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ORIGINAL ARTICLE

The effects of Lactobacillus delbrueckii and Lactobacillus rhamnosus on cytokines and their related molecules: An *ex vivo* study on patients with systemic lupus erythematosus

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ABSTRACT

Objectives: This study aimed to assess the *ex vivo* impact of *Lactobacillus delbrueckii (L. delbrueckii)* and *Lactobacillus rhamnosus (L. rhamnosus)* on inflammatory and anti-inflammatory cytokines as well as their related molecules on the peripheral blood mononuclear cells (PBMCs) of systemic lupus erythematosus (SLE) patients.

Patients and methods: This study was conducted with 20 newly diagnosed SLE patients (18 females, 2 males; mean age: 33.3±12.4 years; range, 18 to 68 years) between September 2017 and September 2018. Extracted PBMCs from each patient were divided into 4 cell groups in our study. Three cell groups act as treatment groups receiving *L. rhamnosus* (10^7 CFU/mL), L. delbrueckii (10^5 CFU/mL) or a mixture of both, and one group act as our untreated control group in the absence of any probiotic agents. All cell groups were cultured in RPMI 1460 medium for 48 h. Then, total RNA was extracted, and cDNA was synthesized.

Results: The gene expression levels of forkhead box P3 (FOXP3), transforming growth factor beta (TGF-β), interleukin (IL)-6, IL-10, and IL-2 were evaluated by a quantitative real-time polymerase chain reaction. The results revealed that expression levels of FOXP3, TGF-β, IL-10, and IL-2 increased and the level of IL-6 decreased in probiotics-receiving groups compared to the control group. *Lactobacillus delbrueckii* and *L. rhamnosus* enhanced the expression of regulatory T cell-related molecules such as FOXP3 and IL-2 and also increased the expression of IL-10. These probiotics also reduced the expression of IL-6 as proinflammatory cytokines in the PBMCs of SLE patients.

Conclusion: The results of the present study show that these probiotics could be effective in regulating the balance of cytokine gene expression *ex vivo* , and due to their beneficial effects, they can be an intriguing option in the production of new complement drugs for SLE

Keywords: Cytokines, immunomodulatory, *Lactobacillus delbrueckii, Lactobacillus rhamnosus*, systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) is a heterogenic, prototypic, and chronic autoimmune disease characterized by breakdowns of immune tolerance and production of autoantibodies against numerous self-antigens.[1] Production of autoantibodies leads to the generation of inflammatory mediators, immune complexes, autoreactive T cells, and vast organ damage.[2] Due to the heterogeneous nature of the disease, there is a broad spectrum of clinical manifestations.[3] The etiology of SLE has not been clarified, but many factors, including genetics, hormones, infections, and environmental factors, play an important role in SLE pathogenesis.[4,5]

Regulatory T cells (Tregs) have a critical role in the maintenance of tolerance and hemostasis in the immune system; thus, reduction and dysfunction of Tregs are associated with the induction and development of autoimmune diseases.[6,7]

Forkhead box P3 (FOXP3) is a transcription factor that is expressed by Tregs and is essential for regulatory functions. Inhibitory cytokines, including transforming growth factor (TGF)-β and interleukin (IL)-10, play critical roles in the maintenance of immune hemostasis. TGF-β cytokine is involved in controlling proliferation and apoptosis.[8,9] Serum levels of TGF-β are reduced in SLE.[10]

Many studies have shown that the levels of IL-10 increased in the plasma of SLE patients. IL-10 is implicated in the apoptosis of cluster of differentiation (CD)4+ and CD8+ T cells. However, IL-10 cytokines contribute to the differentiation and stimulation of B cells. On the other hand, other studies have shown IL-10 is effective in the treatment of SLE disease.[11,12] All these suggest that the function of these cytokines is complicated.[13]

