# *Lactobacillus acidophilus* KBL409 reduced kidney fibrosis via immune modulatory effects in mice with chronic kidney disease

Hyoungnae Kim

Department of Medicine

The Graduate School, Yonsei University

# *Lactobacillus acidophilus* KBL409 reduced kidney fibrosis via immune modulatory effects in mice with chronic kidney disease

Hyoungnae Kim

Department of Medicine

The Graduate School, Yonsei University

# *Lactobacillus acidophilus* KBL409 reduced kidney fibrosis via immune modulatory effects in mice with chronic kidney disease

Directed by Professor Seung Hyeok Han

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Hyoungnae Kim

December 2020

This certifies that the Doctoral Dissertation of Hyoungnae Kim is approved.

---
Thesis Supervisor : Seung Hyeok Han

---
Thesis Committee Member#1: Jung Tak Park

---
Thesis Committee Member#2: Beom Jin Lim

---
Thesis Committee Member#3: Jung Pyo Lee

---
Thesis Committee Member#4: Hyung Jung Oh

The Graduate School
Yonsei University

December 2020

ACKNOWLEDGEMENTS

First of all, I am sincerely grateful to Prof. Seung Hyeok Han who guided me all from start to finish to complete this thesis. His guidance always greatly helps me to improve my research and expand knowledge.

I also thank to Prof. Jung Tak Park, Prof. Beom Jin Lim, Prof. Jung Pyo Lee, and Prof. Hyung Jung Oh, who gave me generous advice to finish my thesis. I also appreciate to Dr. Bo Young Nam and Jimin Park. I can't imagine that I could finish this research without their help. I'd like to thank to Prof. Shin-Wook Kang for being my mentor and having guided me to become a nephrologist.

Last but not least, I'm deeply thankful to my family members. I'm grateful to my parents for supporting my whole life until I become a doctor with finishing this research. I'm also thank to my parents-in-law who give loves to my sons and always support me. Finally, I dedicate this paper to my beloved wife and two sons who always give me happiness.

# <TABLE OF CONTENTS>

# LIST OF FIGURES

# LIST OF TABLES

ABSTRACT

## *Lactobacillus acidophilus* KBL409 reduces kidney fibrosis via immune modulatory effects in mice with chronic kidney disease

Hyoungnae Kim

*Department of Medicine*
*The Graduate School, Yonsei University*

(Directed by Professor Seung Hyeok Han)

**Background:** Intestinal dysbiosis has recently been reported to play an important role in the pathogenesis of various diseases, including chronic kidney disease (CKD). Here, I aimed to evaluate whether probiotic supplements can have protective effects against kidney injury in an animal model of CKD.

**Methods:** Among the candidate strains, *Lactobacillus acidophilus* KBL409 was selected because it can decompose p-cresol *in vitro* and *in vivo*. An animal model of CKD was established by feeding C57BL/6 mice a diet containing 0.2% adenine. These model mice were administered KBL409 at a dose of $1 \times 10^9$ CFU daily for 4 weeks. To clarify the mechanisms underlying the kidney-protecting effects of probiotics *in vitro*, primary mouse tubular epithelial cells (TECs) pretreated with p-cresyl sulfate were treated with butyrate and acetate, two short-chain fatty acids that are the end products of commensal bacteria.

**Results:** CKD model mice showed prominent kidney fibrosis, higher expression levels of profibrotic markers, and higher levels of serum creatinine and albuminuria. Administration of KBL409 significantly improved structural alterations and kidney function and decreased albuminuria. CKD mice also exhibited a disrupted intestinal

barrier and elevated levels of systemic inflammation (TNF-α and IL-6) and oxidative stress (8-hydroxy-2'-deoxyguanosine). These changes were attenuated by KBL409. Administration of KBL409 significantly reduced macrophage infiltration in CKD mice and had immunomodulating effects towards anti-inflammatory state in these mice by promoting a switch to the M2 macrophage phenotype and increasing regulatory T cells. Notably, the NLRP3 inflammasome pathway was activated in the kidneys of CKD mice, as evidenced by the increased expression of NLRP3, ASC, IL-18, and IL-1β, which were significantly decreased by administration of KBL409. In addition, TECs treated with p-cresyl sulfate expressed increased levels of NLRP3 inflammasome pathway components and profibrotic markers. These alterations were reversed by the administration of short-chain fatty acids.

