Review Article

Systemic Sclerosis and Microbiota: Overview of Current Research Trends and Future Perspective

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The commensal microbiota contributes to the maintenance of immune homeostasis in the human body. Autoimmunity can be aggravated or alleviated by the microbiota, which affects both innate and adaptive immune cells. Many studies have demonstrated the role of gut dysbiosis, the alteration of the gut microbiome, in the development and progression of numerous autoimmune diseases. Systemic sclerosis (SSc) is an autoimmune disease of the connective tissue and is characterized by skin and lung fibrosis, as well as injuries in small arteries. Recent studies have shown variable degrees of dysbiosis in SSc patients and the effect of probiotics on these patients, providing evidence for the potential link between microbiota and SSc. However, further research is needed to elucidate the key microorganisms and the mechanisms through which they affect the pathoimmunological process of SSc. This review summarizes the current knowledge regarding the association between microbiota and SSc, and discusses the changing perspectives and potential therapy strategies based on the microbiota and its products. (**J Rheum Dis 2019;26:235-247**)

Key Words. Microbiota, Systemic sclerosis, Autoimmune disease, Dysbiosis

INTRODUCTION

The human body harbors 10∼100 trillion microbes, mainly bacteria, that are found in various organs such as the gut, skin, and oral cavity [1]. The gut microbiota, in particular, plays a crucial role in the function and development of the host immune system [2]. Gut microbiota consistently interacts with the mucosal immune system and the immunologic distinction between steady state and disease conditions is established by networks implicating the microbiota and the host [3]. Intestinal epithelial cells produce antimicrobial proteins through activation of signaling pathways involving pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors [4]. PRRs on intestinal epithelial cells and immune cells influence the microbiota composition by promoting different immune responses based on the recognition of conserved molecular motifs present in bacteria, such as microbe-associated molecular patterns expressed by the resident microbiota and pathogen-associated molecular patterns (PAMPs) expressed by invading microorganisms [1,5]. Being part of the innate immune system, dendritic cells (DCs) located at the intestinal mucosal surface can directly collect antigens from normal microbiota and pathogenic bacteria [2], inducing different immune responses depending on the source of the sampled antigens. Activated DCs promote and regulate T cell differentiation into effector helper T (Th) cells, such as Th1, Th2, and Th17 cells, and regulatory T (Treg) cells [2]. Intestinal DCs can also stimulate the secretion of immunoglobulin A (IgA) in B cells to control the growth of pathogenic strains. In turn, IgA can support intestinal homeostasis by inhibiting bacterial adhesion to epithelial

cells and neutralizing the bacteria [3].

The gut microbiota itself can regulate the intestinal immune system. Commensal bacteria can modulate the function of Treg cells that produce anti-inflammatory cytokines such as interleukin (IL)-10 [1]. Polysaccharide A, an immunomodulatory molecule of the commensal *Bacteroides fragilis*, promotes the secretion of IL-10 by Treg cells via binding to TLR2 on the surface of plasmacytoid DCs (pDCs) during intestinal inflammation [6]. Moreover, Clostridia strains isolated from human fecal samples are able to preferentially induce the expansion of Treg cells in the colon [7]. The phenotype of macrophage populations is also shaped by microbiota, as *Clostridium butyricum* stimulates macrophage polarization toward the IL-10-producing type [8]. Bacterial metabolites, such as short-chain fatty acids (SCFAs) produced by colonic microbiota, can regulate immune responses by inducing T cell differentiation, inhibiting the inflammatory response of macrophages, enhancing epithelial barrier function, and inducing the production of anti-inflammatory mediators [2,9]. SCFA generally enters the lamina propria and activates G-protein-coupled receptors-41, -43, or -109A on antigen-presenting cells, triggering differentiation of peripheral immune cells [10]. Oral administration of SCFAs ameliorated the disease symptoms by increasing the number of Treg cells and the production of IL-10 in animal models of colorectal colitis [11] and experimental autoimmune encephalomyelitis [12]. These observations indicate the important role of bacterial metabolites on the modulation of the host immune system in inflammatory and autoimmune diseases.

Numerous studies have focused on the role of the gut microbiota in the pathogenesis of autoimmune diseases [1]. The microbiome might be a major contributory factor to autoimmunity because microbial composition alteration (dysbiosis) can result in the loss of immune tolerance [1]. Increasing studies have revealed alterations in the gut microbiota of patients with intestinal autoimmune diseases such as inflammatory bowel disease (IBD). Crohn's disease (CD) seems to be associated with the outgrowth of Proteobacteria and the reduction of commensal microbiota, especially SCFA-producing bacteria such as *Bacteroides* and Firmicutes [13,14]. Intestinal microbiota can also be associated with the development of non-intestinal autoimmune diseases, including multiple sclerosis (MS), systemic lupus erythematosus (SLE), and psoriasis. Reduction of microbial diversity was observed in non-intestinal autoimmune disease patients

[15-17]. MS patients exhibited a decreased abundance of *Bacteroides* and *Lactobacillus*, and an increased abundance of *Akkermansia*, *Bifidobacterium*, *Blautia*, and *Ruminococcus* [18,19]. A reduced Firmicutes/Bacteroidetes ratio was observed in the intestinal flora of SLE patients [15]. Microbiota isolated from fecal samples of SLE patients promoted T cell activation and Th17 differentiation [20]. Psoriasis is a chronic inflammatory skin disease and is associated to comorbidity with CD [21], supporting an association between intestinal dysbiosis and autoimmune skin disease. Patients with both psoriatic arthritis and skin psoriasis showed decreased bacterial diversity with a reduction in the relative abundance of *Akkermansia*, *Ruminococcus*, and *Pseudobutyrivibrio* [22].