Interleukin-6 is a pleiotropic cytokine in the regulation of the immune system. IL-6 and TGF-β contribute to the differentiation of naïve CD4+ T cells into T helper (Th)17 cells. Thus, IL-6 is correlated with disease activity.[14] Furthermore, studies have indicated that TGF-β and IL-2 are involved in the differentiation of naïve CD4+ T cells into Tregs,[15] and they are also necessary for function, survival, and development of Tregs.[16] Recent studies have reported that in SLE patients, the level of IL-2 is reduced, which could affect FOXP3 expression and cause dysfunction of Tregs.[9,10,17]

Additionally, the high rate of mortality and morbidity of SLE patients is not only due to the pathogenesis of the disease but also the toxic effects of the drugs used. Probiotics are living microorganisms known as commensal microflora. Two studies have reported the beneficial effects of the probiotics on the host health and regulation of immune responses.[18,19] Today, Lactobacillus is known as the most important species of probiotics that can modulate the immune response.[20] Tolerogenic probiotics, through the regulation of responses of inflammatory cells or promotion of regulatory cell responses, act as a modulator of immune system responses to maintain hemostasis. Some tolerogenic probiotics have a positive effect on increasing the number of Tregs, while negatively reducing the proinflammatory and inflammatory cytokines. The use of Lactobacillus has been effective in the treatment and prevention of rheumatoid arthritis, atopic dermatitis, asthma, allergies, rhinitis, and infectious diseases.[17]

Studies have suggested that *Lactobacillus rhamnosus* has been effective in the treatment and prevention of autoimmune diseases. This probiotic could regulate the balance between Th1/17 cells and Tregs. It also increased IL-10 by increasing CD4+ T cells. The elevated level of IL-10 inhibits excessive inflammatory responses. *Lactobacillus delbrueckii* also showed a tolerogenic function and played a role in increasing tolerogenic immune responses.[20,21] This probiotic increased antimicrobial peptides and decreased proinflammatory cytokines.[22] The regulatory functions of these probiotics were seen in the production of IL-6, IL-12, and TGF-β cytokines in the colon and IL-6 and TGF-β in the spleen. Those effects also enhanced Tregs in the lymph nodes of the colitis mice model.[23,24]

In this study, we evaluated the modulatory effects of *L. rhamnosus* and *L. delbrueckii* on inflammatory and anti-inflammatory cytokines as well as their mediators in the peripheral blood mononuclear cells (PBMCs) of SLE patients *ex vivo*.

PATIENTS AND METHODS

The study was conducted at the Mashhad University of Medical Sciences, Department of Immunology between September 2017 and September 2018. Twenty newly diagnosed SLE patients (18 females, 2 males; mean age: 33.3±12.4 years; range, 18 to 68 years) according to the American College of Rheumatology classification criteria (1997 revised criteria) were enrolled in this study. None of the patients had yet received any treatment or medications. SLE patients were sequentially enrolled in the study from the rheumatology center. The following laboratory data were evaluated: C3, C4, C-reactive protein, erythrocyte sedimentation rate, anti-dsDNA (double-strand DNA) titers, ANA (anti-nuclear antibody), hemoglobin, white blood cell count, red blood cell count, and platelet count. To evaluate the effects of two probiotics on cytokine profile in SLE patients, PBMCs were isolated and cultured in the presence or absence of probiotics. Extracted PBMCs from each patient were divided

into 4 cell groups in our study; Three cell groups act as treatment groups receiving *L. rhamnosus*, *L. delbrueckii* or a mixture of both, and one group act as our untreated control group in the absence of any probiotic agents.

PBMCs Isolation

Peripheral blood samples (10 mL) of SLE patients were collected before any treatment. These samples were collected into EDTA tubes and diluted with phosphate-buffered saline (PBS; pH 7.4) in a 1;1 ratio. PBMCs were separated using Ficoll-Paque (Cedarlane, Toronto, Canada) density gradient centrifugation. After washing twice with PBS, the cells were resuspended at a concentration of 4×10^6 cells/mL in RPMI 1640 medium (BioSera, London, UK), where RPMI contained 10% heat-inactivated fetal bovine serum (Gibco Inc., Billings, USA).

