BPB Reports

Regular Article

Intake of *Lactobacillus Pentosus TJ515* Prevents the Formation of Retinal Edema in Retinal Vein Occlusion Model Mice

Yae Hidaka,[a] Aika Kuriyama,[a] Anri Nishinaka,[a] Kei Takahashi,[a] Seigo Nakaya,[b] Takeshi Kubota,[b] Shinsuke Nakamura,[a] Masamitsu Shimazawa,[a,c] and Hideaki Hara[a,c,*]

[a]*Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, Gifu, Japan;* [b]*Sagami Research Laboratory, Wakamoto Pharmaceutical Co., Ltd.;* [c]*Laboratory of Collaborative research for Innovative Drug Discovery, Gifu Pharmaceutical University, Gifu, Japan*

Received January 13, 2023; Accepted March 19, 2023

Macular edema is a sight-threatening symptom in patients with retinal vein occlusion (RVO) and diabetic retinopathy. It is treated by the intravitreal injection of anti-vascular endothelial growth factor agents, but this has a physical burden on patients. Thus, it is important to develop a treatment that can be administered orally. The probiotic *Lactobacillus pentosus TJ515* can reduce inflammation by regulating host immunity via its induction of interleukin-10 (IL-10). However, its action on inflammatory diseases of the retina remains unclear. This study aimed to investigate the preventive effects of *L. pentosus TJ515* intake on retinal edema using RVO model mice. Occlusion of the retinal vein led to an increase in the thickness of all retinal layers and the inner nuclear layer. Intake of *L. pentosus TJ515* for three weeks suppressed the formation of retinal edema versus vehicle and the control strain (*L. pentosus JCM1558*). The expression of *MCP-1* and *MCP-3* was increased in RVO model mice but suppressed by the intake of *L. pentosus TJ515*. F4/80-positive cells (i.e., activated macrophages) were increased in the retinas of RVO model mice but decreased by the intake of *L. pentosus TJ515*. Therefore, *L. pentosus TJ515* suppressed macrophage migration in the retinas. Treatment of mouse bone marrow-derived macrophages (BMDM) with plasma from mice that had ingested *L. pentosus TJ515* decreased the mRNA expression of IL-6 and increased the mRNA expression of IL-10. Thus, oral intake of *L. pentosus TJ515* can prevent retinal edema through its anti-inflammatory effects on macrophages and inhibiting effects on macrophage migration.

Key words retinal edema, *lactobacillus pentosus TJ515*, macrophage, retinal vein occlusion, interleukin-6, interleukin-10, monocyte chemoattractant protein-1

INTRODUCTION

Macular edema is one of the major pathologies in patients with retinal vein occlusion (RVO)[1] and diabetic macular edema (DME).[2] Macular edema is caused by leakage from damaged blood vessels, which leads to vision loss.[3] The formation of retinal edema is closely associated with the expression of vascular endothelial growth factor (VEGF).[4] Thus, intravitreal administration of anti-VEGF agents is used for the treatment of retinal edema in patients with RVO and DME.[5] However, anti-VEGF therapy is expensive and requires several repeat doses at relapse, placing a significant financial and physical burden on patients.[5,6] In addition, RVO[7] and DR[8] are known to increase with age. In today's aging society, this is expected to result in an increase in healthcare costs. Therefore, it is important to decrease the number of patients by developing a prevention as well as treatment for retinal edema.

Inflammation is a related factor of macular edema formation.[9,10] When cells are stressed or injured, immune cells promote the release of proinflammatory cytokines and the migration of immune cells into tissues, which is followed by inflammation.[11] One of the immune system cells involved in this process is monocytes/macrophages.[11] Monocytes/macrophages are recruited from the systemic circulation following an increase in the permeability of blood vessels, which results in the production of proinflammatory cytokines in experimental RVO model mice[12] and patients with RVO.[13] Monocyte chemoattractant protein (MCP) -1 and MCP-3 play important roles in monocyte infiltration into tissues.[14,15] MCP-1 and MCP-3 are chemotactic factors for monocytes produced mainly by macrophages and endothelial cells.[15,16] Expression of these factors is increased in the vitreous fluid of patients[17] and retinas of animal models[18] with retinal edema. The accumulation of macrophages leads to an increased inflammatory response,[19] which in turn exacerbates the leakage of plasma components into retinas.[20,21] These reports show that monocyte/macrophage migration into retinas is associated with the formation of retinal edema. Thus, inhibition of macrophage accumulation into tissues suppresses inflammation, which is expected to be beneficial for treating retinal edema.

The connection between the gut and the eyes is attracting attention. Previous reports have suggested a possible interaction between gut microbiome dysbiosis and ocular diseases such as DR,[22] uveitis,[23] glaucoma,[24] and age-related macular degeneration.[25,26] Thus, regulation of the intestinal environment could be a therapeutic option or prevention for patients with ocular diseases. Probiotics, which are live microorganisms that positively impact host health by improving the bal-

*To whom correspondence should be addressed. e-mail: hidehara@gifu-pu.ac.jp

ance of intestinal flora,[27] have been reported to have beneficial effects on ocular disease.[28–30] For example, intake of *Lactobacillus paracasei KW3110* suppressed age-related retinal ganglion cell loss and photoreceptor degeneration via activating M2 macrophages in mice.[29] Oral administration of *L. plantarum NK151* and *Bifidobacterium bifidum NK175* also relieved benzalkonium chloride solution-induced dry eye by modulating the expression ratio of pro-inflammatory cytokines.[31] These reports showed that probiotics are expected to be effective in the suppression of inflammation in retinal disease through the regulation of host immunity and alleviating systemic inflammation. *L. pentosus TJ515* is a strain found in fermented Thai foods.[32] In a previous study, *L. pentosus TJ515* produced more anti-inflammatory cytokines such as interleukin-10 (IL-10) and interferon-γ (IFN-γ) than the control stain *L. pentosus JCM1558* in mesenteric lymph node cells.[32] Given that it has been reported that dietary supplementation with *L. pentosus TJ515* and resveratrol could manage the progression of near vision impairment,[33] *L. pentosus TJ515* taken orally could exert an action in the eye. Thus, *L. pentosus TJ515* is expected to prevent retinal inflammatory diseases by regulating systemic inflammation.