Systemic sclerosis (SSc) is an immune-mediated fibrotic disease that affects the gastrointestinal (GI) tract in more than 90% of SSc patients [23]. This clinical observation led to a connection between gut microbiota composition, immune aberrations, and SSc symptoms. Indeed, previous studies have described alterations in the gut microbiota of SSc patients [24,25]. These provide evidence for the dynamic roles of microbiota in the immune-related pathogenetic events of SSc. A thorough understanding of the immunomodulatory mechanisms of microbiota would be of great interest to allow the development of new therapeutic strategies for SSc. This review summarizes the findings of recent reports on SSc-related microbiota and explores the possible mechanisms underlying the effect of microbiota and microbiota-derived molecules on SSc.

MAIN SUBJECTS

Immunopathological mechanisms and dysbiosis in SSc

1) Vascular injuries and fibroblast activation in SSc

Injury of endothelial cells (ECs) in the microvasculature is the earliest events of SSc. The injured ECs experience pathogenic changes such as EC apoptosis or activation, and endothelial-to-mesenchymal transition (EndoMT). The factors that trigger EC damage include infection, circulating cytotoxic factors, anti-endothelial cell autoantibodies, and ischemia-reperfusion injury [26]. Recent studies characterized the potential inducers of EndoMT in SSc pathogenesis. These studies have shown the induction of EndoMT by transforming growth factor (TGF)-β, angiotensin II, endothelin-1, inflammatory cytokines (IL-1β, tumor necrosis factor [TNF]-α, IL-6, inter-

feron [IFN]-γ, and oncostatin M), TLR4 agonist/endotoxin, and reactive oxygen species [27-34].

Fibrosis is characterized by collagen and extracellular matrix (ECM) deposition in rigid connective tissue as the final result of an immune and vascular signaling cascade. Under normal conditions, fibroblasts are capable of both synthesis and degradation of ECM [26]. However, in fibrotic conditions, collagen-producing activated fibroblasts or myofibroblasts are expanded by the proliferation of resident fibroblasts and trans-differentiation of the epithelium and endothelium [35,36]. TGF-β is a potent inducer of trans-differentiation into activated myofibroblasts and collagen secretion [37]. ILs and chemokines are also known to be positive regulators of fibrosis induction. IL-1β, Th2 cytokines (IL-4 and IL-13), and IL-6 were associated with fibrogenesis in SSc patients and a bleomycin-induced fibrosis animal model [38-41]. Meanwhile, mice lacking T-box transcription factor TBX21 (T-bet) showed exaggerated bleomycin-induced skin fibrosis, suggesting the important role of T-bet and Th1 immune responses in preventing fibrosis [42]. Although the Th1 cytokine IFN-γ negatively regulated fibroblast activation and myofibroblast trans-differentiation, it may also have an indirect pro-fibrotic effect by stimulating TGF-β synthesis in ECs [28].

A recent study on the transcriptional network that induces polarization of fibroblasts identified the transcription factor PU.1 as a fundamental regulator of the profibrotic program [43]. The upregulation of PU.1 induced the transcription of profibrotic genes (*ACTA2* and *COL1A1*) and a phenotypic switch to fibrotic fibroblasts. PU.1 is associated with pro-fibrotic signaling pathways, including canonical TGF-β/SMAD, activator protein 1, and transcriptional enhanced associate domain/HIPPO, and is regulated by post-transcriptional and translational control. As found in human fibroblasts, PU.1 was expressed in fibroblasts of a mouse fibrosis model and fibroblast-specific knockout of *Spi1* (the gene encoding PU.1) ameliorated fibrosis in this model. Thus, targeting transcriptional factors may be a therapeutic strategy for fibrotic diseases including SSc.

2) Modulation of the immune system in SSc

Inflammation and immune activation are highly involved in the fibrogenesis of SSc. Inflammatory cells induce fibrosis through the production of mediators capable of initiating and accelerating inflammation, followed by activation of resident fibroblasts [44]. Some chemokines, such as IL-8, macrophage inflammatory protein-1α, and monocyte chemotactic protein 1, stimulate fibrosis via attraction of immune cells toward inflammatory lesions and overproduction of fibrogenic mediators such as TGF-β and IL-6. Although inflammation may be reduced during the later stages, fibrosis may still be sustained.