**Conclusions:** These results demonstrate that supplementation with the probiotic *Lactobacillus acidophilus* KBL409 has beneficial immunomodulating effects and protects against kidney injury.

---

Key words: probiotics, KBL409, fibrosis, macrophage, chronic kidney disease

# *Lactobacillus acidophilus* KBL409 reduces kidney fibrosis via immune modulatory effects in mice with chronic kidney disease

Hyoungnae Kim

*Department of Medicine*
*The Graduate School, Yonsei University*

(Directed by Professor Seung Hyeok Han)

## I. INTRODUCTION

Chronic kidney disease (CKD) is a global health problem, and more than 10% of the world's population is reported to have CKD.[1,2] In Korea, approximately 8.2% of adults over 20 years old have CKD,[3] and the prevalence of CKD is expected to increase as diabetes, hypertension, and obesity, which are well-known risk factors of CKD, become more prevalent. When CKD progresses to end-stage kidney disease (ESKD), kidney replacement therapies, such as dialysis and transplantation, are unavoidable. Notably, the number of patients who require kidney replacement therapy has been steadily increasing nationwide.[4] These patients carry a high risk of cardiovascular disease and mortality, and experience negative socioeconomic effects, such as high medical expenses and social loss. Therefore, delaying the progression of CKD is an important therapeutic goal.

To date, only a limited number of drugs have been used to block the progression of CKD. Renin-angiotensin-aldosterone system (RAAS) inhibitors have been extensively studied and are widely accepted as the cornerstone of treatment to slow disease

progression and reduce proteinuria in patients with CKD.[5,6] Sodium glucose cotransporter 2 (SGLT2) inhibitors are another potential drug, as interventional studies have consistently shown that the use of SGLT2 inhibitors resulted in fewer adverse kidney outcomes and a slower decline in estimated glomerular filtration rate in patients with type 2 diabetes.[7] Despite the use of these drugs, a substantial proportion of patients continue to progress. As kidney function declines, uremic solutes accumulate in the body and exert adverse biological effects. These compounds, known as uremic toxins, are associated with increased systemic inflammation, oxidative stress, and endothelial injury, all of which are associated with a high risk of cardiovascular disease.[8,9] Because some uremic toxins are nitrogen waste products that are produced via degradation of proteins by intestinal bacteria, an absorbent known as AST-120 has been used in clinical practice to promote the excretion of uremic toxins in feces. However, notwithstanding the potential benefits shown in animal studies,[10,11] AST-120 can cause indigestion and other gastrointestinal problems, and its effectiveness has been questioned because in clinical trials, this absorbent failed to attenuate the decline kidney function or to reduce proteinuria and mortality in CKD patients.[12,13] In fact, many drugs have been tested for these purposes,[14-16] but none have yielded any beneficial effects.

It has been suggested that dysregulation of the gut microbiota, so called dysbiosis, is involved in many chronic diseases, such as obesity, type 2 diabetes mellitus, inflammatory bowel disease, and cardiovascular disease.[17] The intestinal microbiota has recently gained much attention in CKD research because dysbiosis has been shown to be associated with many uremic complications and even the progression of CKD.[18-20] A previous analysis of fecal samples from ESKD patients revealed significant

expansion of intestinal bacterial families that produce uremic toxins such as indoxyl sulfate and p-cresyl sulfate.[21] Alteration of the gut microbial composition in CKD contributes to increased levels of uremic toxins in the intestine, consequently resulting in increased levels of these toxins in the circulation.[22] This phenomenon can be mechanistically explained by disruption of the epithelial barrier in the gastrointestinal tract, which is known as a "leaky gut." [23] Importantly, not only uremic toxins but also microbes and endotoxin can cross the intestinal barrier and are recognized by families of pattern recognition receptors, such as membrane-bound Toll-like receptors and cytoplasmic NOD-like receptors. In turn, these lead to the activation and assembly of the NLRP3 inflammasome, which can induce a local immune response and fibrosis in the kidney.[24] Moreover, an important detrimental feature of the gut microbiota in patients with CKD is the reduction in bacterial species producing short-chain fatty acids (SCFAs).[21,22] SCFAs exert their effects by activating G-protein coupled receptors (GPCRs) and inhibiting histone deacetylase. Through these mechanisms, SCFAs can modulate the recruitment of leukocytes and the production of chemokines and inflammatory cytokines.[25] SCFAs also preserve the function of the intestinal barrier by inhibiting the NLRP3 inflammasome pathway.[26,27] Thus, depletion of SCFAs in CKD can lead to pro-inflammatory conditions and exacerbate a leaky gut, creating a vicious cycle of systemic inflammation.

