Chapter 8

Diet and Microbes in the Pathogenesis of Lupus

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Abstract

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder with no known cure. It is characterized by severe and persistent inflammation that damages multiple organs. To date, treatment and prevention of disease flares have relied on long-term use of anti-inflammatory drugs where side effects are of particular concern. There is a need for better understanding of the disease and for better approaches in SLE treatment and management. In this chapter, we delineate the roles of diet and microbes in the pathogenesis of SLE.

Keywords: diet, microbiota, lupus, hygiene hypothesis, epigenetics

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder with complex genetic and environmental etiology. It is characterized by severe and persistent inflammation that leads to tissue damage in multiple organs. The cause is unclear, and there is currently no cure. Current standard of care treatments for SLE are primarily nonselective immunosuppressants, and not all patients respond to these regimens. Although current therapies can treat acute symptoms and reduce the risk of renal failure associated with SLE, side effects are a major cause of concern. Patients taking long-term immunosuppressants are prone to higher incidence of and more severe infections. There is an imperative need to develop new treatment strategies against SLE, for which a better understanding of disease pathogenesis is required. In this chapter, we delineate the roles of diet and microbes in the pathogenesis of SLE.

2. The hygiene hypothesis

Introduction. Environmental factors are known to impact lupus progression in both human and mouse. The role of environmental factors in the etiology of SLE is evidenced by the dramatic difference in disease incidence between West Africans and African-Americans, both derived from the same ethnic group but exposed to different environments. In addition, two remarkable cases of disease amelioration have been reported in SLE patients who had experienced multiple microbial infections. In lupus-prone mice, infections have also been demonstrated to attenuate lupus-like disease. The mechanisms behind these observations are unclear, but improvement in hygiene and absence of certain microbes may have contributed to the higher incidence and faster progression of lupus disease. In this subsection, we will describe the hygiene hypothesis and its relationship with the pathogenesis of SLE.

SLE and the hygiene hypothesis. Proposed by Strachan [1], the hygiene hypothesis initially states that the increased incidence of allergies stems from "declining family size, improvements in household amenities, and higher standards of personal cleanliness." Since then, the scope of the hypothesis has expanded to cover several autoimmune diseases, including type 1 diabetes (T1D), inflammatory bowel disease and multiple sclerosis. Many infectious agents have been described to be protective against autoimmunity [2, 3], whereas mouse models of these autoimmune diseases, such as NOD mice for T1D, are known to develop more progressive clinical signs in cleaner environments. In the case for SLE, it has been long recognized that lupus-prone mice exhibit different disease courses in different animal facilities, suggesting an important role for environmental factors in the etiology of SLE. In addition, the incidence of SLE increased at least threefold in the second half of the twentieth century [4, 5], which is strongly correlated with the increase in hygiene and the decrease of infections, particularly those of the gastrointestinal tract [6]. We therefore have recently extended the hygiene hypothesis to SLE [7].

Protective infectious agents against SLE. The increase in hygiene could reduce infections that are either pathogenic or protective in SLE. Known triggers of lupus development include parvovirus B-19 [8], rubella virus [9], Epstein-Barr virus and cytomegalovirus (CMV) [7, 10–15]. Infectious agents that have been shown to be protective against SLE, on the other hand, include *Helicobacter pylori*, hepatitis B virus (HBV), *Toxoplasma gondii*, malaria parasites such as *Plasmodium berghei* and *Plasmodium chabaudi* and more recently described, helminths. In a large cohort of African-Americans, *H. pylori* seronegativity was found to be associated with an increased risk and earlier onset of SLE [16, 17]. For HBV, only 2.5% of SLE patients were found to be positive for an HBV-specific antibody, suggesting prior infection, whereas in the general population, >10% of people are found positive for the same antibody [9, 18]. Besides human studies, it has been shown that the infection of *T. gondii* protects New Zealand Black (NZB)/New Zealand White (NZW) F1 hybrid (NZB/W F1) lupus-prone mice from developing SLE-like disease, with significantly decreased mortality, ameliorated lupus nephritis and reduced autoantibodies in the serum [19, 20]. Infections with malaria-causing parasites also protected NZB/W F1 mice from developing clinical signs of murine lupus [21–24]. In another classical mouse model of SLE, MRL/Mp-Fas^{lpr} (MRL/lpr), helminthic infection and administration of helminth (worm)-derived molecules

have been recently shown to induce the regulatory functions of the immune system and attenuate SLE-like disease such as glomerulonephritis and development of anti-nuclear antibodies [25–27]. Similar results have also been obtained in NZB/W F1 mice, although with a different worm-derived compound [28]. Interestingly, all these protective infectious agents are highly prevalent in Africa where the incidence of SLE is low. Due to vaccinations and the increase in hygiene such as higher quality of drinking water, people in North America and Europe are much less likely to be infected by these agents. Conversely, SLE is of high prevalence in Western developed countries, especially for people of African descent.

It is most remarkable, however, that multiple infections led to complete amelioration of lupus disease in two female patients previously diagnosed with the most active form of SLE [20]. Both women were of childbearing age, where pregnancy was found to be a trigger of lupus flares. In addition, their disease was refractory to all available treatments. Due to drug-induced immunosuppression, they experienced multiple infections over several months that included CMV and *Staphylococcus aureus*, which unfortunately induced sepsis. One patient also experienced infections of *Pseudomonas aeruginosa* and *Pneumocystis carinii*, whereas the other experienced multiple *Escherichia coli* urinary tract infections. Both were treated with antivirals such as ganciclovir and antibiotics such as vancomycin and trimethoprim-sulfamethoxazole. Remarkably, the course of lupus disease changed dramatically in both women with improved autoantibody titers. They were reported to be symptom free after 3–5 years of follow-ups, and one patient even had a normal pregnancy afterwards. While the authors of the reports suggested that the infections had caused the amelioration of SLE symptoms, it is likely that the combination of infections and antiviral/antibacterial treatments had contributed to the change in disease course.