TLRs are key molecules of the innate immune system, which is the first line of defense against microbial pathogens [45]. Activation of fibroblast TLRs in SSc might be triggered by endogenous TLR ligands, the so-called damage associated molecular patterns (DAMPs). DAMPs are mainly produced by epithelial cells in response to inflammation, mechanical damage, and autoimmunity [26]. Several TLRs (TLR2, TLR3, TLR4, and TLR9) are overexpressed in the skin of SSc patients [46-48]. TLR4 is expressed in macrophages, fibroblasts, and myofibroblasts, and the activation of TLR4 by lipopolysaccharide (LPS) plays a causal role in liver fibrosis. However, the role of TLR3 in the pathogenesis of SSc is controversial, as this receptor exerts both pro- and anti-fibrotic effects. The TLR3 ligand was shown to stimulate type I IFN response gene signature in fibroblasts, while inhibiting TGF-β-induced fibrotic responses [47].

The involvement of macrophages, notably alternatively activated M2 macrophages, in SSc pathogenesis has been unveiled [49]. Soluble CD163, a putative marker for M2 macrophages, was elevated in the blood of SSc patients [50]. The M2 phenotype dramatically releases proinflammatory and fibrogenic mediators including TGF-β. In addition, activated lung resident macrophages show an increased gene expression related to lipid and cholesterol trafficking in SSc [51]. This suggests a metabolism switch and plasticity of macrophages in the disease process of SSc. The mechanisms leading to aberrations in M2 macrophages remain unclear. One study demonstrated that IL-4 receptor α activation by IL-4 and IL-13 induced M2 macrophage polarization, controlling the production of profibrotic molecules in a wound healing mouse model [52]. Interestingly, wound tissue of germ-free (GF) mice showed a high expression of M2 macrophage-related genes [53]. Moreover, gut dysbiosis induced by antibiotic treatment promoted airway inflammation with an overgrowth of *Candida* species in the gut and induction of M2 macrophages in the lung [54]. This suggests the role of the gut microbiota in the regulation of macrophage phenotype in distant organs, postulating the alleviation of skin fibrosis induced by M2 macrophages and related cytokines via the modulation of the gut microbiome.

pDCs spontaneously secrete IFN-α and chemokine (C-X-C motif) ligand-4 in the fibrotic skin or peripheral blood of SSc patients [55]. pDCs were detected in the fibrotic skin but were reduced in the circulation of SSc patients, probably due to their recruitment into the fibrotic skin. Furthermore, pDC depletion ameliorated bleomy- cin-induced skin fibrosis. Although pDCs are considered critical in skin fibrosis, further investigation is required to elucidate the mechanism underlying the effect of pDCs on SSc onset and progression. In SSc, T cells show skewing toward Th2 or Th17 phenotypes, which creates a fibrotic milieu [56]. B cells in SSc patients were found to

Table 1. The alterations of gastrointestinal microbial composition in SSc patients

Bacterial taxa	Sample	Observation	References
Phylum			
Bacteroidetes	Feces	↓ in SSc (vs. HC)	34
Firmicutes	Feces	↑ in SSc (vs. HC)	34,89
Class			
γ-Proteobacteria	Colonic lavage	↑ in SSc (vs. HC)	65
Family			
Clostridiaceae	Feces	↓ in SSc (vs. HC)	71
U.m. of Lachnospiraceae	Feces	↓ in SSc (vs. HC)	89
Genus			
Actinobacillus	Colonic lavage	↑ in severe (vs. less GI symptoms)	65
Akkermansia	Colonic lavage/Feces	↑ in SSc (vs. HC)	34,65,70
	Feces	↑ in SSc/GI+ (vs. SSc/GI-)	
Bacteroides	Feces	↓ in SSc (vs. HC)	34,70
		↓ in SSc/GI- (vs. HC)	
Bifidobacterium	Colonic lavage/Feces	↑ in SSc (vs. HC)	65,70
Blautia	Feces	↑ in SSc/GI+ (vs. SSc/GI- and HC)	70
Butyricimonas	Feces	↑ in SSc (vs. HC)	89
Clostridium	Colonic lavage/Feces	↓ in SSc (vs. HC)	34,65,70
		↓ in severe (vs. less GI symptoms)	
Coprococcus	Feces	↑ in SSc/GI+ (vs. SSc/GI- and HC)	70
Desulfovibrio	Feces	↑ in SSc (vs. HC)	89
		↑ in SSc/GI+ (vs. HC)	
Erwinia	Colonic lavage/Feces	↑ in SSc (vs. HC)	34,65
Faecalibacterium	Colonic lavage/Feces	↓ in SSc (vs. HC)	34,65,70
	Feces	↓ in SSc/GI+ (vs. SSc/GI- and HC)	
Fusobacterium	Colonic lavage/Feces	↑ in SSc (vs. HC)	34,65
	Colonic lavage	↑ in severe (vs. less GI symptoms)	
Lactobacillus	Colonic lavage/Feces	↑ in SSc (vs. HC)	34,65,70,71
	Feces	↑ in SSc/GI+ (vs. HC)	
		↓ in severe (vs. less GI symptoms)	
Parabacteroids	Feces	↑ in SSc (vs. HC)	34,89
		↑ in severe (vs. less GI symptoms)	
Prevotella	Colonic lavage	↑ in SSc (vs. HC)	34,65,70
	Feces	↓ in SSc (vs. HC)	
		↓ in SSc/GI+ (vs. HC)	
		↑ in severe (vs. less GI symptoms)	
Rikenella	Feces	↓ in SSc (vs. HC)	65
Roseburia	Feces	↓ in SSc (vs. HC)	70
		↓ in SSc/GI+ (vs. HC)	
Ruminococcus	Colonic lavage/Feces	↑ in SSc (vs. HC)	34,65
Streptococcus	Feces	↑ in SSc (vs. HC)	70
		↑ in SSc/GI- (vs. HC)	
Turicibacter	Feces	↓ in SSc (vs. HC)	89