Accordingly, many investigators have attempted to test pre- and probiotics to modulate the gut microbiota, which have shown promising results in most preclinical studies. In fact, previous experimental studies have shown that probiotic administration attenuates kidney injury by reducing both gut and systemic inflammation, supporting

the concept of a "leaky gut-inflammation" axis.[28-30] However, which components of the gut microbiota preferentially influence kidney disease is unknown, and few studies have examined the kidney-specific effects of probiotic strains. With this background in mind, my collaborators screened several gut microbiome species and found that KBL409, a strain of *Lactobacillus acidophilus*, specifically scavenged p-cresol, a representative uremic toxin seen in kidney failure. Therefore, this study aimed to investigate whether supplementation with probiotics containing KBL409 can ameliorate kidney damage in an animal model of CKD and explore the molecular mechanisms responsible for the observed beneficial effects. I particularly focused on the effect of *Lactobacillus acidophilus* KBL409 on both renal and systemic immunomodulation, given the significant association between the gut and inflammation.

## II. MATERIALS AND METHODS

### 1. Selection of probiotics

My collaborators first screened probiotics that can decompose p-cresol. Among the tested probiotics, 67 human-derived *Lactobacillus* and *Lactococcus* strains and 33 *Bifidobacterium* strains were evaluated. p-Cresol decomposition was confirmed by measuring the concentration of p-cresol remaining after culturing the strains in MRS medium containing p-cresol. The strains were cultured for 36 hr in MRS medium containing 200 μM p-cresol. Then, concentrated sulfuric acid (2.5 μL) was added to each sample (50 μL) and heated at 90°C for 30 min. An internal standard (0.2 mg/mL 2,6-dimethylphenol [2.5 μL]; Sigma-Aldrich, St. Louis, MO, USA) and ethyl acetate

(50 μL) were added and vortexed for 1 min. After centrifugation at 15,000 rpm for 2 min, the supernatant was transferred to a GC vial (with a 250 μL glass insert), and the concentration of p-cresol in the supernatant was measured by gas chromatography (GC-EI-MS, Agilent 5985; Agilent Technologies, Santa Clara, CA, USA). GC was carried out by increasing the oven temperature from 75°C to 150°C, at a rate of 20°C/min, and then to 250°C, at a rate of 25°C/min, at a flow rate of 1.3 mL/min (post-run: 75°C, 3 min). A DB-5 capillary column (30 m × 0.25 mm, df = 0.25; Agilent Technologies) was used for the analysis. Probiotic strains that decomposed p-cresol in the *in vitro* analysis were selected. These strains were administered to CKD animal models, and the changes in serum p-cresol levels were measured by GC.

## 2. Animal model

For the animal study, a CKD model was established by feeding adenine, which has been widely used as an experimental model of CKD.[31] Six-week-old C57BL/6J mice were housed under a 12 hr light-dark cycle with free access to water and chow. The control group was fed a normal diet, whereas the CKD group was fed a 0.2% adenine-containing diet. The probiotic *Lactobacillus acidophilus* KBL409 was administered via oral gavage at a dose of $1 \times 10^9$ colony-forming units (CFU) once daily. Mice were divided into four groups: control + phosphate-buffered saline (PBS), control + KBL409, CKD + PBS, and CKD + KBL409. After four weeks, the mice were sacrificed. All animal experiments were conducted following a protocol approved by the Committee for the Care and Use of Laboratory Animals of Yonsei University

College of Medicine.