We hypothesized that the intake of *L. pentosus TJ515* may be an effective prevention of retinal edema. To verify this hypothesis in the present study, we investigated the preventive effect of *L. pentosus TJ515* on retinal edema using RVO model mice and mouse bone marrow-derived macrophages (BMDM).

MATERIALS AND METHODS

Animal Treatments 5 weeks-old mice (Male albino ddY mice) were obtained from Japan SLC (Hamamatsu, Japan). The mice were housed under a 12 h light-dark cycle at 24 ± 2°C and 55 ± 15% humidity and fed with free access to water. Before the start of the experiment, mice were weighed and divided into three groups (Control group, *L. pentosus TJ515* treated group, *L. pentosus JCM1558* treated group). Under free feeding, mice in each group were fed a powdered mouse powder feed (MF; Oriental Yeast Co., Ltd., Tokyo, Japan), MF diet containing 3% *L. pentosus TJ515*, and a powdered MF diet containing 3% *L. pentosus JCM1558* for three weeks. Experimental procedures were consistent with the Association for Research in Vision and Ophthalmology Statement (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research. All experiments were approved and monitored by the Institutional Animal Care and Use Committee of Gifu Pharmaceutical University.

Mouse Model of Retinal Vein Occlusion (RVO) The preparation of RVO murine model was previously described.[18] All work was performed under anesthesia, the composition of which is ketamine (120 mg/kg; Daiichi-Sankyo, Tokyo, Japan) and xylazine (6 mg/kg; Bayer, Health Care, Osaka, Japan). 8 mg/mL Rose Bengal (10 mL/kg; Wako, Osaka, Japan) was injected into the tail vein. Then, the 50-mW laser light (532 nm) was applied for 5 seconds (10-15 times to each vein) to occlude three retinal veins. (Meridian AG, Bierigustrasse, Switzerland).

Bacteria Strain *L. pentosus TJ515* was supplied by Wakamoto Pharmaceutical Co., Ltd. *Lactobacillus pentosus JCM 1558* was obtained from the Japan Collection of Microorganisms, Japan.

Preparation of Cells *L. pentosus TJ515* and *L. pentosus JCM1558* were cultured at 27°C, for 20 h in Difco Lactobacilli MRS broth (Becton, Dickinson and company, Sparks, USA). Cells were harvested by centrifugation and were suspended a stabilizer fluid (starch, etc.). Cell suspension was dried with the freeze-dryer. Dried cells were adjusted to 5-7 × 10^{10} CFU/g with starch.

Optical Coherence Tomography (OCT) Imaging OCT images were gained by Micron IV fundus camera, an OCT Scan Head equipped with a mouse objective lens (Phoenix Research Labs, Pleasanton, CA, USA), and StreamPix 6 and Micron OCT commercial software (Phoenix Research Labs). Under anesthesia, mice were mydriatic with 0.5% tropicamide and phenylephrine hydrochloride (Santen Pharmaceutical Co. Ltd., Osaka, Japan). Images were obtained from four mice in each group and quantified using "In Sight" software, which automatically detects the retinal layer and measures its thickness.

Histological Analyses The eyes were enucleated after mice were euthanized by cervical dislocation. The eyes were immersed in a cold fixative of 4% paraformaldehyde (PFA; Wako) in 0.1 M phosphate buffer (PB; pH 7.4) for 48 h. Then, those were embedded in paraffin. The paraffin-embedded sections were cut into 5 μm and stained with hematoxylin and eosin. The retinas were observed and imaged with the All-in-One BZ-X710 fluorescent microscope (Keyence, Osaka, Japan). The inner nuclear layer (INL) thickness was measured every 240 μm from the optic nerve head using the Image-J software (National Institutes of Health, Bethesda, MD, USA).

Bone Marrow-Derived Macrophage (BMDM) Preparation Mice were euthanized by hyperesthesia with isoflurane. The femur, tibia, and humerus were each harvested and the surrounding tissue was removed. One side of each bone was cut and centrifuged at 10,000 × *g* for 10 s. Obtained cells were hemolyzed with Red Blood Cell Lysis Buffer (Thermo Fisher Scientific, Waltham, MA, USA) for 30-60 s. It is suspended in 10 mL of DMEM low glucose including 10% FBS and 100 U/mL penicillin (Meiji Seika Pharma Co., Ltd., Tokyo, Japan) and 100 μg/mL streptomycin (Meiji Seika Pharma) (10% FBS DMEM). Then, it was centrifuged at 1,300 rpm for 5 min. After resuspension, cells were seeded at a density of 100,000 cells/mL in 24-well plates with 10% FBS DMEM including 10 ng/mL macrophage colony-stimulating factor (M-CSF; R&D Systems Minneapolis, MN, USA) and incubated under a humidified atmosphere of 5% CO_2 at 37°C. Two days after seeding, changed the medium and incubated for 2 more days.

Isolation of RNA and qRT-PCR The eyes were enucleated one day after laser irradiation. The retinas were isolated and frozen in liquid nitrogen. BMDM were incubated into 5% FBS DMEM including 25% plasma for 48 h. Each plasma was gained from Control mice, RVO model mice, RVO model mice fed *L. pentosus TJ515*, and RVO model mice *L. pentosus JCM1558*. The RNAs were isolated from retinas and BMDM using NucleoSpin RNA kit (Takara, Shiga, Japan) according to the manufacturer's instructions. RNA concentrations were measured using spectrophotometrically at 260 nm. Then, the cDNA was synthesized using a PrimeScript RT reagent kit (Perfect Real Time; Takara Bio Inc., Shiga, Japan). Quantitative PCR was conducted with TB Green Premix Ex Taq II (Takara, Shiga, Japan). The PCR primer sequences were as flow;

Gapdh Forward: 5′-TGTGTCCGTCGTGGATCTGA-3′ and Reverse: 5′-TTGCTGTTGAAGTCGCAGGAG-3′

Mcp-1 Forward: 5′-CTG AAG CCA GCT CTC TCT G-3′ and Reverse: 5′-CAG GCC CAG AAG CAT GAC A-3′

Mcp-3 Forward: 5′-GCT GCT TTC AGC ATC CAA GTG-3′and Reverse: 5′- CCA GGG ACA CCG ACT ACT G-3′