Table 1. Continued

Bacterial taxa	Sample	Observation	References
Species			
Bacteroides fragilis	Colonic lavage	↓ in severe (vs. less GI symptoms)	65
Candidatus arthromitus	Colonic lavage	↓ in severe (vs. less GI symptoms)	
Faeclibacterium prausnitzii	Feces	↓ in SSc (vs. HC)	70,71
		↓ in SSc/GI+ (vs. SSc/GI- and HC)	
Lactobacillus reuteri	Feces	↑ in SSc/GI+ (vs. HC)	70
Lactobacillus salivaris	Feces	↑ in SSc/GI+ (vs. SSC/GI- and HC)	
Roseburia faecis	Feces	↓ in SSc/GI+ (vs. HC)	
Streptococcus salivarius	Feces	↑ in SSc/GI- (vs. HC)	

SSc: systemic sclerosis, HC: healthy control, GI: gastrointestinal, SSc/GI+: SSc patients with gastrointestinal symptoms, SSc/GI-: SSc patients without gastrointestinal symptoms.

generate autoantibodies, secrete IL-6 and TGF-β in peripheral blood, and activate fibroblasts in vitro [57].

Recent studies using RNA sequencing of skin cells from SSc patients classified them into subsets, such as inflammatory, fibroproliferative, and normal-like subsets, based on the gene expression signature [58-61]. Molecular features of SSc patients were associated with immune activation, proliferation, and lipid signaling. The inflammatory subset was characterized by activated chemokine, TLR, T cell, B cell, and TGF-β signaling. The fibroproliferative subset was involved in major fatty acid metabolism with minor immune activation, while fatty acid signaling without immune activation was indicative of a normal-like subset.

Many lines of evidence have shown that diverse immune cells and immune mediators are involved in abnormal vascular changes and induction of a profibrotic environment. It remains to be determined whether these immune aberrations observed in SSc are associated with gut dysbiosis. Understanding the immunopathogenesis of SSc is essential to unravel the microbiome-host interplay and the role of the microbiota in the SSc disease status. Microbial metabolites may be potential therapeutic agents acting in the gut-skin axis as these metabolites can circulate via blood-stream and modulate the immune cell function.

3) Gut dysbiosis in SSc patients

Several studies have identified changes in the gut microbial composition in SSc (Table 1). In the first study that characterized the lower gut microbiota of SSc patients, colonic lavage specimens showed a decrease in commensal bacteria (e.g., *Faecalibacterium*, *Clostridium*, and *Rikenella*)

and an increase in pathobionts (e.g., *Fusobacterium*, *Prevotella*, and γ-Proteobacteria) compared with those from healthy controls [62]. Interestingly, SSc patients had increased *Bifidobacterium* and *Lactobacillus*, which are known to be decreased during the inflammatory condition [62]. As commensal bacteria, *Lactobacillus* species are generally used as probiotics with anti-inflammatory properties [63]. The relationship between the abundance of *Lactobacillus* and SSc remains to be examined. *Fusobacterium* species are considered pathobionts capable of causing systemic disease by entering the blood [64]. Similar to *Fusobacterium*, the *Prevotella* genus is also deemed a pathobiont. *Prevotella copri* was increased in patients with early rheumatoid arthritis (RA); administration of fecal samples from these patients induced severe arthritis with increased Th 17 cells in GF mice [65].

Another observational study using stool specimens reported similar results [25]. In this study, SSc patients exhibited lower abundance of commensal genera (*Bacteroides*, *Faecalibacterium*, and *Clostridium*), and higher abundance of pathobiont genera (*Fusobacterium*, *Ruminococcus*, and *Erwinia*) and commensal genus *Lactobacillus* than healthy controls [25]. In both studies, *Ruminococcus* and *Akkermansia* were enriched in SSc patients relative to healthy controls. These two genera are associated with a fibrotic phenotype of CD [66] and may also be involved in SSc fibrosis. In conclusion, gut dysbiosis in SSc is characterized by low abundance of *Faecalibacterium* and *Clostridium*, and high abundance of *Fusobacterium*, suggesting a potential pathogenic role of microbiota in SSc.