Preparing probiotics

Lyophilized *L. delbrueckii* subsp. *lactis* (PTCC: 1743 [DSM20072]) was prepared by the Iranian Research Organization for Science and Technology and *L. rhamnosus* GG (ATCC: 9595) was purchased from the Pasteur institute.

Lyophilized *L. delbrueckii* subsp. lactis and *L. rhamnosus* were cultured in De Man, Rogosa, and Sharpe (MRS) broth (Biolife, Milano, Italy) under microaerobic conditions for 1 h at 37°C. Next, the probiotics were centrifuged for 2 min at 2,500 rpm. Afterward, the pellet was cultured on MRS agar (Biolife Italiana, Milan, Italy) under microaerobic conditions for 24 h at 37°C.

Thereafter, a specified colony was transferred to the MRS broth medium and placed into the incubator for 2 h at 37°C under an anaerobic condition until reaching the logarithm phase (the log phase time was characterized following previous research in our laboratory). Colony-forming units (CFU/mL) of probiotic-receiving groups were determined. Then, 10^8 CFU/mL of probiotics were washed twice with PBS solution and suspended in complete RPMI medium, before being added to the cell cultures.

The optimization of probiotic concentration for PBMCs treatment

To find the optimized probiotic concentration, 10^6 PBMCs of three SLE patients were cultured in the presence of 10^3, 10^5, 10^7, and 10^9 CFU/mL of probiotics. The cytotoxicity effects of probiotic doses were determined by MTT assay, and the apoptotic effects of various doses of probiotics on the PBMCs were also obtained using Annexin-V/PI (Abcam, Cambridge, MA, USA) via flowcytometry. Based on the results, we chose the concentration of 10^7 CFU/mL for *L. rhamnosus* and 10^5 CFU/mL for *L. delbrueckii* in this *ex vivo* study.

Culture of PBMCs in the presence of probiotics

Patient-isolated PBMCs were cultured (1×10^6 cells/well) in the presence of *L. rhamnosus* (10^7 CFU/mL), *L. delbrueckii* (10^5 CFU/mL), a mixture of both probiotics (*L. rhamnosus* 10^7 CFU/mL and *L. delbrueckii* 10^5 CFU/mL), and in the absence of probiotics, in a total volume of 1 mL RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (BioSera, London, UK), 100 IU/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine. The cells were incubated for 48 h at 37°C in a 5% carbon dioxide humidified incubator.

RNA extraction and cDNA synthesis

Total RNA was extracted from PBMCs (1×10^6) of patients using Tripure reagent (Roche, Mannheim, Germany) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using 9 µg of total RNA, random hexamer primer (100 µm), and reverse transcriptase enzyme (10 µm; Fermentas, Vilnius, Lithuania) according to the manufacturer's protocol. Next, synthesized cDNAs were diluted at a 1:10 ratio for use in real-time polymerase chain reaction (PCR).

Gene expression determined by quantitative real-time PCR

Real-time PCR was performed using the SYBR Green PCR Master Mix (Takara, Shiga, Japan) using a Rotor-Gene 6000 thermal cycler (QIAGEN, Hilden, Germany) with forward and reverse specific primers according to the manufacturer's instructions (Table 1). In a clean 9 µL tube of SYBR Green Master mix, 1 µL diluted cDNA and 3 µL of RNase-free water mix were added and underwent cycling conditions; 95°C for 15 min, followed by 40 cycles of 15, 30, and 30 sec at 94°C, 55 °C, and

70°C, respectively. Thereafter, 0.5 mM of each specific primer was used for FOXP3, TGF-β, IL-6, IL-10, IL-2, and glyceraldehyde-3-phosphate dehydrogenase as an endogenous housekeeping internal control. Quantification of the target gene in the probiotics treated sample was expressed as a fold change compared to the untreated sample.