### 3. Primary culture of renal tubular epithelial cells

Renal tubular epithelial cells (TECs) were isolated from C57BL/6J mice. After extracting the kidneys from mice, the adjacent adrenal gland and ureter were removed, and the kidney capsule was peeled off. Then, the kidneys were dissected, placed in 1 mL of ice-cold Dulbecco's PBS (DPBS), and minced into pieces (~1 $mm^3$). The pieces were digested by incubation with collagenase in DPBS and centrifuged at 180 rpm for 30 min at 37°C. The supernatant was sieved through a 100-μm nylon mesh and centrifuged for 10 min at 3,000 rpm. The pellet was resuspended in red blood cell lysis buffer (8.26 g of $NH_4Cl$, 1 g of $KHCO_3$, and 0.037 g of EDTA per liter of double-distilled $H_2O$) and incubated at 4°C for 3 min. After repeated centrifugation at 3,000 rpm for 10 min, the supernatants were discarded. Cells were washed twice with DPBS and seeded in culture dishes. TECs were cultured in RPMI 1640 containing 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin G, 2.5 μg/mL amphotericin B, and 20 ng/mL epidermal growth factor (all from Sigma-Aldrich, St. Louis, MO, USA). To examine the protective effect of SCFAs under uremic conditions, primary TECs were cultured in medium with or without p-cresyl sulfate (0.5 mM, Sigma-Aldrich, St. Louis, MO, USA) and two SCFAs (acetate at 5, 10, and 20 mM and butyrate at 1, 5, and 10 mM, both from Sigma-Aldrich, St. Louis, MO, USA). TECs were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ and harvested after 48 hr of stimulation.

### 4. Total RNA extraction

Whole kidney samples were rapidly frozen in liquid nitrogen and homogenized using a mortar and pestle thrice with 700 μL of RNAiso reagent (Takara Bio Inc., Otsu, Japan). For TECs, 700 μL of RNAiso was added to the cell culture dish, and the cell suspension was collected and homogenized for 5 min at room temperature. Then, 160 μL of chloroform was added to the homogenized samples of kidney tissue or cells. Next, the mixture was shaken vigorously for 30 sec, incubated for 3 min at room temperature, and centrifuged at 12,000 rpm for 15 min at 4°C. The aqueous phase, located at the top of the three phases, was transferred to a fresh tube while carefully avoiding contamination with the other phases. The extracted RNA was precipitated with 400 μL of isopropanol and centrifuged at 12,000 rpm for 30 min at 4°C. The RNA pellet was washed with 70% ethanol, air-dried for 2 min, and dissolved in sterile DEPC-treated distilled water. The quantity and quality of the extracted RNA were assessed by spectrophotometric measurements at wavelengths of 260 and 280 nm.

### 5. Reverse transcription

A Takara cDNA synthesis kit (Takara Bio Inc., Otsu, Japan) was used to obtain first-strand cDNA. The obtained RNA extracts (containing 2 μg of RNA) were reverse transcribed with 10 μM random hexanucleotide primers, 1 mM dNTP, 8 mM $MgCl_2$, 30 mM KCl, 50 mM Tris-HCl, pH 8.5, 0.2 mM dithiothreitol, 25 U of RNase inhibitor, and 40 U of PrimeScript reverse transcriptase. The mixture was incubated for 10 min

at 30°C and for 1 hr at 42°C, and then incubated for 5 min at 99°C to inactivate the enzyme.

### 6. Quantitative real-time polymerase chain reaction (PCR)

Total RNA from mouse kidneys was prepared using a commercial kit. The quality of the obtained RNA was examined by separation on agarose gels, and the quantity was measured using a NanoDrop. Total RNA (1 μg) was reverse transcribed using a cDNA kit. Then, quantitative real-time PCR was performed in a total volume of 20 μL, containing 10 μL of SYBR Green PCR master mix (Applied Biosystems, Foster City, CA, USA), 5 μL of cDNA, and 5 pM sense and antisense primers. The primer concentrations were determined in preliminary experiments designed to find the optimal concentrations of each primer. The PCR conditions were as follows: 35 cycles of denaturation for 30 min at 94.5°C, annealing for 30 sec at 60°C, and extension for 1 min at 72°C with an initial heating step for 9 min at 95°C and a final extension for 7 min at 72°C. The sequences of the primers used in this study are shown in Table 1. Each sample was run in triplicate, and a control without cDNA was included with each assay. After real-time PCR, the temperature was increased from 60°C to 95°C at a rate of 2°C/min to construct a melting curve. The cDNA content of each specimen was determined using the comparative CT method ($2^{-\Delta\Delta CT}$). The results were determined as the relative expression normalized to that of 18s rRNA and are expressed in arbitrary units. Using real-time PCR, the transcript levels of genes related to fibrosis (*Col1a1*, *Fn1*, and *Acta2*), macrophage infiltration (*Adgre1* and *Cd68*), monocyte chemoattractant protein-1 (MCP-1; *Ccl2*), M1 macrophages