4) Relationship between SSc symptoms and gut dysbiosis

The variations in the microbial composition may be due to differences in factors such as genetics, age, gender, disease duration, and dysfunction of internal organs among cohorts [4]. Some studies have elucidated the correlation between GI symptoms and abundance of specific bacteria in SSc (Table 1). The fecal bacterial composition in SSc patients with GI symptoms was higher with respect to the levels of *Lactobacillus, Blautia,* and *Coprococcus,* and lower with respect to levels of *Roseburia* and *Faecalibacterium* in comparison to the fecal bacterial composition in healthy controls [24].

Moreover, specific genera were linked to the GI symptom severity in SSc [4,25]. SSc patients with moderate/severe GI symptoms had increased abundance of *Fusobacterium* and decreased abundance of *B. fragilis* in colonic lavage specimens compared with SSc patients with none/mild GI symptoms [62]. In another study, *Clostridium* and *Blautia* were more abundant in patients with less se-

vere GI symptoms than in those with more severe symptoms, while *Fusobacterium* and *Parabacteroides* were positively associated with GI symptom severity [25,62,67]. Regarding the categories of GI symptoms, *Prevotella* increased in SSc patients with moderate/severe diarrhea relative to SSc patients with less severe diarrhea. *Lactobacillus* decreased in SSc patients with moderate/severe constipation relative to SSc patients with less severe constipation [25]. At the species level, *Roseburia faecis* and *Faecalibacterium prausnitzii* were decreased in SSc patients with GI symptoms compared with healthy controls. The two genera are major producers of butyrate, which is predominantly detected in the healthy human gut [24]. Besides, dysbiosis (low abundance of *F. prausnitzii* and *Clostridiaceae* and high abundance of *Lactobacillus*) was associated with pulmonary fibrosis, malnutrition, and esophageal dysfunction [68]. These findings suggest that fecal bacterial communities can show a high degree of individual variability depending on the SSc symptoms and
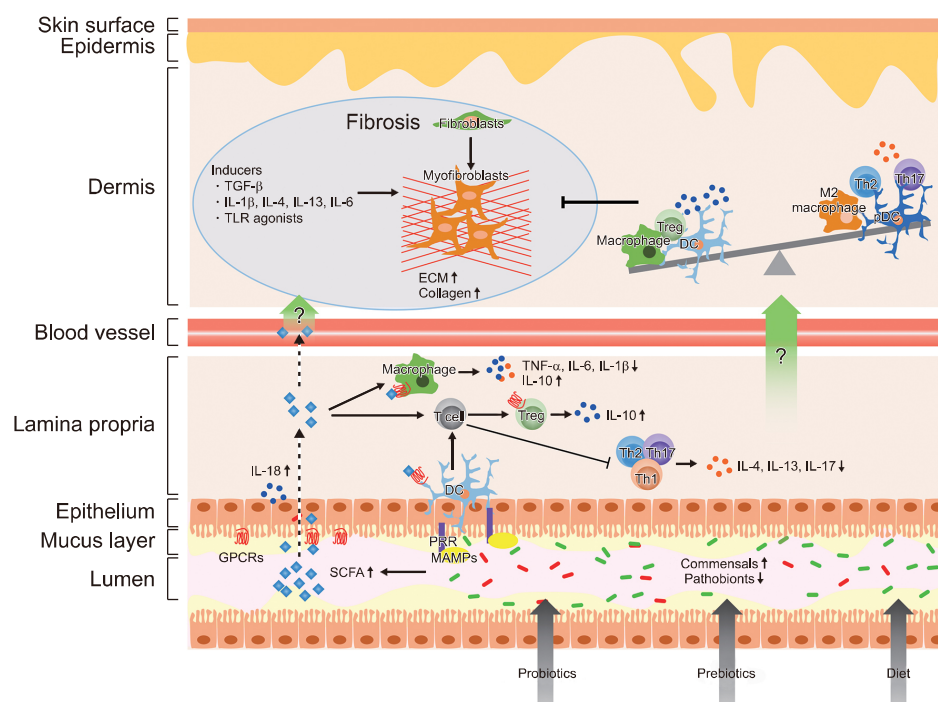

Figure 1. Potential effects of microbiota-targeted challenges in systemic sclerosis (SSc). Probiotics, prebiotics, and dietary fibers can help produce microbial metabolites, such as short-chain fatty acids (SCFAs), via the recovery of commensal bacteria. SCFAs bind to G protein-coupled receptors (GPCRs) on intestinal epithelial cells and immune cells. In turn, SCFAs regulate intestinal barrier integrity by inducing secretion of interleukin (IL)-18 by epithelial cells, differentiation of T cells into regulatory T (Treg) cells, and proinflammatory cytokine production by macrophages. Dendritic cells (DCs) also regulate T cell differentiation by both SCFAs and microbe-associated molecular patterns (MAMPs) sensing through pattern-recognition receptors (PRRs). Presumably, the circulation of SCFAs and anti-inflammatory cytokines might prevent fibrosis by modulating the dysregulated immune system in SSc. TGF-β: transforming growth factor-β, TLR: toll-like receptor, ECM: extracellular matrix, pDC: plasmacytoid DC, TNF-α: tumor necrosis factor-α.

indicate the importance of cohort selection based on clinical phenotypes in SSc-microbiota studies. Overall, *Blautia* and *Coprococcus* were closely related to the development of GI symptoms and *Fusobacterium* and *Parabacteroides* were positively correlated with the severity of GI symptoms in SSc.