$\Delta CT_T = CT_{Target} - CT_{HKG}$

$\Delta CT_C = CT_{Control} - CT_{HKG}$

$\Delta\Delta CT = \Delta Ct_T - \Delta Ct_C$

Fold change= $2^{-\Delta\Delta Ct}$

Statistical analysis

All results were presented as mean ± SEM (standard error of the mean) and were analyzed with Graph Pad Prism 6 software (Graph Pad Software Inc., San Diego, CA, USA). Statistical analyses for significant differences were performed according to parametric and nonparametric tests. A p-value <0.05 was considered statistically significant.

RESULTS

The results showed that the Systemic Lupus Erythematosus Disease Activity Index score was 21.3±11.7, which indicated a high level of disease activity in the study population. Skin rashes were the most common clinical disorders (75%), followed by joint involvement (65%). ANA was present in 70% of the patients, and 90% of the patients were positive for anti-dsDNA. A low level of C4 (<15 mg/dL) and C3 (<85 mg/dL) was detected in 55% (11/20) of patients. In addition, fever, headache, oral wound, renal diseases, and hematological disorders were reported to a lower extent in patients (Table 2).

The expression level of FOXP3 in all probiotic-receiving groups increased compared to the untreated group, and this increase was significant in the treatment group of *L. delbrueckii*. The expression amount of TGF-β in *L. rhamnosus* and the mixture of probiotics-receiving groups increased

Table 1. Sequence of primers used in this study

Genes	Sequences	Base pair
FOXP3		
Forward primer	5′-ACATGGACTACTTCAAGTTCC-3′	21
Reverse primer	5′-AACCAGTGGTAGATCTCATTG-3′	21
TGF-β		
Forward primer	5′-GCAACAATTCCTGGCGATACC-3′	21
Reverse primer	5′-GCCCTCAATTTCCCCTCCAC-3′	20
IL-6		
Forward primer	5′-CTTCGGTCCAGTTGCCTTCTC-3′	21
Reverse primer	5′-ATTCGTTCTGAAGAGGTGAGTGG-3′	23
IL-10		
Forward primer	5′-GGACTTTAAGGGTTACCTGG-3′	20
Reverse primer	5′-GTCTGGGTCTTGGTTCTC-3′	18
IL-2		
Forward primer	5′-AGGGATCTGAAACAACATTCA-3′	21
Reverse primer	5′-GATGCTTTGACAAAAGGT-3′	18
GAPDH		
Forward primer	5′-AGCCGGGCATGTTCTTCAAC-3′	20
Reverse primer	5′-AGGGAGCTTCACGTTCTTGTAT-3′	21

FOXP3: Forkhead box P3; TGF-β: Transforming growth factor beta; IL: Interleukin; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

in the untreated group. Furthermore, the level of TGF-β in the group treated with *L. rhamnosus* and mixed probiotics (*L. rhamnosus* and *L. delbrueckii*) was higher than the *L. delbrueckii*-receiving group, but these increases were not significant. In the PBMCs groups receiving probiotics, we did not observe any significant decrease in the level of IL-6 compared to the untreated group. The expression level of IL-2 in all probiotic-receiving groups increased in comparison to the untreated group, and this rise was significant in the *L. delbrueckii*-treated group.

Expression of FOXP3 in the presence of probiotics

Peripheral blood mononuclear cells treatment with *L. delbrueckii* significantly raised the expression levels of FOXP3 mRNA in PBMCs of the treatment group compared to the untreated group of SLE patients (p=0.02, Figure 1). Additionally, treatment with *L. rhamnosus* and a mixture of probiotics increased the amount expression of FOXP3 mRNA in PBMCs of the treatment group compared to the untreated group of SLE patients, but it was not significant (p=0.5 and p=0.4, respectively; Figure 1).

Expression of TGF-β in the presence of probiotics

Treatment with *L. rhamnosus* and a mix of both probiotics increased the expression levels of TGF-β mRNA in PBMCs of the treatment group compared to the untreated group of SLE patients (not significant; p=0.3 and p=0.5, respectively; Figure 2).