Il-6 Forward: 5′-TCT GCA AGA GAC TTC CAT CCA GT-3′and Reverse: 5′-TCT GCA ACT GCA TCA TCG TTG T-3′

Il-10 Forward: 5′-TGG CCC AGA AAT CAA GGA GC-3′and Reverse: 5′- CAG CAG ACT CAA TAC ACA CT-3′

Immunofluorescence Staining The enucleated eyes were fixed in 4% PFA for 24 h and transferred in 5, 10, 15, and 20 (for 2 h each) and placed in 25% sucrose (24 h) at 4°C. The eyes were embedded in an optimal cutting temperature (OCT) compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan) and cut 15 μm thickness with a cryostat (Leica Microsystems, Bensheim, Germany). The tissue sections were incubated with PBS containing 5% goat serum (Vector Laboratories, Burlington, VT, USA) and 0.3% Triton-X 100 (BioRad Laboratories, Hercules, CA, USA) for 1 h. Then the sections were rinsed in PBS three times and then incubated with F4/80 rat monoclonal antibody (1:50; BioRad Laboratories) overnight at 4°C. They were incubated with Alexa Fluor-546 goat anti-rat IgG (1:1000, Thermo Fisher Scientific) for 1 h and Hoechst 33342 (1:1000, Thermo Fisher Scientific) for 10 min. The images were photographed with a FLUOVIEW FV3000 (Olympus, Tokyo, Japan).

Statistical Analyses Statistical analyses were performed using the 16 Statistical Package for the Social Sciences 15.0 J for Windows software (SPSS Japan Inc, Tokyo, Japan). Comparisons between group means were performed using Student' *t*-test and Tukey's test (Fig. 1C and Fig. 3). All results are presented as mean ± SEMs, and $p < 0.05$ were considered significant.

RESULTS

The Intake of *L. Pentosus TJ515* Prevents the Formation of Retinal Edema We fed mice the control feeds, feeds containing *L. pentosus TJ515*, and feeds containing *L. pentosus JCM1558* (control strain) for three weeks to evaluate the inhibitory effects of *L. pentosus TJ515* on retinal edema (Fig. 1A). *L. pentosus TJ515* and *L. pentosus JCM1558* had no effect on body weight or phenotype (Data are not shown). The thickness of all retinal layers measured by OCT was increased in RVO model mice fed the control feeds one day after occlusion (Fig. 1B). The increase in retinal layer thickening was prevented by taking *L. pentosus TJ515* (Fig. 1B). Because the retinal edema forms in the INL in RVO model mice, not only the thickness of all retinal layers but INL can be used for the evaluation of retinal edema. The thickness of INL was also increased in RVO model mice fed the control feeds; however, this was prevented by the intake of L. *pentosus TJ515* (Fig. 1C). We evaluated the difference between *L. pentosus TJ515* and *L. pentosus JCM1558* (control strain) by all retinal layer thicknesses measured using OCT. Intake of *L. pentosus TJ515* prevented the retinal edema formation, whereas intake of *L. pentosus JCM1558* did not decrease it (Fig. 1D). We found that the intake of *L. pentosus TJ515* had inhibitory effects on retinal edema after RVO.

The Intake of *L. Pentosus TJ515* Suppressed the Expression of Chemokines Involved in Macrophage Migration Next, we investigated the effects of *L. pentosus TJ515* on the mRNA expression of chemokines in retinas from RVO model mice fed with each feed one day after RVO. The expression of MCP-1 and MCP-3 was increased in retinas from RVO model mice fed the control feeds (Fig. 2A, B). The intake of *L. pentosus TJ515* significantly inhibited the increase in expression of MCP-1 and tended to decrease the expression of MCP-3 (Fig. 2A, B). *L. pentosus JCM 1558* has no significant effect on the expression of either MCP-1 or MCP-3 (Fig. 2A, B).

The Intake of *L. Pentosus TJ515* Inhibited Macrophage Migration To determine the involvement of *L. pentosus TJ515* in macrophage migration, we investigated the localization of macrophages in retinas from RVO model mice fed with each feed one day after RVO by the immunofluorescence of F4/80 (the monocyte/macrophage marker). F4/80-positive cells accumulation was increased in RVO model mice fed the control feeds compared with normal mice (Fig. 3A, B). Intake of *L. pentosus TJ515* decreased F4/80-positive cells in INL, but *L. pentosus JCM1558* had no effect on it (Fig. 3A, B).

***L. Pentosus TJ515* Acts as an Anti-Inflammatory on BMDM *via* Plasma Components** To explore the mechanism by which *L. pentosus TJ515* taken orally acts on retinas, we focused on plasma components from mice fed with *L. pentosus TJ515* for three weeks. We examined the expression of cytokines produced by macrophages treated by plasma from control mice and plasma from RVO mice fed the vehicle feeds, feeds including *L. pentosus TJ515*, or feeds including *L. pentosus JCM1558*. The mRNA level of IL-6 produced in BMDM was increased by treatment with plasma from RVO mice compared with plasma from control mice (Fig. 4A). However, the increase was suppressed by treatment with plasma from mice fed with *L. pentosus TJ515* (Fig. 4A). On the other hand, there was no significant difference between the plasma from the vehicle group and the *L. pentosus JCM1558*-treated group (Fig. 4A). The expression of IL-10 (an anti-inflammatory cytokine) was increased by treatment with plasma from mice fed with *L. pentosus TJ515* compared with the vehicle-treated group (Fig. 4B). Plasma components from mice fed with *L. pentosus TJ515* had anti-inflammatory effects on BMDM (Fig. 4B).

DISCUSSION

Here, we showed that the intake of *L. pentosus TJ515* had a suppressive effect on retinal edema. The mechanism underlying this effect was the inhibition of macrophage migration to the inflammatory site through a decrease of MCP-1 and MCP-3 expression and the regulation of the effect of plasma components on IL-6 and IL-10 production in macrophages. In order to reduce the physical and financial burdens of intravitreal administration of anti-VEGF drugs in patients, it is important for preventive methods to be developed that can be administered more simply. Our study suggested that the intake of *L. pentosus TJ515* is effective for the prevention in patients at risk for retinal edema.