(*Ifng* and *Ccl5*), M2 macrophages (*Egr2* and *Retnla*), macrophage chemokines (*Ccr2* and *Cx3cr1*), M2 polarization factors (*Csf1*, *Csf2*, and *Ntn1*), inflammation suppression proteins secreted by M2 macrophages (*Lcn2*, *Arg1*, *Retnlb*, and *Socs3*), the NLRP3 inflammasome pathway (*Tlr4*, *Nlrp3*, *Asc*, *Il18*, and *Il1b*), and colonic tight junctions (*Tjp1*, *Ocln*, *Cldn1*, and *Cldn2*) were assessed. All primers were obtained from Applied Biosystems (Foster City, CA, USA).

**Table 1. Primer sequences**

| Mouse Gene | | Sequence (5'→ 3') |
|---|---|---|
| *Col1a1* | Forward | GCC AAG AAG ACA TCC CTG AA |
| | Reverse | GTT TCC ACG TCT CAC CAT TG |
| *Fn1* | Forward | TGA CAA CTG CCG TAG ACC TGG |
| | Reverse | TAC TGG TTG TAG GTG TGG CCG |
| *Acta2* | Forward | CTG ACA GAG GCA CCA CTG AA |
| | Reverse | CAT CTC CAG AGT CCA GCA CA |
| *Adgre1* | Forward | GAG ATT GTG GAA GCA TCC GAG AC |
| | Reverse | GAG ATT GTG GAA GCA TCC GAG AC |
| *Cd68* | Forward | TCT GAT CTT GCT AGG ACC GC |
| | Reverse | CAT CGT GAA GGA TGG CAG GA |
| *Ccl2* | Forward | TTA AAA ACC TGG ATC GGA ACC AA |
| | Reverse | GCA TTA GCT TCA GAT TTA CGG GT |
| *Ifng* | Forward | AAG ACT GTG ATT GCG GGG TT |
| | Reverse | GCA CCA GGT GTC AAG TCT CT |
| *Ccl5* | Forward | CCC TCA CCA TCA TCC TCA CT |
| | Reverse | GAG CAC TTG CTG CTG GTG TA |
| *Egr2* | Forward | TTA ATA GCT GGG CGA GGG GA |
| | Reverse | TGA CTC TCT CCT GCC TGT GA |
| *Retnla* | Forward | CTG CTA CTG GGT GTG CTT GT |
| | Reverse | TGG TCC AGT CAA CGA GTA AGC |
| *Ccr2* | Forward | AGG TCC CTG TCA TGC TTC TG |

| | | |
|---|---|---|
| | Reverse | TCT GGA CCC ATT CCT TCT TG |
| *Cx3cr1* | Forward | TCA CCG TCA TCA GCA TCG AC |
| | Reverse | CGC CCA GAC TAA TGG TGA CA |
| *Csf1* | Forward | CCG AGA GGC TCC AGG AAC T |
| | Reverse | TGG AAA GTT CGG ACA CAG GC |
| *Csf2* | Forward | CAG GGT CTA CGG GGC AAT TT |
| | Reverse | ACA GTC CGT TTC CGG AGT TG |
| *Ntn1* | Forward | TTG CAA AGC CTG TGA TTG CC |
| | Reverse | TTG TCG GCC TTC AGG ATG TG |
| *Tlr4* | Forward | CCT GTA GAG ATG AAT ACC TC |
| | Reverse | TGT GGA AGC CTT CCT GGA TG |
| *Nlrp3* | Forward | CTC CGG TTG GTG CTT AGA CT |
| | Reverse | TCC CAG ACA CTC ATG TTG GC |
| *Asc* | Forward | GCA CAG GCA AGC ACT CAT TG |
| | Reverse | ACG AAC TGC CTG GTA CTG TC |
| *Il1b* | Forward | ATC TCG CAG CAG CAC ATC AA |
| | Reverse | AAG GTC CAC GGG AAA GAC AC |
| *Il18* | Forward | CGG CCA AAG TTG TCT GAT TCC |
| | Reverse | ACT CTT GCG TCA ACT TCA AGG |
| *Tjp1* | Forward | GCC AGA GAA AAG TTG GCA AG |
| | Reverse | TTG GAT ACC ACT GCG CAT AA |
| *Ocln* | Forward | TGG CAA AGT GAA TGG CAA GC |
| | Reverse | GTC AGA GGA ATC TCC TGG GC |
| *Cldn1* | Forward | CCT GCC CCA GTG GAA GAT TTA |
| | Reverse | CTT TGC GAA ACG CAG GAC AT |
| *Cldn2* | Forward | TCC TTT GCT TTT CCT GCT CGC |
| | Reverse | TTG GAG AGC TCC TAG TGG CAA |
| *18s* | Forward | CGC TTC CTT ACC TGG TTG AT |
| | Reverse | GGC CGT GCG TAC TTA GAC AT |