Mechanisms of protection. A couple of mechanisms could explain the protective effects of infectious agents against SLE. The first mechanism is competition. It is likely that the strong immune responses elicited by infectious agents can compete successfully for homeostatic signals (cytokines, growth factors, etc.) against the autoimmune response elicited by weaker autoantigens. The second mechanism is that regulatory cells stimulated by infectious agents can dampen autoimmune response. For example, type I interferons induced by some infectious agents can induce the generation of IL-10-producing regulatory T cells (Treg or Tr1 cells) [29, 30] and limit the production of IL-17 from T cells [31], both of which are mechanisms to suppress autoimmunity. Besides regulatory T cells, anti-inflammatory dendritic cells (DCs) that produce IL-10 and transforming growth factor β (TGF-β) and drive T helper (Th)-2 responses can be also induced by parasitic infections [3]. In addition, parasitic infections can promote IL-4 secretion from basophils and NKT cells, leading to a Th2-biased response to dampen Th1/Th17-induced autoimmunity [3]. Bacterial products, on the other hand, have been shown to directly induce IL-10-producing Treg cells [32]. Finally, toll-like receptors (TLR) appear to mediate the effects of protective infectious agents, which are TLR agonists, in preventing autoimmunity [6]. It is worth noting that these mechanistic studies were performed with regard to T1D.

Specifically for SLE, several studies have tried to pinpoint the mechanisms by which protective infectious agents prevent lupus-like disease in mice. In response to *T. gondii* infection,

the levels of interferon (IFN)-γ and IL-10 decreased in NZB/W F1 mice, suggesting the repression of disease-facilitating Th1 and Th2 cytokines in the development of lupus-like nephritis [19]. In addition, alterations of the redox state in kidney and liver tissues may explain the protective effects of malarial infections against lupus nephritis [21]. A parasite-derived compound tuftsin-phosphorylcholine, on the other hand, was able to enhance the expression of TGF-β and IL-10 as well as the expansion of Treg cells in NZB/W F1 mice, while at the same time inhibiting the expression of IFN-γ and IL-17 [28]. This suggests a shift of the balance between Th1/Th17 and Treg toward a Treg-biased phenotype that may in turn attenuate lupus-like disease. Moreover, infection of *Schistosoma mansoni* skewed the Th1:Th2 balance to a Th2 phenotype in MRL/lpr mice, which significantly changed the pathophysiology of glomerulonephritis from diffuse proliferative lupus nephritis (more severe) to membranous lupus nephritis (less severe) [25]. Furthermore, it has been found that a parasitic worm product ES-62 targets MyD88-dependent effector functions of B cells (promoting regulatory B cells and inhibiting plasmablast differentiation) to suppress auto-antibody production and proteinuria in MRL/lpr mice [26, 27]. Altogether, these studies suggest that infectious agents can protect against the development of SLE by inducing the regulatory functions of immune cells.

3. Diet and SLE

Introduction. Environmental factors beyond microbial infections contribute to the pathogenesis and severity of disease progression in mouse models, as well as lupus patients. Nutritional components including vitamins, caloric excess or restriction, polyunsaturated fatty acid composition, excess sodium intake and exogenous hormone containing compounds all influence the clinical signs and immune system cellular responses. Nutritional compounds such as vitamin D, omega-3 fatty acids and conjugated linoleic acid appear to have beneficial effects on some parameters of lupus, while high levels of vitamin E, omega-6 fatty acids, a modern "western diet," and high levels of circulating adipokines may exacerbate disease flares. Various commonly consumed phytoestrogens exert complex and differential effects, leading to regulation or exacerbation of SLE clinical signs. Modulating the immune system through dietary intervention to promote regulation of the immune response may form an adjunctive treatment to reduce signs of SLE both for maintenance as well as during disease flare-ups, while decreasing necessary dosages of systemic medications. In this section, we will discuss the role of diet in the pathogenesis of SLE.

Vitamin A. The three main isoforms of the ubiquitously expressed nuclear retinoic acid receptor (RAR), α, β and γ, heterodimerize with the retinoic X receptor (RXR) isoforms. Retinoic acid binding to these heterodimers leads to promotion of retinoic acid-responsive genes through retinoic acid response elements (RAREs). The RXR receptors are also associated with multiple nuclear receptors, including peroxisome proliferator-activated receptor (PPAR) and vitamin D receptor (VDR). Retinoic acid and 1,25-dihydroxyvitamin D3 (D3), the active hormonal metabolite of vitamin D, have been described to potentially antagonize each other's effects due to the similar binding patterns of RAR and VDR to RXR [33].

Retinoic acid has many roles in the development of both innate and cell-mediated immunity. Exposure of antigen presenting cells (APCs) to retinoic acid is critical for cell development and establishment of normal cellular function, leading to an increase in co-stimulatory molecules and induction of the expression of CD11b, a critical adhesion marker, on murine splenocytes and human monocytes [34, 35]. Retinoic acid appears to have differential effects on the induction of immune responses. 9-cis retinoic acid decreases the production of IL-12 in macrophages. However, when combined with lipopolysaccharide (LPS), it augmented the production of nitric oxide, leading to promotion of the Th2 phenotype through regulation of cytokine production by APCs [34, 36, 37].

The development and differentiation of B and T cells are also influenced by the exposure to retinoic acid. Administration of retinoic acid contributes to increased $CD19^+$ B cell numbers in the bone marrow and spleen, accelerated B cell maturation and increased expression of Pax-5, a transcription factor promoting early B cell development and expansion [33, 34]. Conversely, retinoic acid is reported by several groups to decrease mature B cell expansion by arresting B cells in the G0/G1 phase through increased expression of p27 (Kip1). This restricted proliferation may be necessary for the increased expression of CD38 leading to increased plasma cell numbers and production of IgG1 [34, 38–40]. Retinoic acid has also been reported to increase the proliferation of memory B cells when stimulated with the TLR9 ligand CpG and retinoic acid. Stimulation of B cells with both molecules led to a threefold increase in immunoglobulin secretion compared with cells stimulated with CpG alone [34].

Vitamin A deficiency has been associated with a decrease in $CD4^+$ T cells, a decrease in the $CD4^+$ to $CD8^+$ ratio, decreased splenic germinal center formation, decreased total splenocytes, as well as splenic and thymus organ mass [41–43]. Deficiency of vitamin A also contributed to an increase in a Th1-driven immune response, with increased IFN-γ and IL-12 and a decrease in IL-5 and IL-10 production [41]. Administration of retinoic acid contributed to an increase in CD4:CD8 ratio and the promotion of a Th2-driven immune response with an increase in IL-5 production, while repressing IFN-γ and the Th1 response [37, 44]. The administration of a single dose of all-*trans*-retinoic acid in a murine model of lupus nephritis reduced the disease severity and production of Th1-associated cytokines IL-2 and IFN-γ [45]. Another study in the same NZB/W F1 model of lupus reported prolonged survival with decreases in total IgG, IgG2a and anti-dsDNA autoantibodies in the serum [46]. A major contribution to the development of lupus nephritis is the deposition of immunoglobulins and complement proteins in the glomeruli. Multiple groups have reported the protective effect of retinoic acid on the development of glomerular disease, leading to decreased proteinuria and glomerular damage, while maintaining similar levels of IgG and C3 deposition within the glomeruli [47]. Another study reported that retinoic acid inhibits Th1-phenotype-related genes T-bet and IRF-1, leading to a decrease in Th1-related cytokines and altering the Th1/Th2 balance, skewing toward a type-2 response [48].