Accumulated macrophages induce the disruption of endothelial barrier function through the production of inflammatory cytokines in retinal edema.[9,10] It has been reported that an accumulation of macrophage-like cells tends to cause more severe edema in RVO patients.[13] Our data indicated that *L.*

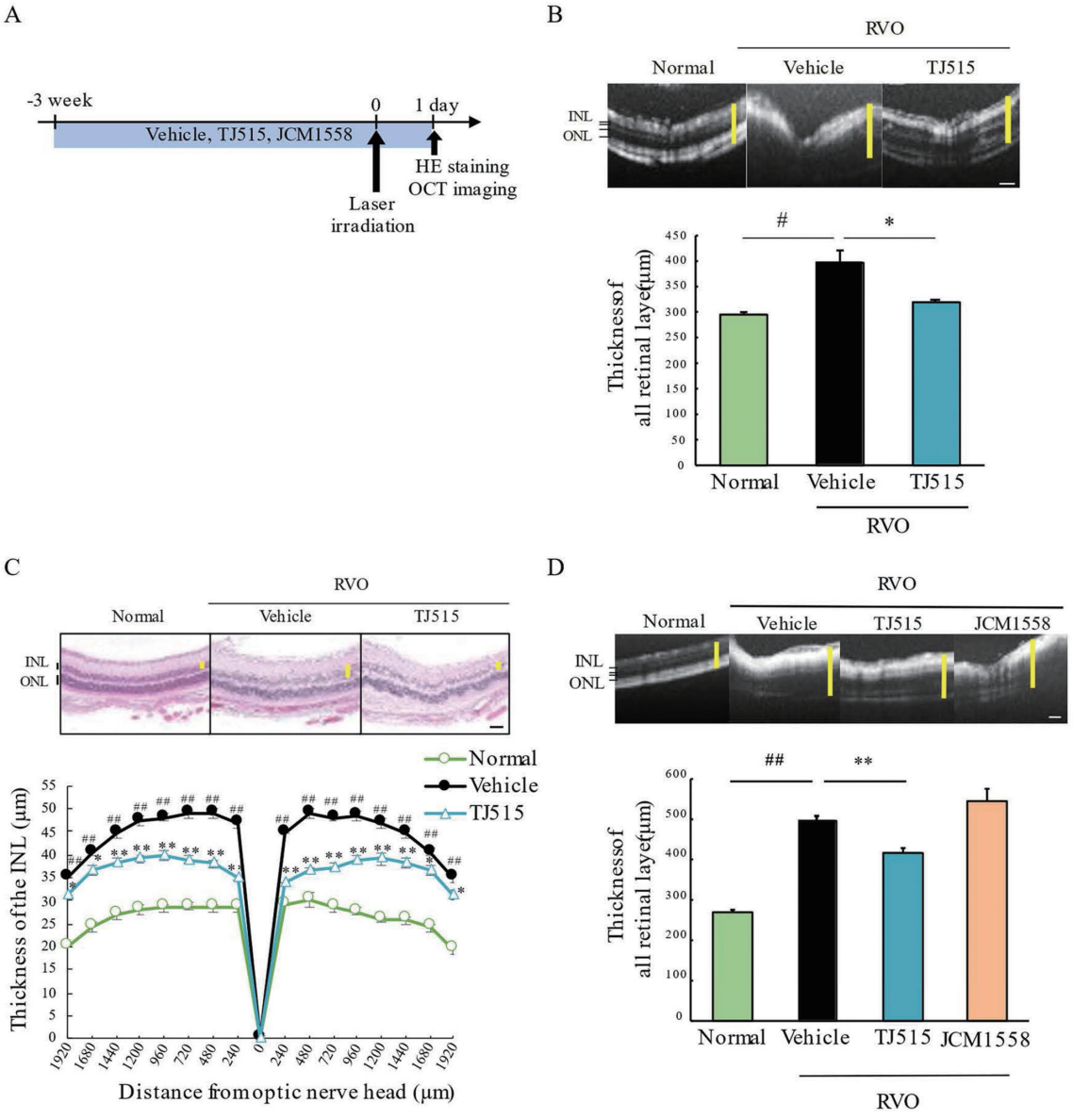

Fig. 1. Intake of *Lactobacillus Pentosus TJ515* Prevented the Retinal Formation in RVO Murine Model.

(A) The protocol for investigating the effects of *lactobacillus pentosus TJ515* on the retinal edema in RVO model mice. (B) The OCT images of the retinal layer. The graph shows the thickness of the whole retinal layer of normal, vehicle, and *lactobacillus pentosus TJ515* intake group. The data represent the mean ± SEM (n = 4). $^{\#}P < 0.05$ versus Normal group and, $^{*}P < 0.05$ versus Vehicle group (Welch's *t*-test). Scale bar = 50 μm. (C) Typical images of retinas stained with hematoxylin and eosin. The graph shows the INL thickness in the retinas of normal, vehicle, and orally administered *lactobacillus pentosus TJ515* groups. The data represent the mean ± SEM (Normal; n = 10, Vehicle; n = 10, and TJ515; n = 14). $^{\#\#}P < 0.01$ versus Normal group, $^{*}P < 0.05$, and $^{**}P < 0.01$ versus Vehicle group (Tukey's test). Scale bar = 50 μm. (D) The OCT images of the retinal layer. The graph shows the thickness of the whole retinal layer of normal, vehicle, *lactobacillus pentosus TJ515* intake group, and *lactobacillus pentosus JCM1558* intake group. The data represent the mean ± SEM (n = 6). $^{\#\#}P < 0.01$ versus Normal group and, $^{**}P < 0.01$ versus Vehicle group (Welch's *t*-test). Scale bar = 50 μm. INL, inner nuclear layer. ONL, outer nuclear layer.

pentosus TJ515 inhibited macrophage migration into RVO retina. Because inhibiting macrophage accumulation also reduces inflammatory cytokines released from them, inhibiting macrophage accumulation could prevent retinal edema. The migration of macrophages is induced by MCP-1 and MCP-3, potent chemotactic factor for monocytes.[14,15] Following an increase in MCP-1 and MCP-3 expression in the inflammation site, the monocytes/macrophages are recruited into there and induce more inflammation and permeability reactions.[14,15] Previous results has shown that the suppression of *MCP-1* expression ameliorated retinal endothelial dysfunction in DR model mice.[34] Therefore, the regulation of MCP-1 and MCP-3 expression might lead to the treatment of retinal edema.

