mice (41). Comparing the predicted gut microbiome of lupus-prone mice to that of the control, categories representing genes important for genetic information processing (e.g., DNA replication and repair, protein synthesis) and nucleotide metabolism were significantly lower in MRL/lpr microbiome (Fig. 4A), indicating reduced turnover of bacterial DNA, RNA, and protein synthesis (58). This was accompanied by a significant increase in cell motility genes (e.g., bacterial chemotaxis and flagellar assembly) in the gut microbiome of lupus-prone mice.

FIG 3 Changes of bacterial abundance with vitamin A treatments. (A) Taxonomy breakdown at the family level abundant (>0.1%) bacterial OTU. HC, MRL healthy control treated with canola oil; vehicle, lupus-prone MRL/lpr treated with canola oil; RA, MRL/lpr treated with retinoic acid (6 mg/kg of body weight); VARA, MRL/lpr treated with retinol (6 mg retinol/kg of body weight) and retinoic acid (0.6 mg/kg of body weight, which facilitates retinol storage). All mice were female and treated orally from 6 to 14 weeks of age. Mice were sacrificed at 14 weeks of age and colonic contents were collected (n = 4 per group). (B) Comparison of individual families shown in panel A. Statistical significance was determined by one-way ANOVA, followed by a pairwise t test with TukeyHSD-adjusted significance. (C) Correlation between bacterial abundance and lupus disease indices. Spleen, spleen weight in grams; MLN, mesenteric lymph node weight in grams; renal, renal function estimated from proteinuria and glomerular scores as described in Materials and Methods. Correlation coefficients are shown on each individual plot. For example, the correlation between Lactobacillaceae and Lachnospiraceae was −0.66. Spearman’s correlation tests were used for pairs involving ranked renal function data; all other correlations were calculated by using the Pearson method. Raw data can be found in the supplemental material.
sug­gest­ing that the bacteria may be ac­tive­ly ac­cess­ing sub­strates and/or ad­just­ing their lo­ca­tions (59). Spo­ra­tion gen­es also in­creased with lu­pus dis­ease, pos­si­bly due to the in­creased of spore-for­ming clo­stridia (Fig. 2A). In the car­bo­hy­drate me­tab­ol­is­m cat­e­gory, gal­actose me­tab­ol­is­m and gly­col­y­sis de­creased, while gly­oxyl­ate and di­car­boxy­late me­tab­ol­is­m in­creased with lu­pus dis­ease (Fig. 4A). Lu­pus-as­so­ci­ated in­creases in me­m­brane trans­port (e.g., ATP-bind­ing cas­sette trans­port­ers), sig­nal trans­duc­tion (two-com­ponent sys­tem), and a­mino acid me­tab­ol­is­m were also ob­served (Fig. 4A).

To as­sess whether vi­to­min A treat­ments could af­flect gut mi­cro­bi­ome func­tions in lu­pus-prone mice, we per­formed a sim­i­lar anal­y­sis be­tween ve­hi­cle- and RA-treat­ed MRL/lpr mice (Fig. 4B), as well as be­tween ve­hi­cle- and VARA-treat­ed MRL/lpr mice (Fig. 4C). Me­tab­ol­ic func­tions that over­lap­ped with those in Fig. 4A were iden­ti­fied and color-coded in blue. In­ter­est­ing­ly, we found that RA re­versed many lu­pus-as­so­ci­ated changes in mi­cro­bi­ome func­tions that de­vayed from the MRL con­trol (Fig. 4B). These in­clud­ing genes in car­bo­hy­drate me­tab­ol­is­m (e.g., gal­actose me­tab­ol­is­m, gly­oxyl­ate and di­car­boxy­late me­tab­ol­is­m) and a­mino acid me­tab­ol­is­m (e.g., his­ti­dine me­tab­ol­is­m, and pheno­ly­n, ty­ros­ine and tryp­to­phan bio­syn­the­sis). In con­trast, VARA wors­ened lu­pus-as­so­ci­ated changes on all over­lap­ped func­tion­al cat­e­gories, in­clud­ing cell mo­til­ity, me­m­brane trans­port, DNA rep­lica­tion and re­pair, pro­tein syn­the­sis, sig­nal trans­duc­tion, and spo­ra­tion (Fig. 4A). To­gether, these re­sults sug­gest that mi­cro­bi­ome me­tab­ol­ic func­tions in the gut could change with lu­pus dis­ease. RA treat­ment, which attenu­ated symp­toms, re­versed lu­pus-as­so­ci­ated changes in mi­cro­bi­ome func­tions. VARA, on the other hand, ex­ac­erbated the changes of me­ta­gen­omic pro­files in lu­pus-prone mice while wors­en­ing the dis­ease, sug­gest­ing a pos­si­ble cor­re­la­tion be­tween gut mi­cro­bi­ome and dis­ease symp­toms in lu­pus.