Retinoic acid administration to MRL/lpr mice contributes to the sustained function of natural T regulatory (Treg) cells and promotes the differentiation of inducible Treg cells in the peripheral tissues [36]. Enhanced differentiation of T cells to Treg cells was shown in the small intestine lamina propria, as well as upregulation of gut-homing receptors on Treg cells by the

binding of retinoic acid [36, 49]. There is discrepancy as to whether DCs or macrophages are the predominant cell type to induce Treg cell differentiation in the small intestinal lamina propria, while DCs are known to contribute to Th17 cell differentiation. It has been shown that human SLE patients exhibit a defective induction of Treg cells through TGF-β induction and retinoic acid expansion [37]. This impaired induction led to increased numbers of Treg cells with defective regulatory function abilities, causing the inducible Treg cells to be ineffective at controlling the autoimmune inflammatory response. The studies described above clearly show that cells of both the innate and adaptive immune system are regulated by vitamin A/retinoic acid. Therefore, it is conceivable that vitamin A and its metabolites may have an effect on patients suffering from SLE.

Vitamin D. Vitamin D3 and its metabolites have been extensively studied and have shown to influence multiple functions in the body, including the abilities to affect bone density and strength, and to modulate both the innate and adaptive immune branches. In this chapter, we will focus on the evidence of vitamin D's metabolites influence on the human immune system as it pertains to lupus. Vitamin D3 administration contributes to decreased pro-inflammatory cytokine production by human monocytes, along with decrease dendritic cell maturation, while increasing IL-10, impairing the differentiation of T cells into Th1 cells [50]. D3 is also able to decrease the expression of MHC class II, CD40, CD80 and CD86 co-stimulatory molecules, decreasing the ability of DCs to present antigens to T and B cells. The ability of D3 to activate human monocytes and macrophages in vitro led to increased cathelicidin and IL-1 production [48].

D3 decreases the expression of IL-2, IFN-γ and T cell proliferation in vitro, as well as decreasing $CD8^+$ cytotoxic activity. It is important in the regulation of many genes, including IL-2 and IFN-γ in T cells, through interaction with the vitamin D receptor-RXR heterodimer. Similar to retinoic acid, D3 inhibits the induction of the Th1-mediated immunity response in favor of the Th2 phenotype, through enhancing the production of IL-4 and decreasing IFN-γ. The Th17 cell response is also mitigated by vitamin D through the inhibition of IL-6 and IL-23 production, leading to the promotion of the Treg cell phenotype through expansion of Foxp3 [51, 52].

It has not yet been determined if D3 acts directly on B cells to decrease B cell proliferation and induction of apoptosis. D3 is also associated with decreased plasma cell differentiation and a subsequent decrease in IgG secretion. In one study, the authors showed that D3 has the ability to upregulate p27 mRNA; however, p18 and p21 were not similarly upregulated in human purified B cells from patients with SLE. The p27 gene is associated with inhibition of the cell cycle, leading to decreased proliferation in activated human B cells, especially memory B cells [53].

Mouse models of SLE have shown decreased proteinuria and prolonged survival when supplemented with D3. A commonly used therapy for human SLE patients is the administration of glucocorticoids to reduce inflammation and to broadly dampen the immune response [54]. Glucocorticoids have been shown to decrease the expression of the vitamin D receptor in multiple cell types. Conversely, D3 interferes with both glucocorticoid receptor and androgen receptor responsiveness through reduced receptor expression and impaired translocation to

the nucleus due to alterations in phosphorylation status [55]. When administered as a medication, D3 can lead to adverse effects, including hypercalcemia and bone resorption [48].

Though in vitro evidence strongly supports an immune-regulatory role of vitamin D and its metabolites in the adaptive immune system, there are conflicting reports regarding vitamin D deficiency in SLE patients and the supportive role of vitamin D supplementation. The majority of studies describe decreased serum levels of D3 in patients with SLE compared to healthy patients, which correlated with increased disease activity. The current consensus is that vitamin D serum levels correlate inversely with disease activity, and vitamin D deficiency is associated with increased disease activity or severity [51, 56].

Vitamin E. Vitamin E is a potent antioxidant supporting the normal structure and function of cells by reducing damaging free radical reactive oxygen species (ROS). ROS have been implicated in tissue damage and an increase in pro-inflammatory cytokine production [57]. These factors support the development of autoimmune and degenerative disease severity. The preferentially absorbed form of vitamin E is α-tocopherol, which contributes to the normal function of the immune system in humans and mice. In one study, lowered antioxidant ability was found to precede the diagnosis of SLE, and oxidative damage was increased prior to diagnosis of SLE. This suggests that the decreased ability to control oxidative damage is a potential risk factor for development of SLE [58, 59].

Multiple studies in mouse models of SLE have shown that the dose of vitamin E had differential effects on the disease. Low-dose α-tocopherol increases the lifespan of NZB/W F1 mice while decreasing proteinuria, anti-dsDNA autoantibody IgG in the serum, IL-6 and IFN-γ production from splenocytes [60]. In the MRL/lpr mouse model, low-dose administration of vitamin E increases lifespan and IL-2 production. In contrast, high-dose vitamin E increases anti-DNA and cardiolipin IgM, as well as IL-4 and IL-10 from activated splenocytes. The authors conclude that the decreased survival in high-dose groups was due to the imbalance of Th1/Th2 cytokines, with Th2 cytokines leading to hyperactivation of the B cells in the MRL/lpr model [61]. Vitamin E also increases the pro-inflammatory chemokine MIP-1α in MRL/lpr mice, which activates granulocytes and promotes pro-inflammatory cytokine production [62]. A study comparing plasma antioxidant status between healthy patients and patients diagnosed with SLE showed that SLE patients have impaired plasma antioxidant status as well as a decreased antioxidant intake. The study was small and did not take into account dietary restrictions or food intolerance, limiting the ability to draw firm conclusions for a large set of SLE patients [58]. The above studies demonstrate that the dose of vitamin E is a critical determinant in amelioration or exacerbation of murine lupus. Response to vitamin E supplementation in human SLE patients needs to be thoroughly investigated to determine whether vitamin E supplementation is appropriate.