Table 2. Patient data and disease characteristics of the study population

	n	%	Mean±SD
Age (year)			33.3±12.4
Sex			
Female	18	90	
White blood cell			5.0±2.1
Red blood cell			4.2±0.6
Platelets			232.6±82.7
Erythrocyte sedimentation rate			40.0±31.5
SLEDAI			21.3±11.7
ANA positive (>1 U)	14	70	
Anti dsDNA positive (>10 IU/mL)	16	80	
Low level C4 (<15 mg/dL)	11	55	
Low level C3 (<85 mg/dL)	11	55	
C-reactive protein positive	9	45	
Leukopenia (<3.5×10^9/L)	6	30	
Lymphopenia (<1.0×10^9/L)	3	15	
Thrombocytopenia (<150×10^9/L)	2	10	
Renal disease	7	35	
Skin rash	15	75	
Joint involvement	13	65	
Fever	6	30	
Headache	10	50	
Oral wound	5	25	
Heart involvement	1	5	

SD: Standard deviation; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; ANA: Anti-nuclear antibody.

Expression of IL-6 in the presence of probiotics

Treatment with *L. delbrueckii*, *L. rhamnosus*, and a mixture of both of them reduced the expression levels of IL-6 mRNA in PBMCs of the treatment group compared to the untreated group of SLE patients, but these reductions were not statistically significant (p=0.4, p=0.2, and p=0.3, respectively; Figure 3).

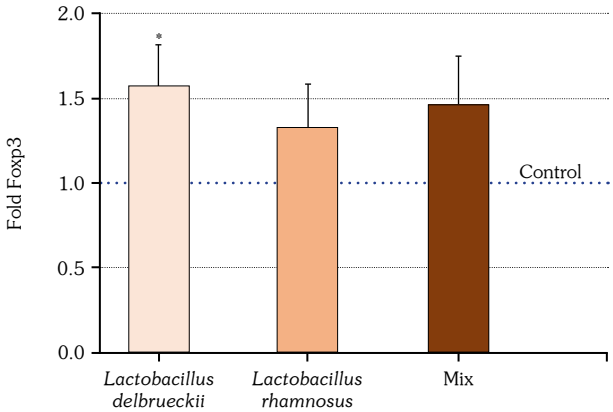

Figure 1. Relative mRNA level of FOXP3 in the PBMCs of SLE patients compared to the (untreated) control group. The gene expression was studied by real-time PCR, and the results are expressed as fold change.

* Significant difference compared to the control group; mRNA: Messenger ribonucleic acid; FOXP3: Forkhead box P3; PBMCs: Blood mononuclear cells; SLE: Systemic lupus erythematosus; PCR: Polymerase chain reaction.

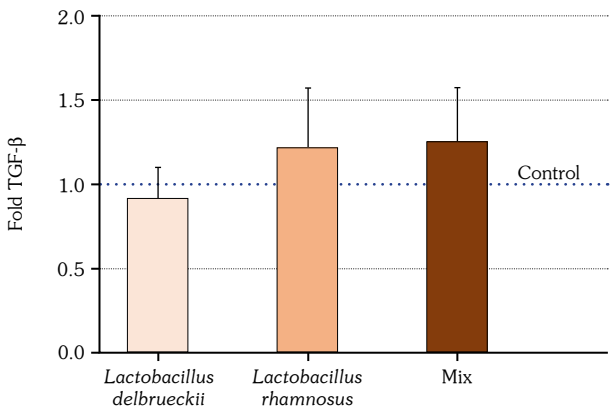

Figure 2. Relative mRNA level of TGF-β in the PBMCs of SLE patients compared to the (untreated) control group. The gene expression was studied by real-time PCR, and the results are expressed as fold change.

mRNA: Messenger ribonucleic acid; TGF-β: Transforming growth factor beta; PBMCs: Blood mononuclear cells; SLE: Systemic lupus erythematosus; PCR: Polymerase chain reaction.