Taken together, even though the precise molecular mechanism is yet undetermined, the potential causality and related microbiota-mediated disease mechanisms in SSc are illustrated in Figure 1.

Therapeutic intervention through microbial manipulation in SSc

1) Probiotics

Probiotics are viable and non-pathogenic microorganisms that can improve disease symptoms by modulating the microbiome and the immune response [69]. The administration of the probiotic *Lactobacillus acidophilus* can correct Treg/Th17 cell imbalance and reduce colonic collagen deposition in an acute colitis mouse model [70]. One small study evaluated the effects of the probiotics Align (*Bifidobacterium infantis*) and Culturelle (*Lactobacillus GG*) in SSc patients with GI symptoms [71]. The probiotics treatment of SSc patients improved the total GI scores, reflux, and distention/bloating, suggesting that probiotics may be able to treat GI symptoms in SSc. In another study, *Lactobacillus casei* improved the reflux and abdominal distension but not the total GI score in SSc patients [72].

In a recent study, probiotics (*Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *L. acidophilus*, and *Bifidobacterium lactis*) decreased the Th17 cell population, with no difference in Th1, Th2, and Treg cells, in SSc patients compared to the placebo group [69]. Although these probiotics had an immunomodulatory effect, they did not improve GI symptoms in SSc patients. Lactobacilli-containing probiotics have improved the symptoms of MS, allergic rhinitis, and IBD [73,74]. However, previous studies described an increased abundance of *Bifidobacterium* and *Lactobacillus* in SSc patients relative to healthy controls [62], which renders the efficacy of probiotics, such as *Bifidobacterium* and *Lactobacillus*, in SSc uncertain. Thus, further selection and evaluation of probiotics should consider the replenishment of genera that are depleted in SSc or genera associated with improved GI symptoms in SSc.

2) Prebiotics and diet

Principal prebiotics include inulin, galacto-oligosaccharides, fructo-oligosaccharides, lactulose, mannan-oligosaccharides, and xylo-oligosaccharides [75]. These prebiotics can promote colonization of SCFA-producing bacteria and increase beneficial microbiota [76]. However, the effects of prebiotics have not been studied in SSc.

Changing the diet can dramatically alter the GI microbial composition and its metabolites [77]. Low-fat, high-fiber diet is associated with a more diverse microbiota [78], whereas high-fat diet can lead to microbial changes, followed by an increase of IFN-γ and TNF-α cytokines in C57BL/6 mice [79]. Furthermore, a low-carbohydrate diet results in low production of SCFAs. Alteration of SCFA levels is common in type 2 diabetes, obesity, and celiac disease [80]. In addition, a low-fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) diet has been reported to be beneficial for irritable bowel syndrome patients with symptomatic fructose intolerance [81]. The improvement of symptoms by low-FODMAP diet may be due to decreased microbial fermentation, resulting in lower gas production and, consequently, decreased luminal distension [82]. The low-FODMAP diet was also effective in improving clinical manifestations in SSc patients with symptomatic fructose malabsorption [83]. However, placebo effect might have been observed in this study, as it was a non-randomized controlled study. Despite the beneficial effect of dietary modifications, diet might also potentiate unhealthy microbial and metabolic changes. Future research on dietary intervention should focus on long-term and randomized studies that include the evaluation of disease symptoms.

Future considerations in SSc-related microbiota research

1) Limitations in characterizing precise microbiota features in SSc patients

Dysbiosis might be a secondary event to pharmacological therapies or SSc disease [84]. Antibiotics, including the immunosuppressive ones, can be used to prevent the progress of autoimmune diseases due to their anti-inflammatory and immunomodulatory properties [85]. For instance, the oral administration of antibiotics alleviated the disease symptoms by increasing IL-10 and decreasing IL-17 in a lupus-prone mouse model [86]. However, despite the positive effects of antibiotics under specific circumstances, the use of antibiotics greatly impacts the gut microbiota. Antibiotics can non-selectively eliminate both beneficial commensal and pathogenic bacteria, thereby allowing the growth of other pathobionts and causing an imbalance in the microbiota composition [2].

Subsequently, antibiotic overuse may cause disruptions of the microbiota-host interactions and modulation of immune responses [87]. The early-life administration of streptomycin could exacerbate skin and lung fibrosis in combination with microbial dysbiosis (increased Bacteroidetes/Firmicutes ratio) in SSc-like mice using topoisomerase I peptide-loaded DC immunization [88]. Therefore, the identification of gut microbiota related to SSc needs to be investigated in an early-stage disease patients, especially prior to the administration of medication.