Omega 3:6 PUFA. Poly-unsaturated fatty acids (PUFAs) have been a popular topic of research in health due to their wide range of effects that can be exerted on the body. Omega-3 PUFAs generally have an anti-inflammatory role, lowering the severity of autoimmune disease and increasing survival in mouse models of SLE. In contrast, diets that are high in saturated fatty acids and omega-6 PUFAs lead to higher anti-dsDNA antibody, proteinuria and inflammatory cytokines in mice [63, 64]. Mouse survival rates were not measured in these studies.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the main bioactive constituents of omega-3 rich fish oil. SLE patients were shown to have decreased EPA in erythrocytes along with a decrease in the EPA to pro-inflammatory arachidonic acid ratio [65]. The evidence in human studies and clinical trials using omega-3 PUFAs in SLE patients is inconclusive at this time, with multiple studies showing no demonstrable effect on SLE disease activity index or other clinical scores and no effect of omega-3 fatty acids on glucocorticoid requirements when used as immunosuppressive medication.

In a mouse study using NZB/W F1 female mice, higher on concentrations of EPA and DHA led to increased lifespan, decreased glomerulonephritis, decreased anti-dsDNA antibodies, as well as a reduction in pro-inflammatory cytokines IL-1β, IL-6 and TNFα in splenocytes, relative to controls. The authors also demonstrated a reduction in nuclear factor-κB (NF-κB) and p65 nuclear translocation in mice fed higher concentrations of both EPA and DHA [66]. In studies using the MRL/lpr mouse model, fish oil altered pro-inflammatory chemokine production, leading to a decrease in RANTES and MCP-1 from splenocytes [62]. In NZB/W F1 mice, omega-6 PUFAs, conversely, led to an increase in IL-6 and TNFα production as well as prostaglandin E2 from macrophages, a decrease in TGF-β mRNA from splenocytes and lower anti-dsDNA IgG in the serum [67].

There is evidence that n-3 PUFAs remodel the lipid rafts in T cells, which can lead to a decrease in intracellular signaling through the T cell receptor, as well as binding to multiple PPARs. Omega-3 PUFAs bind to PPARα in T and B cells, whereas they bind to PPARγ in cells of myeloid lineage, leading to alterations in gene expression. The binding capacity to PPARs by n-3 and n-6 PUFAs is equal, suggesting that gene modulation between the different families is unlikely, though the possibility exists for differences in cellular metabolism of n-3 and n-6 PUFAs to allow for distinct PPAR activation [65]. In certain instances, the evidence is contradictory between ex vivo and in vivo studies, and cytokine levels have been shown to be opposite in mouse models compared to human data [65]. While there is inconclusive evidence for the anti-inflammatory effects of n-3 PUFAs in human clinical trials, it is still recommended by some that rheumatic patients supplement their diet with long-chain n-3 PUFA and gamma linoleic acid [65].

Conjugated linoleic acid. Conjugated linoleic acid (CLA) has been described to provide health benefits in humans and makes up a group of isomers of linoleic acid. These fatty acids occur naturally in dairy and beef. CLA is considered to have anti-carcinogenic, anti-atherosclerotic and immune-enhancing abilities, with respect to lymphocytic cytotoxic function, macrophage activation and lymphocyte proliferation [68]. The isomer of CLA that demonstrates the strongest anti-inflammatory effect is *cis*9, *trans*11-CLA, which accounts for up to 90% of the dietary CLAv [68].

Supplementation with CLA leads to a reduction in the loss of body mass during end-stage disease, along with an increase in survival after the onset of proteinuria in the NZB/W F1 mouse model [69, 70]. In a non-autoimmune mouse model and cell lines, CLA has a profound effect on macrophage functions. In BALB/c mice, CLA ameliorates LPS-induced body wasting and anorexia in vivo, decreases the production of nitric oxide from macrophages, and decreases the serum concentration of TNFα. Splenocytes from BALB/c mice fed the CLA supplement

also have a lower level of IL-4 production and an increase in IL-2 production after stimulation with concanavalin A [71]. CLA also decreases macrophage adhesion as well as downregulating multiple atherogenic genes associated with leukocyte adhesion, while inducing the antagonist IL-1Rα in human umbilical cord vein endothelial cells and the RAW macrophage cell line [72].

CLA is able to exert immune-modulatory effects on dendritic cells and subsequent differentiation of T cells in mice. DCs produce multiple cytokines after activation and express MHC II on the cell surface to facilitate interactions with T cells and B cells. Exposure of DCs to CLA led to a decrease in IL-12 and an increase in IL-10 production, along with decreased migration to the lymph nodes [73]. CLA decreased the expression of MHC II and costimulatory molecules CD80 and CD86 on the surface of DCs, reducing the DC's ability to trigger T cell and B cells responses [74]. When exposed to LPS stimulation in mice fed a CLA rich diet, serum levels of IFN-γ, IL-1β and IL-12p40 were decreased [75]. CLA decreases the ability of DCs to induce the T helper cell differentiation into Th1 and Th17 cells, as well as directly suppressing the production of IL-17 and IL-2 by Th17 cells [74]. The mechanism for CLA influencing the DC response after activation with LPS was shown to be through suppression of both NF-κB and IRF3 downstream of TLR4 [75]. The immune-modulatory effects have been well described in mouse models, though data for the effects of CLA in human SLE patients are lacking at this time.

Western diet and obesity. Considerable attention has recently been given to the attributes of dietary components that make up the typical diet in the developed countries, termed the "western diet." These factors include high-fat, high-protein, high-sugar and high-salt, as well as consumption of a large portion of processed foods. These nutritional components promote obesity, metabolic syndrome and cardiovascular disease. In addition, a recent study has shown the role of the "western diet" in promotion of autoimmune disorders [76]. White adipose tissue is now considered to be a major endocrine organ, secreting more than 50 adipokines including leptin, adiponectin, resistin and visfatin, along with pro-inflammatory cytokines IL-6 and TNFα [76, 77]. Excess white adipose tissue drives a low-level steady state inflammation, which may contribute to various autoimmune and inflammatory-mediated diseases.