Expression of IL-10 in the presence of probiotics

Treatment with *L. delbrueckii* significantly enhanced the expression levels of IL-10 mRNA in PBMCs of the treated group compared to the untreated group of SLE patients (p=0.03, Figure 4). Moreover, treatment with *L. rhamnosus* and a mixture of both probiotics augmented the expression levels of IL-10 mRNA in the treatment group compared to the untreated group of SLE patients (not significant; p=0.8 and p=0.9, respectively; Figure 4).

Expression of IL-2 in the presence of probiotics

The treatment with *L. delbrueckii* significantly enhanced the expression levels of IL-2 mRNA in PBMCs of the treatment group compared to the untreated group of SLE patients (p=0.05, Figure 5). Additionally, treatment with *L. rhamnosus* and a mixture of both probiotics elevated the expression level of IL-2 mRNA in PBMCs of treated groups compared to untreated groups of SLE patients (not significant; p=0.8 and p=0.7, respectively; Figure 5).

DISCUSSION

Systemic lupus erythematosus is a chronic inflammatory autoimmune disease where the

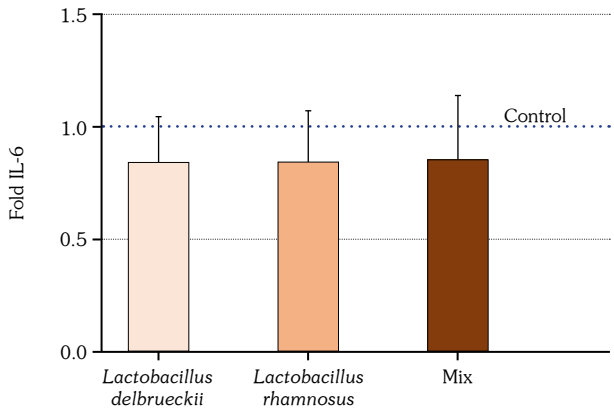

Figure 3. Relative mRNA level of IL-6 in the PBMCs of SLE patients compared to the (untreated) control group. The gene expression was studied by real-time PCR, and the results are expressed as fold change.

mRNA: Messenger ribonucleic acid; IL-6: Interleukin 6; PBMCs: Blood mononuclear cells; SLE: Systemic lupus erythematosus; PCR: Polymerase chain reaction.

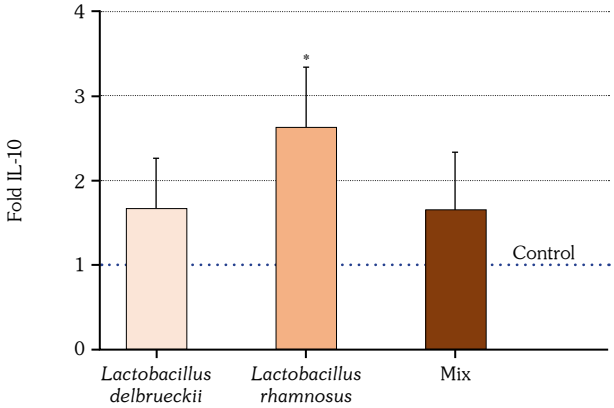

Figure 4. Relative mRNA level of IL-10 in the PBMCs of SLE patients compared to the (untreated) control group. The gene expression was studied by real-time PCR, and the results are expressed as fold change.

* Significant difference compared to the control group; mRNA: Messenger ribonucleic acid; IL-10: Interleukin 10; PBMCs: Blood mononuclear cells; SLE: Systemic lupus erythematosus; PCR: Polymerase chain reaction.