Moreover, the structure of various organs, including skin and gut, is deeply altered in SSc [84]. This indicates that perhaps the organs are susceptible to the colonization of pathogenic bacteria during SSc development, merely reflecting a phenomenon that result from alterations in gut motility and fermentation rather than the role of microbiota in SSc pathogenesis [4]. Longitudinal microbial studies, particularly in early-stage SSc patients, may help to find answers to these questions.

Recent work demonstrated that different species and even different strains within the same species could have a different effect on the host. Current data using 16S rRNA sequencing provide information at the genus level, which may not be enough to find candidates for microbial-based therapies against SSc. Microarray for bacterial antibody detection enables the identification of bacteria at the species level. Some research is underway to discover disease-specific bacteria at the species level by identifying and constructing the libraries of 521 microorganisms constituting the human intestinal microbiota (Figure 2, unpublished data). These attempts might succeed in advancing microbiome research by accurately sorting out the microbial species specifically related with SSc and other diseases.
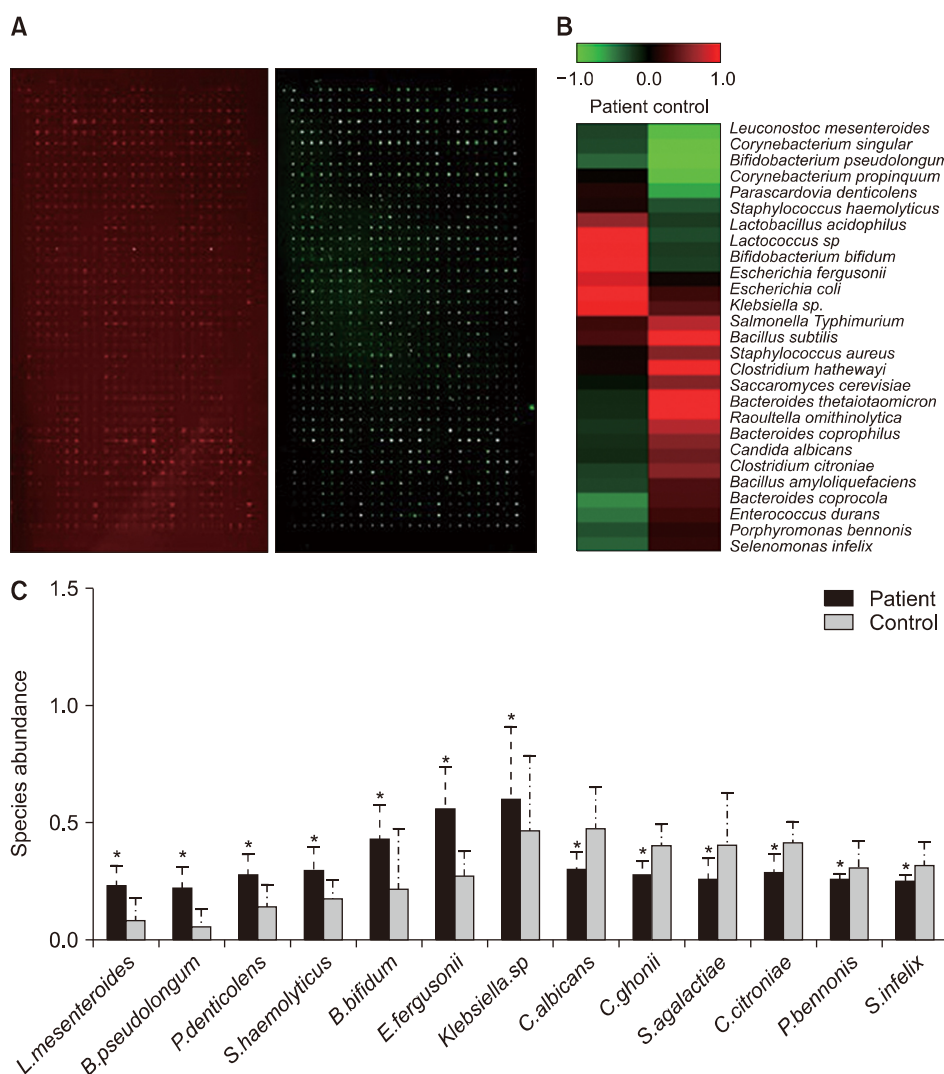

Figure 2. Detection of disease-specific bacterial species using bacterial antibody microarray. (A) Representative images of gut bacterial antibody microarray in serum. (B) Differential expression at the bacterial species level between healthy controls and patients. (C) Species abundance analysis between healthy controls (n = 11) and patients (n = 8). Green and red colors indicate high and low expression of bacterial antibodies, respectively. *p < 0.01.