The connection between obesity and the development of SLE is complex, and contradictory information exists among studies. There was no correlation found in Danish women between obesity and the incidence of SLE after 11 years [77, 78]. This cohort study had multiple limitations, such as reliance on survey questionnaires regarding pre-pregnancy weights and diagnoses, potential socio-economic bias with women of lower socioeconomic status being underrepresented in the Danish National Birth Cohort, and the confounding factor of pregnancy and childbirth during the study, which do not allow for conclusions to be drawn regarding the link between obesity and SLE [78]. Childhood obesity rates correlated positively with incidence of childhood-onset SLE. The adipokine leptin has been extensively studied and exerts multiple effects on the body [79]. Leptin promotes satiety and stimulation of energy expenditure, by acting on the hypothalamic nuclei, as well as contributing to fertility, bone metabolism and having a profound effect on the immune system. Lack of leptin, due to

starvation, testosterone or glucocorticoids, can lead to immunosuppression, while upregulation of leptin by inflammatory cytokines or female sex hormones, 17β-estradiol and progesterone, can lead to inflammation [79]. Leptin can induce the production of IL-6, IL-12 and TNFα pro-inflammatory cytokines from macrophages, while stimulating the proliferation of naïve T cells and promoting a Th1 immune response. Leptin inhibits the production of Th2 cytokines IL-4 and IL-10, along with inhibiting T regulatory cell proliferation [80]. The formation of a pro-inflammatory state through increased leptin levels may contribute to autoimmune disease development.

Leptin is commonly elevated in obese patients as well as SLE patients, which contributes to the survival of autoreactive T cells, and a decrease in the functional T regulatory cells, while promoting the proliferation of Th17 cells in a mouse model of SLE [81, 82]. Circulating serum leptin levels as well as leptin secretion have been shown to be higher in females compared to males, both in obese and non-obese subjects. The sex difference in leptin secretion may be a contributing factor in the development of female-predominant SLE. Leptin deficiency in murine models of SLE has led to decreased anti-dsDNA and a decrease in severity of SLE symptoms [83, 84]. The increased level of circulating leptin may also contribute to the exacerbation of cardiovascular damage in some SLE patients [85, 86]. Adiponectin deficiency was correlated with an increase in SLE disease severity [87, 88]; however, another study found increased levels of adiponectin in the serum and urine of human patient with lupus nephritis [89]. Little information is available regarding other adipokines and their involvement with SLE development.

Obesity is correlated with a predisposition to metabolic syndrome, with increased rates of hypertension, dyslipidemia and atherosclerosis, as well as subsequent cardiovascular disease. Atherosclerosis and cardiovascular disease are highly prevalent in patients with SLE, and a significant number of patient deaths occur due to cardiovascular disease, while obesity has been linked to worsening renal disease and cardiovascular disease in patients suffering from SLE [77]. High levels of inflammatory markers and elevated leptin concentrations found in SLE patients, and mice have been correlated with decreased cognitive function, increased renal damage and increased cardiovascular risks [90, 91].

Diets high in salt (sodium chloride) are found in multiple regions throughout the world, mainly in developed countries where processed foods and "fast food" are prevalent. Excess sodium intake has been attributed to cardiovascular disease and hypertension, as well as stroke, and is now being studied in the development of autoimmune diseases [76]. T cells in a hyperosmotic environment show an increase in p38/MAPK as well as transcription factor NFAT5, leading to an altered cellular response [92]. Sodium can be stored in the human body in various tissues, leading to hyper-osmotic environments in multiple tissues during high-dietary salt intake. Human and murine T cells were investigated under high salt conditions *in vivo* that led to the promotion of Th17 differentiation in vitro [93]. High salt intake has been associated with a decrease in glucocorticoid therapy response in a study of 260 Chinese SLE patients treated with prednisone and followed for 12 weeks [94]. Further studies need to be performed to determine the mechanism for hyperosmotic conditions to promote the highly pro-inflammatory Th17 cell differentiation.

Phytoestrogens. Endogenously produced estrogen contributes significantly to the development of SLE and disease progression. Estrogen is able to suppress IL-2, enhancing the effect of autoantigens. 17β-estradiol implants led to an increase in IFN-γ, nitric oxide and a range of cytokines and chemokines in murine splenocytes [95]. The estrogen receptors on cells comprise two subtypes, α and β. Estrogen receptor-α (ERα) mRNA was increased in the peripheral blood mononuclear cells of SLE patients, while estrogen receptor-β (ERβ) was decreased. ERα was shown to be predominantly pro-inflammatory in a mouse model of SLE, leading to increased proteinuria and decreased survival time when activated, while ERβ showed an immunosuppressive phenotype by decreased autoantibody anti-DNA IgG2 [96, 97].

Phytoestrogens are plant compounds that exert an estrogenic or an anti-estrogenic effect on the human body through interaction with both estrogen receptor α and β, with a preferential binding to ERβ [97]. Phytoestrogens can also block the binding of more potent estrogenic compounds to the ER and regulate target genes. Many types of phytoestrogens are found naturally, with soy-based phytoestrogens, isoflavones, present in many of the foods consumed in industrialized societies. Due to the ubiquity of phytoestrogens in normal diets, controlled human trials are lacking with regard to the specific effects of phytoestrogens on SLE disease development. Data from mouse experiments involving supplementation of isoflavones to MRL/lpr mice show a decrease in IFN-γ from splenocytes with a decrease in anti-dsDNA and cardiolipin levels in the serum, along with a reduction in proteinuria and renal damage through preferential binding of the ERβ [98]. Exclusion of dietary isoflavones through diets using casein as the protein source rather than soy led to amelioration of glomerulonephritis [99]. Alfalfa sprout extract supplementation to MRL/lpr mice produced similar results with decreased renal damage and increased survival, while decreasing IFN-γ and IL-4 production by splenocytes. Alfalfa sprout extract was also able to reduce TNFα, IL-6 and IL-1β pro-inflammatory cytokines in a mouse model of endotoxic shock [100]. Coumestrol, one phytoestrogen in alfalfa, was able to decrease anti-dsDNA IgG and decrease proteinuria in the NZB/W F1 mouse model [101]. However, these findings have not been observed in human SLE patients, where ingestion of alfalfa tablets, which contain all phytoestrogen components of the alfalfa plant, exacerbated disease severity [102].