The increase of *MCP-1* and *MCP-3* is induced by pro-inflammatory cytokines, such as IL-6 and TNF-α in endothelial cells.[35,36] IL-6 enhanced MCP-1 production through binding to receptors on the membrane of vascular endothelial cells and activating the PI3K/AKT, MEK/ERK, and JAK/STAT3 pathways.[37,38] IL-6 is also known to activate astrocytes and promote astrocyte-derived MCP-3 release.[39] On the other hand, IL-10 is reported to inhibit astrocyte activation and MCP-3 release.[39] These reports suggested that changes in *MCP-1*and

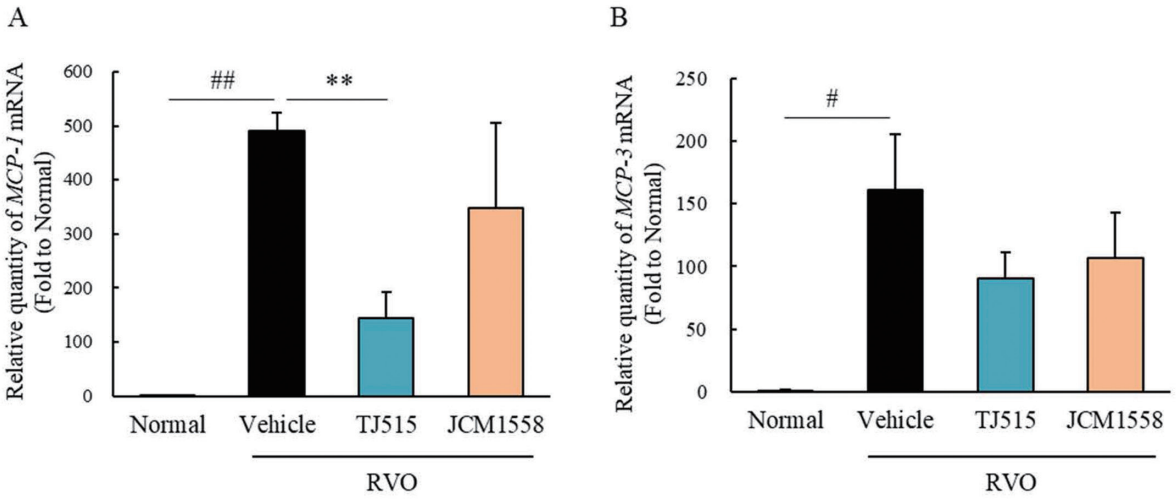

Fig. 2. *Lactobacillus Pentosus TJ515* Intake Inhibited the Increase of Macrophage Migration-Associated Factors in the Retina of a Model of RVO.

The expression levels of (A) MCP-1 mRNA and (B) MCP-3 mRNA were examined in the retina. Data are the means ± SEM. (Normal; n = 5, Vehicle; n = 5, TJ515; n = 6, and JCM1558; n = 6). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus Normal group, $^{**}P < 0.01$ versus Vehicle group (Student's *t*-test).

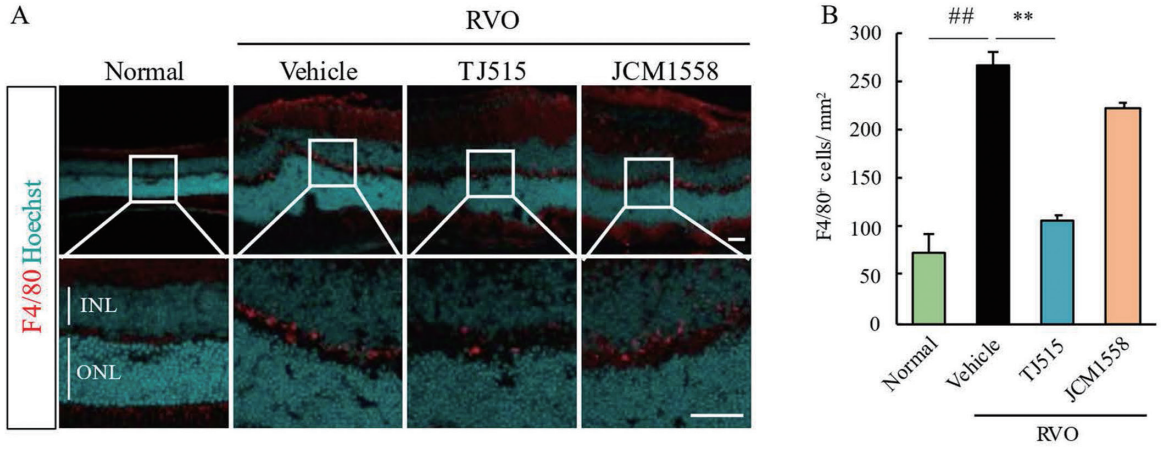

Fig. 3. Oral Administration of *Lactobacillus Pentosus TJ515* Inhibited Macrophage Migration into the Inner Retina.

(A) F4/80 immunostaining and (B) graphical representation of the F4/80 positive cells. The data represent the mean ± SEM (Normal; n = 3, Vehicle; n = 3, TJ515; n = 4, and JCM1558; n = 4). $^{\#\#}P < 0.01$ versus Normal group, and $^{**}P < 0.01$ versus Vehicle group (Tukey's test). Scale bar = 50 μm.

MCP-3 expression are related with the stimulation of endothelial cells and astrocytes by IL-6 and IL-10. Our results showed that the expression of *MCP-1* expression was increased in retinas from RVO model mice, but intake of *L. pentosus TJ515* decreased it. A similar trend to the changes in MCP-1 was also observed for MCP-3 expression. Our study also showed that *IL-6* expression was increased and *IL-10* expression was decreased in BMDM treated with serum from RVO model mice; however, these effects were suppressed by treatment with plasma derived from RVO model mice fed with *L. pentosus TJ515*. These results showed that the suppressing activation of circulating macrophages might decrease *MCP-1* and *MCP-3* expression in retinas. Suppression of macrophages accumulation in the retina by the suppression of MCP-1 and MCP-3 mRNA expression may be the mechanism by which L. *pentosus TJ515* has a suppressive effect on retinal edema.