2) Predicting roles of skin microbiota in SSc

The commensal microbiota colonizing the skin surface is essential to skin physiology and immunity [89]. Disruptions in skin microbial communities may contribute to triggering skin autoimmune diseases [90]. Skin resident microbes play an important role in skin health through interactions with other microbes, host epithelium, and the immune system. Commensal microbes produce bioactive metabolites that kill pathogens and stimulate keratinocytes and immune cells to modulate innate and adaptive immune responses, protecting our body against toxic and foreign substances. Keratinocytes sample the microbiota through PRRs that recognize PAMPs such as flagellin and LPS from gram-negative bacteria, and peptidoglycan and lipoteichoic acid (LTA) from gram-positive bacteria [91]. In turn, this process induces the secretion of antimicrobial peptides (AMPs) and immune mediators by keratinocytes, and the activation of epidermal and dermal immune cells. Indeed, *Staphylococcus epidermidis*, a skin commensal, triggers AMP expression by keratinocytes, and LTA produced by *S. epidermidis* mitigates the cutaneous inflammation through TLR2 signaling [92]. These findings show that the skin microflora can modulate cutaneous immune responses.

Thus, skin specimens can provide direct information about the microbial community and the immune system in SSc. In studies using skin samples from SSc patients, no significant differences in the bacterial microbiome were found, except for the increased expression of *Rhodotorula glutinis* [93]. Moreover, Johnson et al. [59] discovered substantial changes in the skin microbial composition, with decreased lipophilic taxa (*Propionibacterium*) and increased gram-negative taxa (*Burkholderia*, *Citrobacter*, and *Vibrio*). Skin microbial dysbiosis is associated with an increased inflammatory gene signature, which might help to explore components of the immune system that are relevant to SSc pathogenesis [84]. Nevertheless, there are no data on immune-skin microbe interactions and its mechanisms in SSc. Further skin microbial studies are required to elucidate the molecular mechanisms and potential therapeutic strategies for SSc.

Furthermore, some limitations exist in current skin microbial research. Firstly, it may not be clinically feasible because the collection of skin specimens is invasive. Secondly, the composition of skin microbiota shows considerable variation depending on the body site. Lastly, it is difficult to distinguish between transient and resident bacteria from results obtained using 16S rRNA sequenc-

ing [91]. Furthermore, although the 16S rRNA sequencing is a robust method to identify bacterial communities, it provides limited information about bacterial composition and microbe-host interactions. Although whole-genome shotgun (WGS) sequencing can solve the limitations of 16S rRNA sequencing, WGS metagenomic sequencing of skin microbiota also faces some obstacles due to the lack of reference genome sequences for skin bacteria and the high amount of skin DNA. Therefore, skin microbiome research needs caution and further verification from sampling to data interpretation.

Challenges in attempts to correct gut dysbiosis using microbes have been reported in numerous studies, whereas microbial therapy for the skin is still absent. Interestingly, subcutaneous and epicutaneous administration of butyrate reduced contact hypersensitivity reaction, with an increase of forkhead box P3 (Foxp3)-positive cells and upregulation of IL-10 in the skin of mice. This suggests the therapeutic role of SCFA on inflammatory skin reactions through Foxp3-expressing Treg cells in the skin [94]. Microbiota-derived products, such as SCFA, might have potential as therapeutic agents for SSc treatment due to their immunomodulatory function in both skin and gut.

3) Adverse effects of probiotics

Probiotics are generally regarded as safe, with reduced infection-related complications in several diseases. However, probiotic prophylaxis with the combination of probiotic strains was associated with an increased risk of mortality in patients with severe acute pancreatitis [95]. Indeed, the toxicity of probiotics has been described in many cases and include excessive immune stimulation, systemic infection, and deleterious metabolic activity [96]. SSc patients in an immunocompromised state and generally taking prescribed immunosuppressive drugs could be a potential risk group for probiotics administration. Therefore, the safety of probiotics should be thoroughly evaluated, taking in consideration the disease status, before clinical application.

CONCLUSION

Gut dysbiosis, which is characterized by low abundance of *Faecalibacterium* and *Clostridium*, and high abundance of *Fusobacterium* and *Lactobacillus*, is observed in SSc patients. Several microbes, such as *Blautia*, *Coprococcus*, *Fusobacterium*, and *Parabacteroides*, are also related to GI symptoms in

SSc. Furthermore, probiotics, such as *B. infantis*, *L. GG*, and *L. casei*, improved various GI symptoms in SSc patients. Thus, a certain modification of the human microbiota can deteriorate or improve SSc by modulating innate and adaptive immune cells or directly affecting ECs and skin fibroblasts. Well-defined human studies using metagenomic or 16S rRNA sequencing methods, coupled with multi-omics analyses or microbiota antibody array, are needed to identify how and when the microbiota composition in SSc patients differs from healthy controls. More experimental studies are also necessary to establish the potential causation and to elucidate the intricate microbiota-host relationship in SSc. The advances in the rapidly developing field of microbiota research will enable researchers to further characterize the mechanisms of modulation of disease by microbiota and its potential use in diagnostics, therapeutics, and precision medicine.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

S.I.L. designed the study. S.I.L. and S.K. drafted the manuscript. S.H.C. and H.J.P. contributed to the acquisition and analysis of data. All authors approved the final manuscript.

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