Multiple phytoestrogens have been shown to upregulate VDR expression in various human cell lines. Genistein and glycitein upregulated VDR transcription and translation in colon cancer cells, while resveratrol and genistein also increased VDR expression in breast cancer cells [103, 104]. Along with receptor expression, genistein decreased CYP24, an enzyme responsible for metabolism of the vitamin D metabolite D3 [105]. Phytoestrogens are abundant in many diets throughout the world and are able to contribute to the modulation of the immune system through interaction with estrogen receptors, potentially ameliorating or exacerbating patients' clinical signs.

4. The role of gut microbiota

Introduction. The mammalian gut harbors trillions of microorganisms known as the microbiota. Increasing evidence in recent years suggests that host microbiota and immune system

interact to maintain tissue homeostasis in healthy individuals. The importance of microbiota on the host is highlighted by altered immune responses in the absence of commensal bacteria. Higher susceptibility to infectious pathogens and in some cases, attenuated symptoms in autoimmune disorders, has been observed in mice raised under germ-free conditions. Indeed, perturbation of the host microbiota, especially that in the gut, has been shown to be associated with many autoimmune diseases, including SLE. Changes of gut microbiota in nephritic lupus mice versus healthy controls have recently been described, where a decrease of *Lactobacillaceae* and an increase of *Lachnospiraceae* were observed in lupus-prone mice. A cross-sectional study has also shown that a lower *Firmicutes* to *Bacteroidetes* ratio was present in the fecal microbiota of SLE patients with inactive disease, which is consistent with observations in other autoimmune diseases. In addition, oral antibiotics are known to trigger lupus flares, again suggesting a role for commensal bacteria in SLE. In this section, we will describe the existing data and a proposed role of gut microbiota in the pathogenesis of SLE.

Gut microbiota and SLE. Evidence is rapidly growing that mutualistic bacteria contribute to the development of a healthy functioning immune system, as well as the development of aberrant immune responses. The past 10 years have seen a rapid growth in the understanding of how the commensal microbiota is able to contribute to health and disease with the exploration of 16S rRNA sequencing along with the use of gnotobiotic animals. These newer methods of DNA sequencing have allowed researchers to investigate the effects of specific species or strains of organisms and how they interact with the immune system. Gnotobiotic animals are born into a sterile environment and develop under sterile conditions so that a specific group of bacteria, viruses, eukaryotes or parasites can be introduced to the animal, and the resulting effects can be studied without the interference of other organisms [106]. Intestinal microbial dysbiosis can lead to immune system effects at distant sites of the body [49, 107–112]. The systemic immune system is influenced by microbiota in the intestines, suggesting that microbiota at other sites of the body can also have a systemic effect on the host's immune system.

The host's genetic make-up is the primary factor in determining the composition of one's adult microbiota. Other contributing factors include mother's health in utero, the method of delivery during birth, if one is either breast fed or fed formula, the use of antibiotics during early childhood and types of foods consumed prior to, and during, weaning. The adult microbiota is not a static entity, however, and can be modified through many different environmental factors, and the composition will also change with age. Many of the previously discussed contributions to SLE are associated with alterations in the microbiota. Obese patients have an increased *Firmicutes* to *Bacterioidetes* ratio, with members of *Firmicutes* leading to increased energy harvest from dietary nutrients [113]. The "western diet" is associated with alterations in the gut microbiota as well. Children from a rural village in the West African country of Burkina Faso had increased microbial richness and greater *Prevotella* with decreased *Bacteroides* levels compared to European children. The microbiota of the Burkina Faso children also produced more short chain fatty acids than the intestinal microbiota of the European children [114]. These findings of agrarian societies with increased *Prevotella* and decreased *Bacteroides* have been supported in studies comparing rural South Americans and people from Bangladesh to people living in industrialized regions [115]. The source of dietary fat can impact microbiota composition as well, as mice fed diets with a fat source

from either milk-fat, lard, safflower oil or a low-fat diet, all resulted in distinct phylogenetic profiles [116, 117].

Murine studies on how the microbiota affects the immune system have emerged recently. At the level of the intestinal lamina propria, bacteria compete with host defenses and establish homeostasis. T cells are kept in check between pro-inflammatory Th1, Th2 and Th17 cells as well as innate lymphoid cells, and the anti-inflammatory T regulatory cells [118–120]. Specific bacterial groups can elicit distinct immune responses, as evidenced by mice colonized with segmented filamentous bacteria developed elevated levels of Th17 cells in the lamina propria [121, 122]. Colonization of previously germ-free mice with clostridial strains from cluster IV and XIVa resulted in an expansion of the T regulatory cell population in the lamina propria and systemically [123]. Polysaccharide A derived from *Bacteroides fragilis* is able to induce IL-10 production in the lamina propria through binding to TLR2 on T regulatory cells, inhibiting the expansion of the local Th17 cell population [124]. Only a single study, reported in 1999, to date has explored the development of SLE in a germ-free mouse strain. That study used MRL/lpr mice and showed no difference in disease progression between conventionally raised mice and germ-free mice [125]. Due to the *Fas* mutation and the underlying cause of lupus disease in this strain of mice, the MRL/lpr strain may not be the best strain to understand the impact of gut microbiome in a germ-free setting on lupus. Few murine studies investigating the microbiota's role in SLE have been published to date. Regardless, gut microbiome in MRL/lpr mice has recently been shown to impact lupus. In the MRL/lpr mouse model of SLE, young female mice had lower numbers of *Lactobacilli* species and increased numbers of *Lachnospiraceae* density in the murine feces. The administration of retinoic acid restored the *Lactobacilli* density and correlated with reduced clinical signs and a reverse of multiple lupus-associated microbial functions. The increased density of *Lachnospiraceae* was also overrepresented in female lupus-prone mice along with butyrate producing *Clostridiaceae* species. There is potential that use of *Lactobacilli* containing probiotics and vitamin A may benefit lupus patients [49, 107].

There have been few human trials to determine the impact on intestinal microbiota specifically on SLE development. In 20 human SLE patients in remission compared to healthy controls, a decreased *Firmicutes* to *Bacterioidetes* ratio was found in fecal samples. This change in fecal microbiota is associated with an increase in oxidative phosphorylation and glycan utilization by the host microbiota [126, 127]. In contrast to the murine studies, *Lachnospiraceae* and *Clostridia* were associated with healthy patients, rather than lupus patients [126]. The intestinal microbiota is a complex conglomeration that can contribute to modulation of the local and systemic immune system. Current and future work will begin to target specific groups and mechanisms of microbial contribution to systemic autoimmune disease pathogenesis.