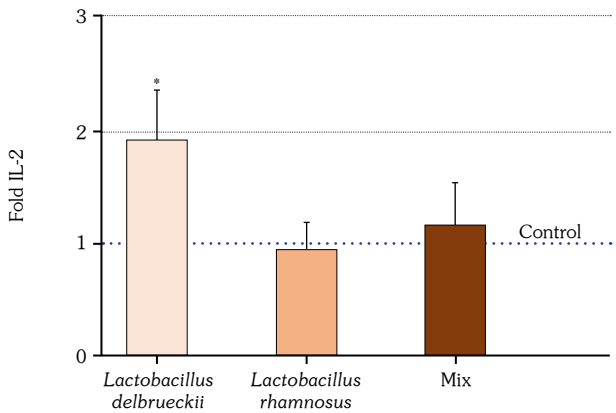

Figure 5. Relative mRNA level of IL-2 in the PBMCs of SLE patients compared to the (untreated) control group. The gene expression was studied by real-time PCR, and the results are expressed as fold change.

* Significant difference compared to the control group; mRNA: Messenger ribonucleic acid; IL-2: Interleukin 2; PBMCs: Blood mononuclear cells; SLE: Systemic lupus erythematosus; PCR: Polymerase chain reaction.

production of proinflammatory and inflammatory cytokines has a determinative role in pathogenesis and severity of the disease.[25] The immunomodulatory effects of some probiotics, particularly the Lactobacillus species, on T cells and regulatory effects on immune and inflammatory responses have been shown in previous studies.[26,27]

In the present study, we showed the effects of *L. rhamnosus* and *L. delbrueckii* on inflammatory and anti-inflammatory cytokines related to Tregs in newly diagnosed SLE patients. Tregs cells express the FOXP3 transcription factor and play an important role in the development of these cells.[28,29] Tregs are also involved in maintaining tolerance and preventing autoimmune diseases.[30] Some studies have reported that the expression level of FOXP3 molecule in patients with active lupus was reduced,[31,32] while the increased expression level of FOXP3 gene has been shown in a Lactobacillus-treated induced colitis murine model.[33] Another study revealed that treatment with Lactobacillus in the experimental autoimmune encephalomyelitis mice model and experimental myasthenia gravis increased in CD4+ CD25+ FOXP3+ Tregs.[30] Researchers reported that treatment with *Lactobacillus reuteri* in entering the colitis mice model increased the number of Tregs and tolerogenic dendritic cells and decreased the levels of the proinflammatory cytokines.[34] In another study, *L. rhamnosus* and *L. delbrueckii* enhanced the expansion of CD4+ CD25+ FOXP3+ Tregs cells in the spleen and also decreased the frequency of Th1 cell and inflammatory cytokines in a pristane model mice.[18,35] Additionally, a mixture of *Lactobacillus paracasei* and *L. reuteri* increased Tregs in a SLE NZB/WF1 mice model.[36] Furthermore, the expansion of Tregs occurred in the atopic dermatitis mice model by *Lactobacillus acidophilus*.[37] Moreover, treatment with *L. rhamnosus* increased Tregs in allergic diseases.[38] This study showed that *L. rhamnosus*, *L. delbrueckii*, and the combination of both increase the expression of the FOXP3 molecule in the culture of SLE patients' PBMCs.

According to previous studies, TGF-β plays an important role in the differentiation of Tregs and inhibition of inflammation.[16,18] The drop of this cytokine in lupus patients is associated with a reduction of disease activity.[39,40] A study found that the administration of *L. rhamnosus* increased the level of TGF-β in children with allergic rhinitis.[41] In addition, Nawaz et al.[42] showed that in mice with inflammatory bowel disease *Lactobacillus casei* treatment increased the level of TGF-β and lowered the production of inflammatory cytokines in the intestinal tissue. Furthermore, treatment with *L. delbrueckii* led to an increased TGF-β level in the spleen and intestinal tissue of the colitis mice model.

Lactobacillus also increased the number of Tregs in the mesenteric lymph nodes of the animal.[23] Contrary to these results, in several studies evaluating the antiallergic effect of *L. rhamnosus* in an experimental murine model, the antiallergic effects of this probiotic reduced the level of TGF-β.[42] Furthermore, treatment with *L. rhamnosus* in the allergic disease reduced the levels of TGF-β cytokines in infants.[43] In this study, our results showed the expression level of TGF-β cytokines was higher in patients receiving *L. rhamnosus*, *L. delbrueckii*, and their mixture compared to the untreated control group.