Plasma includes various vital information carriers such as cytokines and proteins and plays a role in communication with other cells and regulating cellar function.[40,41] It has been reported that serum inflammation and oxidative stress markers are increased in patients with RVO, suggesting that local ocular inflammation might lead to systemic inflammation.[42,43] It has been suggested that changes in plasma miRNAs are associated with a decrease in mRNA expression.[44] Upregulation of miR-106a has been reported in the serum of patients with diabetic retinopathy, a disease associated with retinal edema.[45,46] miR-106a is known to be one of the miRNAs that directly inhibit IL-10 mRNA expression.[47] Although we did not examine miRNA changes in the plasma of RVO model mice, it is possible that miRNAs may be involved in some of the mechanisms of reduced IL-10 mRNA expression.

L. pentosus TJ515 is known to increase IL-10 expression

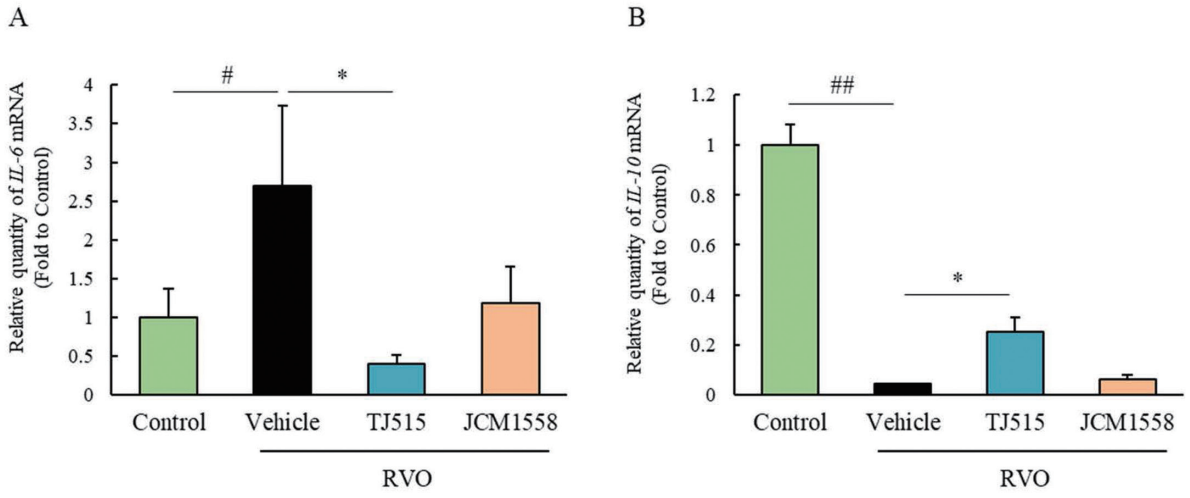

Fig. 4. Plasma Components of Orally Administered *Lactobacillus Pentosus TJ515* Affected Cytokine Expression in BMDM.

The expression levels of (A) IL-6 and (B) IL-10 mRNA were examined in BMDM. Data are the means ± SEM. (Control; n = 5, Vehicle; n = 4, TJ515; n = 4, and JCM1558; n = 4). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus Control group, $^{*}P < 0.05$ versus Vehicle group (Student's *t*-test).

in the intestinal immune cells.[32] Clinical studies[48] and studies using mice[49] have reported that probiotics, which strongly induce IL-10 expression in immune cells in the intestine, increase plasma IL-10 levels. IL-10 inhibits the proliferation and activation of macrophages and the production of inflammatory cytokines including *IL-6* and *TNF-α* in MBDM.[50–52] Thus, plasma from RVO model mice fed with *L. pentosus TJ515* seems to have induced an anti-inflammatory effect in macrophages *via* an increase in IL-10 levels. Also, it has been reported that probiotic-derived exosomes and miRNAs can regulate cytokine expression in plasma and tissues.[53–55] Thus, probiotics may have anti-inflammatory effects *via* diverse pathways. This study revealed that *L. pentosus TJ515* could be a new prevention for retinal edema. However, our study had the following several limitations. We were not able to determine the changes in the intestinal microbiota caused by oral intake of *L. pentosus TJ515*. In addition, the detailed mechanisms of the anti-inflammatory effects of plasma components and cytokines remain unclear. These topics should be considered in future research.

In conclusion, these findings suggested that *L. pentosus TJ515* has anti-inflammatory effects by inhibiting macrophage accumulation by inducing IL-10 production. Probiotics can be taken daily because there is little harm caused by long-term ingestion.[56] Probiotics can be used for minimally and invasively treating retinal edema as an oral administration. Moreover, *L. pentosus TJ515* could be apply treatment of other inflammation diseases because this study indicated it suppressed systemic inflammation. We suggested that the intake of *L. pentosus TJ515* could become an effective therapeutic and prophylactic strategy to suppress retinal edema in RVO and DR patients.

Conflict of interest This work was financially supported by Wakamoto Pharmaceutical Co., Ltd. Nakaya and Kubota are employees of Wakamoto Pharmaceutical Co., Ltd.