FIG 4 Bacterial me­ta­genomes pre­dicted from mi­cro­bial com­munity iden­ti­ties. Gene func­tion­al cat­e­gories were from level 3 of KEGG path­ways. Gene func­tions with a sig­nif­i­cant dif­ference are shown (P < 0.05). (A) Differences be­tween MRL con­trol and lu­pus-prone MRL/lpr mice. The effect size was 0.10%. ABC trans­port­ers, ATP-bind­ing cas­sette trans­port sys­tems. (B) Effect of RA treat­ment on lu­pus-prone bacterial me­ta­genomes. The effect size was 0.02%. (C) Effect of vi­to­min A treat­ment (VARA) on lu­pus-prone bacterial me­ta­genomes. The effect size was 0.08%.

DISCUSSION

In this study, we re­ported the dy­nam­ics of gut mi­cro­bi­ota in mu­rine lu­pus. By us­ing lu­pus-prone mice car­ry­ing the Fas
supr
muta­tion, we showed that the com­po­si­tion and di­ver­sity of gut mi­cro­bi­ota dif­fered be­tween con­trol and dis­eased mice (Fig. 1). Many dif­fer­ences were sex spe­cific and var­ied over time du­ring lu­pus pro­gres­sion (Fig. 2). In ad­di­tion, we found that the use of dif­fer­ent vi­to­min A treat­ments that in­creased abun­dance of Lactobacil­laeae in the in­testine corre­lated with im­proved lu­pus symp­toms, whereas greater col­o­ni­za­tion of Lach­nor­is­caeae corre­lated with dis­ease de­terio­ra­tion (Fig. 3). Through pre­dict­ion anal­y­sis of me­ta­gen­omes (Fig. 4), we also showed that cer­tain func­tions, such as bacte­rial mo­til­ity, in­creased with lu­pus dis­ease in mice. In ad­di­tion, gut mi­cro­bi­ome me­tab­ol­ic func­tions ap­peared to cor­re­late with dis­ease se­ver­ity in lu­pus.

Mem­bers of the Lactobacil­laeae fam­i­ly have been re­com-
mended as probiotics with anti-inflammatory functions (60). Lactobacillus rhamnosus GG, for example, suppresses proinflammatory responses by increasing interleukin-10 and regulatory T (Treg) cells (61, 62). Several other members of Lactobacillaceae, including L. acidophilus and L. reuteri, were also found to induce anti-inflammatory Treg cells (63, 64). Although colonization of Lactobacillaceae in the guts of both humans and mice has been shown to suppress inflammation (65, 66), a direct relationship between intestinal Lactobacillaceae and autoimmune lupus has not been established. In the present study we show that lower colonization of lactobacilli in the gut is associated with disease state in lupus, an autoimmune disease characterized by chronic inflammation.

Several groups have recently reported that short-chain fatty acids produced by gut microbiota, especially butyrate produced by Clostridia, promote the differentiation of Treg cells to suppress inflammation (67–70). However, in the present study, we found that both young (5-week-old) and old (14-week-old) female MRL/lpr mice had higher levels of Lachnospiraceae, where butyrate-producing Clostridium XIVa belongs, than their MRL counterparts (Fig. 1D and Fig. 2A). In the B6/lpr model, the levels of Lachnospiraceae, as well as of another butyrate-producing family, Clostridiaceae, were also higher in lupus-prone mice compared to B6 controls (Fig. 2C). Moreover, we found strong positive correlations between the abundance of Lachnospiraceae and lupus disease parameters, including lymphadenopathy and renal pathology (Fig. 3C). All of these results suggest that Lachnospiraceae, or butyrate-producing bacteria in general, may not be able to suppress inflammation in the lupus-prone MRL/lpr and B6/lpr models. Indeed, a recent report has shown that in mice that are deficient in Fas signaling (e.g., Fas<sup>−/−</sup> mutation), butyrate-induced apoptosis of T cells is abolished (71). Thus, even though Lachnospiraceae accumulated in MRL/lpr and B6/lpr mice, the butyrate that they produced would lack the mechanism required for T-cell apoptosis. This would likely lead to uncontrolled proinflammatory T-cell responses and thereby lupus progression. The notion is supported by our observation that further changes of the abundance of Lachnospiraceae through vitamin A intervention of MRL/lpr mice failed to alter the percentages of different T-cell populations in the periphery (data not shown).