The level of IL-6 in patients with lupus is associated with the severity and activity of the disease and the production of anti-dsDNA.[44] Thus, a decrease in inflammatory cytokine is a way to decrease inflammation in SLE patients. Furthermore, a study on a SLENZB/W mice model revealed that mixture strains of *L. paracasei* and *L. reuteri* reduced the level of IL-6 and tumor necrosis factor.[36] In the same vein, the treatment of inflammatory bowel disease with *L. paracasei* decreased the level of IL-6 and reduced the production of inflammatory cytokines.[45] We also showed that the expression level of IL-6 cytokine decreased in all groups receiving probiotics compared to the control group.

IL-10, as an anti-inflammatory cytokine, has an important effect on the commitment and function of Tregs, which can be effective in treating SLE.[40] Lavasani et al.[46] stated in 2010 that the administration of *L. rhamnosus* increased the level of IL-10 and induction of Tregs in the experimental autoimmune encephalomyelitis mice model. Another study has shown that *L. rhamnosus* treatment in rotavirus infected BALB/c mice reduced the level of IL-10.[47] In our study, IL-10 cytokine expression level was higher in PBMCs of patients receiving *L. rhamnosus* compared to the untreated control group.

Additionally, IL-2 cytokine plays an important role in the survival and function of Tregs. This cytokine along with TGF-β can differentiate CD4+T cells to Tregs.[15] Some studies found that the level of IL-2 cytokine in lupus patients was reduced.[48] Treatment with mixture strains of Lactobacillus in the atopic dermatitis mice model increased the level of IL-2.[49] Furthermore, the administration of *L. rhamnosus* as a solution in healthy people enhanced the level of IL-2.[27] The results of the current study showed that IL-2 cytokine expression level was significantly higher in PBMCs of patients receiving *L. delbrueckii* and a mixture of both probiotics compared to the untreated control group.

As these probiotic agents affect a wide array of immunoregulator cells and cytokines, understanding their mechanism of action is key to unlocking their full therapeutic potential. Although such studies are lacking for a large portion of probiotic agents, several studies have demonstrated the effects on key pathways and modulators, including nuclear factor kappa B, mitogen-activated protein kinase, Wnt/BAR-1, Toll-like receptor 2, FOXP3, and dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin, altering the inflammatory profile and immune cell populations.[45,50-53]

It is important to note that although the current literature predominantly supports the beneficial effects of probiotics, their safety should be thoroughly investigated before widespread use as a therapeutic agent. Sporadic studies have reported that these agents may cause severe life-threatening infections, specifically in immunocompromised patients. This shows that despite the obvious advantages of probiotic use, universal guidelines for their dosage, handling, administration, and possible severe side effects are desperately needed.[54-57]

In conclusion, the results of our study indicated that the use of probiotics can be helpful to elevate the level of the immunomodulatory molecules, such as FOXP3 (transcription factor of Tregs), TGF-β and IL-10 (cytokines related to Tregs), IL-2 (key cytokine for production and function of Tregs), and to reduce the level of IL-6 (a proinflammatory cytokine) in highly active naïve SLE patients. Note that Tregs function by secreting cytokines and transcription factors, and they are also effective in controlling the disease. Thus, these probiotics could be effective in regulating the balance of gene expression of cytokines profile *in vitro*, and they can be used to produce new complement drugs due to their beneficial effects. Further *in vivo* studies are needed to clarify the exact mechanisms, while animal and clinical trial studies are also suggested in order for these results to be confirmed.

Ethics Committee Approval: The study protocol was approved by the Mashhad University of Medical Sciences Ethics Committee (date: 14.06.2017, no: IR.MUMS.sm.REC.1396.158). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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