REFERENCES

1) Rogers S, McIntosh RL, Cheung N, Lim L, Wang JJ, Mitchell P, Kowalski JW, Nguyen H, Wong TY. The Prevalence of Retinal Vein Occlusion: Pooled Data from Population Studies from the United States, Europe, Asia, and Australia. *Ophthalmology*, **117**, 1–14 (2010).
2) Bodnar ZM, Desai A, Akduman L. Diabetic macular edema. *Spectr. Domain Opt. Coherence Tomogr. Macular Dis.*, **59**, 117–127 (2016).
3) Ouyang Y, Heussen FM, Keane PA, Pappuru RKR, Sadda SR, Walsh AC. Evaluation of the axial location of cystoid spaces in retinal vein occlusion using optical coherence tomography. *Retina*, **33**, 1011–1019 (2013).
4) Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am. J. Pathol.*, **146**, 1029–1039 (1995).
5) Cox JT, Eliott D, Sobrin L. Inflammatory complications of intravitreal anti-vegf injections. *J. Clin. Med.*, **10**, 1–16 (2021).
6) Hoeh AE, Ach T, Schaal KB, Scheuerle AF, Dithmar S. Long-term follow-up of OCT-guided bevacizumab treatment of macular edema due to retinal vein occlusion. *Graefes Arch. Clin. Exp. Ophthalmol.*, **247**, 1635–1641 (2009).
7) Song P, Xu Y, Zha M, Zhang Y, Rudan I. Global epidemiology of retinal vein occlusion: A systematic review and meta-analysis of prevalence, incidence, and risk factors. *J. Glob. Health*, **9**, (2019).
8) Varma R, Bressler NM, Doan QV, Gleeson M, Danese M, Bower JK, Selvin E, Dolan C, Fine J, Colman S, Turpcu A. Prevalence of and risk factors for diabetic macular edema in the United States. *JAMA Ophthalmol.*, **132**, 1334–1340 (2014).
9) Funatsu H, Noma H, Mimura T, Eguchi S, Hori S. Association of Vitreous Inflammatory Factors with Diabetic Macular Edema. *Ophthalmology*, **116**, 73–79 (2009).
10) Haller JA, Bandello F, Belfort R, Blumenkranz MS, Gillies M, Heier J, Loewenstein A, Yoon YH, Jiao J, Li XY, Whitcup SM. Dexamethasone intravitreal implant in patients with macular edema related to branch or central retinal vein occlusion: twelve-month study results. *Ophthalmology*, **118**, 2453–2460 (2011).
11) Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. *Nat. Immunol.*, **6**, 1182–1190 (2005).
12) Ebneter A, Kokona D, Schneider N, Zinkernagel MS. Microglia activation and recruitment of circulating macrophages during ischemic experimental branch retinal vein occlusion. *Invest. Ophthalmol. Vis. Sci.*, **58**, 944–953 (2017).