5. Bacterial metabolites and mechanisms of action

Introduction. Bacterial metabolites produced by the gut microbiota may have profound effects on immune function. Recently, several groups have found that short-chain fatty acids (SCFAs) produced by gut microbiota, especially butyrate produced by Clostridia, can promote the

differentiation of regulatory T (Treg) cells in the colon, spleen and lymph nodes and suppress inflammation [128–131]. Treg deficiency leads to autoimmunity, while re-introduction of Treg cells can rescue animals from the disease. In human SLE, a pathogenic role of dysfunctional Treg cells has been suggested. In particular, the imbalance between Treg and Th17 cells and a bias toward IL 17-producing cells (both Th17 cells and double-negative T cells can produce IL-17 in SLE) are widely recognized for SLE. In the gut, the number and diversity of butyrate-producing bacteria are subject to factors related to age, disease and to diet. Butyrate and SCFAs are inhibitors of histone deacetylases (HDACs). HDAC inhibition can result in altering gene expression by making the chromatin more accessible to transcription factors by acetylating histone proteins at specific lysine residues and may also lead to post-translational modification of several transcription factors that reside both cytosolic and nuclear. Below, we have outlined the influence of SCFAs (predominately butyrate) on HDAC activity on the epithelia in the gut microbiota as it is likely to play an important role in regulation of the gut microbiota and lupus.

Role of epigenetics in immunity. Although genome-wide association studies have identified many genes that may play a role in the initiation or progression of SLE [132–134], these studies do not account for risk attributed to heritable factors [135] and have failed to identify a unifying switch. Epigenetics is the process in which alterations in gene expression and phenotype occur which are heritable but do not alter the DNA sequence [136]. There is increasing evidence that epigenetics plays a key role in SLE pathogenesis and epigenetically targeted therapies may be efficacious [137, 138]. In regard to epigenetics, interactions between DNA and core histone proteins are important in regulating the accessibility of transcription factors to bind promoter regions and thus regulate gene expression [139]. Histone acetyltransferases (HATs) and HDACs can alter the charge and subsequent binding affinity of core histone proteins to the chromatin through removal or addition of acetyl groups on lysine residues, respectively [140–142]. Recent investigations have revealed that HATs and HDACs are also capable of modifying lysine residues on numerous non-histone nuclear and cytosolic proteins [141, 143], which has driven some researchers to alternatively refer to the enzymes as lysine (K) acetyltransferases (KATs) and lysine deacetylases (KDACs).

HDAC enzymes. There are 18 mammalian HDACs, which remove acetyl groups from lysine residues in histones and other proteins to control multiple cellular functions including transcription, cell cycle kinetics, cell signaling and cellular transport processes [144]. HDACs are classified based on structure, homology to yeast HDACs and function into classes I–IV [145, 146]. Class I HDACs (HDAC-1, -2, -3 and -8) are nuclear exclusive enzymes found in a wide range of tissues and cells lines where they are known for histone modification and repression of transcription [147, 148]. Class II HDACs are further subdivided into class IIa (HDAC-4, -5, -7 and -9) and class IIb (HDAC-6 and -10) based on domain organization [149] and exhibit selective tissue expression, nucleocytoplasmic shuttling and function through recruitment of distinct cofactors [148]. Class III comprises the sirtuins, which act through a distinct NAD^+-dependent mechanism and are not considered "classical" HDACs [147]. HDAC11 is the sole member of class IV as phylogenetic analysis revealed very low similarity to HDACs in the other classes [150].

In addition to their initial relevance in cancer biology [151], HDAC enzymes are now increasingly being investigated as regulators of inflammation and immunity [147]. As reviewed by Shakespear et al., HDACs are documented to play a role in myeloid development, toll-like receptor (TLR) and interferon (IFN) signaling in innate immune cells, antigen presentation and development and function of B and T lymphocytes [147]. Subsequently, pharmacologic inhibition of HDACs has been evaluated as a possible treatment modality in a wide spectrum of diseases, including inflammatory and autoimmune diseases [152].

SCFAs. Intestinal bacteria provide the hosts with nutrients and confer resistance to infection. The delicate balance between pro- and anti-inflammatory mechanisms, essential for gut immune homeostasis, is affected by the composition of the commensal microbial community and has been reviewed by others [153]. Recent studies have shown that metabolism and immunology are intertwined and the field of immunometabolism has emerged which examines how gut metabolites influence immune cell function [154]. In the gut, carbohydrates resistant to breakdown in the stomach and small intestine are subject to colonic fermentation to result in the production of SCFAs, containing1–6 carbon atoms. Anaerobic bacteria generate the major SCFAs and include acetate, propionate and butyrate. The SCFA butyrate is produced by fermentation of dietary fiber by the intestinal microbiota and is the primary energy source of colonocytes [155]. Recently, Imhann and coworkers showed that patients with inflammatory bowel disease (IBD) had a decrease in the genus *Roseburia*. Furthermore, they noted that *Roseburia* spp. is acetate-to-butyrate converters suggesting that a lack of butyrate may contribute to IBD [156]. Furthermore, a reduction on butyrate producing *Roseburia* ssp. has been associated with chronic kidney disease progression [157]. On the other hand, butyrate has seen shown to aggravate dextran sulfate sodium induce colitis in an animal model [158]. In regard to HDAC inhibition, butyric acid has been reported to specifically inhibit the class I HDACs (1, 2, 3 and 8) [159].

T cells in gut immunity. Balanced mucosal immunity in the gut is critical for host homeostasis and defense. Naïve $CD4^+$ T cells when activated differentiate into T helper cell [Th1, Th2, Th17 or follicular helper (Tfh)] depending on cytokine exposure and B cell or antigen presenting cell influence. One specific subset of T cells (Treg cells) acts to suppress effect T cell function. The majority of Treg cells develop in the thymus (nTregs) and are selected for by strong or intermediate T cell receptor (TCR) signals while escaping negative selection [160]. Additionally, Treg cells can also be induced under certain circumstances from naïve T cells in the periphery (iTregs). This can happen systemically and has been shown to occur at interface with the environment in both whole animal and in in vitro assays [161]. Treg differentiation is strongly influenced by the presence of the anti-inflammatory cytokine transforming growth factor β (TGF-β). Furthermore, expression of the transcription factor Foxp3 is essential for Treg development and function and is regulated by genomic regulatory elements termed conserved noncoding DNA sequences (CNS). While CNS1 is unnecessary for nTreg differentiation, it has been reported to be crucial for iTreg generation in gut-associated lymphoid tissues (GALT) [162]. CNS2 is required for Foxp3 expression in the progeny of dividing Treg cells. CNS3 controls *de novo* Foxp3 expression and nTreg differentiation [163]. Studies have shown that Treg cells expressing transcription factor Foxp3 have a key role in limiting inflammatory responses in the intestine [164, 165].