Many autoimmune disorders are more prevalent in females (72). Sex-specific differences in gut microbiota have not been reported until recently, where microbiota was found to influence sex bias in type 1 diabetes (19, 20). In nonobese diabetic (NOD) mice, females were found to possess greater microbial diversity than male counterparts (19). In addition, it was found that the levels of Alstipes (family Erysipelotrichaceae) were higher in male NOD mice, although the difference was not statistically significant (20). Our data from the MRL/lpr model agreed with the above reports that female mice had significantly higher microbial diversity (see Fig. S2D in the supplemental material), and Erysipelotrichaceae was significantly enriched in the gut microbiota of male MRL/lpr mice (Fig. 2A). In addition to these differences, we found that in lupus-prone mice, females had significantly higher levels of Lachnospiraceae, whereas males had significantly higher levels of Bifidobacterium (Fig. 2A). Bifidobacterium, like lactobacilli, has been suggested to exert anti-inflammatory functions (73). Thus, a greater abundance of Bifidobacterium in the gut may be associated with attenuated lupus symptoms in males. It was also noted that the variation of the abundance of some bacteria appeared to be greater in females than in males (see, for example, Verrucomicrobiaceae in Fig. 2A). This could be due to the lack of synchronization of the estrus cycle, since microbiota may change according to the female reproductive cycle (74).

Important roles of vitamin A and its derivatives, including RA, in regulating antiviral and/or antibacterial immune responses have been recognized since the 1920s (75). However, whether and how RA affects lupus is not clearly understood. We found here that treatment with RA attenuated lupus-like symptoms while increasing the Lactobacillaceae family of commensal bacteria in the colons of lupus-prone MRL/lpr mice (Fig. 3C). RA can sustain the stability and function of natural Treg cells (76) and promote the differentiation of inducible Treg cells in the periphery (77–79), which may in turn attenuate lupus. Lactobacillus spp. can also suppress proinflammatory responses by increasing the number of inducible Treg cells (61, 62). Thus, it is likely that RA and Lactobacillaceae that RA can enrich in the gut of lupus-prone mice (Fig. 3A and B) could work in concert to induce Treg cells and attenuate inflammatory flares in lupus.

Retinol is the primary ingredient in vitamin A supplements. In this report we found that retinol, represented by VARA treatment, increased the fraction of Lachnospiraceae in the gut of MRL/lpr mice (Fig. 3B) that was already higher than that in MRL mice (Fig. 1D). It did not attenuate lupus (Fig. 3C) or affect members of the Lactobacillaceae family (Fig. 3B). This suggests that unlike RA, retinol may promote intestinal colonization of Lachnospiraceae that does not attenuate lupus pathogenesis. In fact, the percentage of Lachnospiraceae in MRL/lpr mice negatively correlated with that of Lactobacillaceae and positively correlated with lymphadenopathy of the spleen and impaired renal function (Fig. 3C). Therefore, our results suggest that RA, given as a direct dose, may be better than retinol in suppressing inflammation in lupus. Metagenomic predication of microbial metabolic functions, where RA was shown to reverse, while retinol (VARA) exacerbated, lupus-associated changes (Fig. 4), supports this hypothesis.

The composition of gut microbiota can be greatly altered by the housing condition. In the present study, all mice were maintained in the same room under the same specific-pathogen-free housing condition. In addition, mice in each experimental group were randomly selected from different litters to eliminate the cage effect as a potential confounding factor. We kept control and lupus animals in different cages to avoid the possible effect of coprophagy on disease progression. It has been noted, however, after the completion of the study, that the combination of housing condition adaptation and dam cohousing between control and lupus strains prior to their respective breeding could have further equilibrated the baseline microbiota.

Altogether, our study showed several important lupus-associated changes in gut microbiota. Intestinal colonization of Lactobacillaceae, in particular, was found to negatively correlate with lupus activity. This suggests that probiotics containing lactobacilli may be able to decrease the occurrence and/or severity of inflammatory flares suffered by lupus patients, but future studies are required to address whether a causal relationship between lactobacilli and lupus exists. In addition, the potential therapeutic benefit of probiotics against lupus may be enhanced when combined with RA, a dietary supplement that can increase the levels of lactobacilli in the gut and attenuate lupus symptoms.
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We declare that we have no conflict of interest.

REFERENCES