13) Zeng Y, Zhang X, Mi L, Gan Y, Su Y, Li M, Yang R, Zhang Y, Wen F. Characterization of Macrophage-Like Cells in Retinal Vein Occlusion Using En Face Optical Coherence Tomography. *Front. Immunol.*, **13**, 1–9 (2022).
14) Singh S, Anshita D, Ravichandiran V. MCP-1: Function, regulation, and involvement in disease. *Int. Immunopharmacol.*, **101**, 107598 (2021).
15) Luthar S, Cicchetti D, Becker B. Additive Roles for MCP-1 and MCP-3 in CCR2-mediated Recruitment of Inflammatory Monocytes During Listeria monocytogenes Infection. *Child Dev.*, **73**, 543–562 (2000).
16) Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J. Interferon Cytokine Res.*, **29**, 313–325 (2009).
17) Fonollosa A, Garcia-Arumi J, Santos E, MacIa C, Fernandez P, Segura RM, Zapata MA, Rodriguez-Infante R, Boixadera A, Martinez-Castillo V. Vitreous levels of interleukine-8 and monocyte chemoattractant protein-1 in macular oedema with branch retinal vein occlusion. *Eye (Lond.)*, **24**, 1284–1290 (2010).
18) Fuma S, Nishinaka A, Inoue Y, Tsuruma K, Shimazawa M, Kondo M, Hara H. A pharmacological approach in newly established retinal vein occlusion model. *Sci. Rep.*, **7**, 1–14 (2017).
19) Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. *Nat. Rev. Immunol.*, **11**, 762–774 (2011).
20) Bamforth SD, Lightman S, Greenwood J. The effect of TNF-α and IL-6 on the permeability of the rat blood-retinal barrier *in vivo*. *Acta Neuropathol.*, **91**, 624–632 (1996).
21) Van der Wijk AE, Vogels IMC, Van Noorden CJF, Klaassen I, Schlingemann RO. TNFα-induced disruption of the blood-retinal barrier *in vitro* is regulated by intracellular 30‘50’-cyclic adenosine monophosphate levels. *Invest. Ophthalmol. Vis. Sci.*, **58**, 3496–3505 (2017).
22) Jiao J, Yu H, Yao L, Li L, Yang X, Liu L. Recent Insights into the Role of Gut Microbiota in Diabetic Retinopathy. *J. Inflamm. Res.*, **14**, 6929–6938 (2021).
23) Rosenbaum JT, Asquith M. The microbiome and HLA-B27-associated acute anterior uveitis. *Nat. Rev. Rheumatol.*, **14**, 704–713 (2018).
24) Gong H, Zhang S, Li Q, Zuo C, Gao X, Zheng B, Lin M. Gut microbiota compositional profile and serum metabolic phenotype in patients with primary open-angle glaucoma. *Exp. Eye Res.*, **191**, 107921 (2020).
25) Lin P, McClintic SM, Nadeem U, Skondra D. A review of the role of the intestinal microbiota in age-related macular degeneration. *J. Clin. Med.*, **10**, (2021).
26) Andriessen EM, Wilson AM, Mawambo G, Dejda A, Miloudi K, Sennlaub F, Sapieha P. Gut microbiota influences pathological angiogenesis in obesity-driven choroidal neovascularization. *EMBO Mol. Med.*, **8**, 1366–1379 (2016).
27) Williams NT. Probiotics. *Am. J. Heal. Pharm.*, **67**, 449–458 (2010).
28) Morita Y, Miwa Y, Jounai K, Fujiwara D, Kurihara T, Kanauchi O. Lactobacillus paracasei KW3110 prevents blue light-induced inflammation and degeneration in the retina. *Nutrients*, **10**, (2018).
29) Morita Y, Jounai K, Sakamoto A, Tomita Y, Sugihara Y, Suzuki H, Ohshio K, Otake M, Fujiwara D, Kanauchi O, Maruyama M. Long-term intake of Lactobacillus paracasei KW3110 prevents agerelated chronic inflammation and retinal cell loss in physiologically aged mice. *Aging (Albany NY)*, **10**, 2723–2740 (2018).
30) Morita Y, Jounai K, Miyake M, Inaba M, Kanauchi O. Effect of heat-killed Lactobacillus paracasei KW3110 ingestion on ocular disorders caused by visual display terminal (VDT) loads: A randomized, double-blind, placebo-controlled parallel-group study. *Nutrients*, **10**, 1–17 (2018).
31) Yun SW, Son YH, Lee DY, Shin YJ, Han MJ, Kim DH. Lactobacillus plantarum and Bifidobacterium bifidum alleviate dry eye in mice with exorbital lacrimal gland excision by modulating gut inflammation and microbiota. *Food Funct.*, **12**, 2489–2497 (2021).
32) Chaiyasut M kimura; S siriun; and C. Isolation of an efficient lactobacillus pentosus strain capable of stimukating lymphoid cells to produce in-10, International jounal of probiotics and prebiotics, 2017.
33) Nagashima H, Sasaki N, Amano S, Nakamura S, Hayano M, Tsubota K. Oral administration of resveratrol or lactic acid bacterium improves lens elasticity. *Sci. Rep.*, **11**, 1–6 (2021).
34) Rangasamy S, McGuire PG, Nitta CF, Monickaraj F, Oruganti SR, Das A. Chemokine mediated monocyte trafficking into the retina: role of inflammation in alteration of the blood-retinal barrier in diabetic retinopathy. *PLoS One*, **9**, 1–10 (2014).
35) Maekawa T, Takahashi N, Honda T, Yonezawa D, Miyashita H, Okui T, Tabeta K, Yamazaki K. Porphyromonas gingivalis antigens and interleukin-6 stimulate the production of monocyte chemoattractant protein-1 via the upregulation of early growth response-1 transcription in human coronary artery endothelial cells. *J. Vasc. Res.*, **47**, 346–354 (2010).
36) Murao K, Ohyama T, Imachi H, Ishida T, Cao WM, Namihira H, Sato M, Wong NCW, Takahara J. TNF-α stimulation of MCP-1 expression is mediated by the Akt/PKB signal transduction pathway in vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **276**, 791–796 (2000).
37) Zegeye MM, Lindkvist M, Fälker K, Kumawat AK, Paramel G, Grenegård M, Sirsjö A, Ljungberg LU. Activation of the JAK/STAT3 and PI3K/AKT pathways are crucial for IL-6 trans-signaling-mediated pro-inflammatory response in human vascular endothelial cells. *Cell Commun. Signal.*, **16**, 1–10 (2018).
38) Kang S, Tanaka T, Inoue H, Ono C, Hashimoto S, Kioi Y, Matsumoto H, Matsuura H, Matsubara T, Shimizu K, Ogura H, Matsuura Y, Kishimoto T. IL-6 trans-signaling induces plasminogen activator inhibitor-1 from vascular endothelial cells in cytokine release syndrome. *Proc. Natl. Acad. Sci. USA*, **117**, 22351–22356 (2020).
39) Li J, Deng G, Wang H, Yang M, Yang R, Li X, Zhang X, Yuan H. Interleukin-1β pre-treated bone marrow stromal cells alleviate neuropathic pain through CCL7-mediated inhibition of microglial activation in the spinal cord. *Sci. Rep.*, **7**, 1–15 (2017).
40) Müller W. Dissecting the cytokine network. *Cell. Immunol.*, **244**, 162–164 (2006).
41) Xia P, Wadham C. Sphingosine 1-phosphate, a key mediator of the cytokine network: juxtacrine signaling. *Cytokine Growth Factor Rev.*, **22**, 45–53 (2011).
42) Özata Gündoğdu K, Doğan E, Çelik EAG. Serum inflammatory marker levels in serous macular detachment secondary to diabetic macular edema. *Eur. J. Ophthalmol.*, **32**, 3637–3643 (2022).
43) Chen KH, Hsiang EL, Hsu MY, Chou YC, Lin TC, Chang YL, Tsai CY, Li TH, Woung LC, Chen SJ, Peng CH, Hwang DK. Elevation of serum oxidative stress in patients with retina vein occlusions. *Acta Ophthalmol.*, **97**, e290–e295 (2019).
44) Ananthi JA, William JM, Thomas BT. miRNA regulation of cytokine genes. *Cytokine*, **45**, 58–69 (2009).
45) Zhao X, Ling F. Zhang G wei, Yu N, Yang J, Xin X yang. The Correlation Between MicroRNAs and Diabetic Retinopathy. *Front. Immunol.*, **13**, 1–11 (2022).
46) Satari M, Aghadavod E, Mirhosseini N, Asemi Z. The effects of microRNAs in activating neovascularization pathways in diabetic retinopathy. *J. Cell. Biochem.*, **120**, 9514–9521 (2019).
47) Quinn SR, O’Neill LA. The Role of microRNAs in the Control and Mechanism of Action of IL-10 BT - Interleukin-10 in Health and Disease. *Interleukin-10 Heal. Dis.*, **380**, 145–155 (2014).
48) Sierra S, Lara-Villoslada F, Sempere L, Olivares M, Boza J, Xaus J. Intestinal and immunological effects of daily oral administration of Lactobacillus salivarius CECT5713 to healthy adults. *Anaerobe*, **16**, 195–200 (2010).
49) Gardlik R, Palffy R, Celec P. Recombinant probiotic therapy in experimental colitis in mice. *Folia Biol. Czech Repub.*, **58**, 238–245 (2012).
50) O’Farrell AM, Liu Y, Moore KW, Mui ALF. IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: evidence for Stat3-dependent and -independent pathways. *EMBO J.*, **17**, 1006–1018 (1998).
51) Murray PJ. The primary mechanism of the IL-10-regulated antiinflammatory response is to selectively inhibit transcription. *Proc. Natl. Acad. Sci. USA*, **102**, 8686–8691 (2005).
52) Ip WKE, Hoshi N, Shouval DS, Snapper S, Medzhitov R. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. *Science (80-.).*, **356**, 513–519 (2017).
53) Zhao Y, Zeng Y, Zeng D, Wang H, Zhou M, Sun N, Xin J, Khalique A, Rajput DS, Pan K, Shu G, Jing B, Ni X. Probiotics and MicroRNA: Their Roles in the Host–Microbe Interactions. *Front. Microbiol.*, **11**,

1–12 (2021).
54) Wang C, You Q, Cao X, Guo H, Gao X, Peng X. Micro RNA-19a suppresses interleukin-10 in peripheral B cells of patients with diabetic retinopathy. *Am. J. Transl. Res.*, **9**, 1410–1417 (2017).
55) Molina-Tijeras JA, Gálvez J, Rodríguez-Cabezas ME. The immunomodulatory properties of extracellular vesicles derived from probiotics: A novel approach for the management of gastrointestinal diseases. *Nutrients*, **11**, (2019).
56) Doron S, Snydman DR. Risk and safety of probiotics. *Clin. Infect. Dis.*, **60**, S129–S134 (2015).