Treg-Th17 balance. Th17 is a subset of T helper cells and serves to maintain the mucosal barrier and contribute to pathogen clearance at mucosal surfaces. However, they have also been implicated in autoimmune and inflammatory disorders as the loss of Th17 cells at mucosal surfaces has been shown to allow chronic inflammation and microbial translocation. In the gut, there exists a balance of Treg-Th17 cells as the signals that cause Th17s to differentiate actually inhibit Treg differentiation [166]. Since both Treg and Th17 cells are both pertinent to gut homeostasis and immune regulation, the balance of these T cells is critical for homeostasis [167]. Treg cells prevent systemic and tissue-specific autoimmunity and inflammatory lesions at mucosal interfaces [165]. Mice deficient in iTregs spontaneously developed pronounced Th2-type pathologies at mucosal sites including in the gastrointestinal tract and lungs and shows hallmarks of allergic inflammation and asthma [168]. Studies have shown that in the gut, iTreg cells are the prominent phenotype and are rapidly induced following naïve T cell activation which is dependent on Notch2-singling and may be somewhat independent of TGF-β [165]. This suggests that whereas nTreg cells generated in the thymus appear sufficient for control of systemic and tissue-specific autoimmunity, extrathymic differentiation of iTregs affects the commensal microbiota composition and serves a distinct, essential function in maintaining the inflammatory response at mucosal interfaces. Furthermore, when the animals were given the SCFA (butyrate), Treg cell differentiation in the gut increased and this was dependent on CNS1 expression [129]. In addition to butyrate, de novo iTreg generation in the periphery was potentiated by propionate, another SCFA of microbial origin capable of HDAC inhibition, but not acetate, which lacks this HDAC-inhibitory activity, suggesting that bacterial metabolites mediate communication between the commensal microbiota and the immune system. Other studies have also reported that a butyrate-mediated increase in the Treg cell subset in vivo was due to increased extrathymic generation of Treg cells and not due to their increased nTreg cells [169]. In addition to butyrate inducing a Treg phenotype, butyrate has also been shown to increases macrophage phagocytosis and killing of bacteria. When Treg cells were cultured with stimulated macrophages exposed to IL-4 and butyrate, less inflammatory cytokines were produced compared to macrophages treated with IL-4 indicating that microbial-derived butyrate decreases inflammatory mediator production in the gut [170]. In other studies using the antibiotic vancomycin, which targets Gram-positive bacteria, the level of iTregs was reduced. This could be due to the decrease in *Roseburia* spp. that is the butyrate-producing, Gram-positive anaerobic bacteria that inhabit the human colon. However, when specific pathogen-free mice were treated with a combination of vancomycin and SCFAs, the reduction in iTregs was completely restored suggesting that SCFAs play a role in iTreg homeostasis [128].

HDAC and Treg-Th17 balance in lupus. HDAC inhibition has been shown to decrease disease in lupus-prone MRL/lpr and NZB/W F1 mice [171–174]. Mechanisms by which HDAC inhibition decreases SLE disease have previously been reviewed by Reilly and others [142, 175]. HDAC inhibition may act in several ways including correction the hypoacetylation states of histones H3 and H4 [176], increased $CD4^{+}CD25^{+}Foxp3^{+}$ Treg cells [172, 174], reduced Th1- and Th17-inducing cytokines (IL-12 and IL-23) as well as Th1-attracting chemokines [142], and inhibition of germline and post-switch immunoglobulin transcripts in splenic B cells [177]. More importantly in SLE, decreased renal disease (glomerulonephritis and proteinuria) has been consistently reported in studies investigating the use of HDAC inhibitors to

treat lupus in various mouse models [171–174]. Pan and selective HDAC inhibitors are being evaluated in the clinic for inflammatory diseases with some mixed results and adverse effects such as fatigue, nausea, vomiting, diarrhea, thrombocytopenia, neutropenia and cardiac irregularities [178]. Investigations of specific functions for each HDAC isoform in knockout mice have revealed that elimination of class I and class IIa HDACs result in embryonic lethal phenotypes or fatal cardiac, vascular, musculoskeletal or neural crest defects, and specific HDAC isoform activity is required for normal cells development [178, 179]. The observed butyrate-mediated increase in the Treg cell subsets in vivo due to increased extrathymic generation of Treg cells and not due to their increased thymic output would support a role of HDACi in gut homeostasis [169]. In studies involving lupus patients, while Treg or Th17 cells alone were not correlated with SLE development, the ratio of Treg to Th17 cells in active SLE patients was significantly lower than that in inactive SLE patients and healthy controls. Moreover, corticosteroid treatment increased the ratio of Treg to Th17 cells in active SLE patients. Indeed, the Treg/Th17 cell ratio is inversely correlated with the severity of active SLE, indicating that in active SLE, there appears to exist an imbalance between Treg and Th17 cells [180]. Inducible Tregs cells are dependent on the expression of the transcription factor Foxp3 which may be transiently expressed allowing for plasticity of the Tregs/Th17 phenotype [181]. Interestingly, when Foxp3 is acetylated, the transcription factor becomes more stable and has greater propensity to bind DNA yielding a more stable and effective Treg population [182]. In addition to the regulation of Treg differentiation, butyrate has also been shown to increase the tri-methylation of lysine 27 on histone 3 (H3K27me3) in the promoter of nuclear factor-κB1 (NF-κB1) in colon tissue resulting in repression of inflammation [183]. In our lupus mouse studies, we found a marked depletion of lactobacilli in our lupus animals and increases in *Lachnospiraceae* compared to age-matched health controls. Interestingly, we also found that *Lachnospiraceae*, butyrate-producing genera, was more abundant in the gut of lupus-prone mice at specific time points during lupus progression [49]. Whether this was causative or in response to disease pathogenesis is an active area of investigation in our laboratory. Nonetheless, our results and others demonstrate the dynamics of gut microbiota as that bacterial production of SCFAs and butyrate may play a role in the initiation and progression of inflammation and autoimmunity.

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