### 7. Western blot analysis

Using western blot analysis, I compared the expression levels of proteins related to fibrosis, macrophages, the NLRP3 inflammasome pathway, and colonic tight junctions. Harvested cultured cells and mouse kidneys were prepared using sodium dodecyl sulfate (SDS) sample buffer [2% SDS, 10 mM Tris-HCl, pH 6.8, 10%

(vol/vol) glycol]. Lysates were centrifuged at 10,000 × *g* for 10 min at 4°C, and the supernatant was stored at -70°C until use. Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Laemmli sample buffer was added to aliquots of the extracts containing 50 μg of protein and were heated for 5 min at 100°C prior to SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred to a Hybond-ECL membrane using a Hoeffer semidry blotting apparatus (Hoeffer Instruments, San Francisco, CA, USA). The membrane was incubated in blocking buffer A (1× TBS, 0.1% Tween 20, and 5% nonfat milk) for 30 min at room temperature, and incubated overnight at 4°C with primary antibodies against the following proteins: fibronectin (DAKO, Carpentaria, CA, USA), type I collagen (Southern Biotech, Birmingham, AL, USA), NLRP3 (AdipoGen Life Sciences, San Diego, CA, USA), ASC (Cell Signaling Technology, Boston, MA, USA), IL-1$\beta$ (Abcam, Cambridge, MA, USA), IL-18 (Abcam, Cambridge, MA, USA), and β-actin (Sigma-Aldrich, St. Louis, MO, USA). Horseradish peroxidase-conjugated anti-goat (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-rabbit (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or anti-mouse (Santa Cruz Biotechnology, Santa Cruz, CA, USA) IgG was used as the secondary antibody. After several washes, membrane was developed with chemiluminescent reagent (ECL, Amersham Life Science, UK). The density of the bands was measured with ImageJ software ver. 1.49 (National Institutes of Health, Bethesda, MD, USA; online at http://rsbweb.nih.gov/ij). The changes in the optical densities of bands of the treated groups relative to the control cells or tissues were analyzed.

## 8. Measurement of intestinal permeability

Intestinal permeability was analyzed using fluorescein isothiocyanate-conjugated dextran 4000 (FITC-dextran; Sigma-Aldrich, St. Louis, MO, USA). At the beginning of the experiment, food and water were withdrawn for 4 hr, and the mice were subjected to oral gavage with FITC-dextran at 60 mg/100 g body weight in a volume of 200–300 μL. After 4 hr, serum was collected and FITC dextran measurements were performed in duplicate by fluorometry (excitation, 490 nm; emission, 530 nm) with a Cytofluor 2300 (Millipore, Burlington, MA, USA). Serial dilutions of FITC-dextran in PBS were used to calculate a standard curve.

## 9. Flow cytometry

To determine the fractions of various immune cell populations, such as regulatory T cells (Tregs), M1/M2 macrophages, and dendritic cells, in the kidney and spleen, flow cytometry was performed using a commercial staining kit (BioLegend, San Diego, CA, USA). Spleens were triturated in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) with sterile syringes, and the resulting cell suspension was filtered through a 40 μm cell strainer (BD Biosciences, Australia). Kidneys were finely minced and incubated in collagenase D (2 mg/mL; Sigma) solution for 30 min at 37°C. A single cell suspension was generated from the kidney tissues by mechanical disruption of the tissues using a 70 μm cell strainer (BD Biosciences, Franklin Lakes, NJ, USA). For flow cytometry, the macrophages were incubated at 4°C for 20 min with Fc Block

(BioLegend, San Diego, CA, USA) and were used for double staining of the cell surface and intracellular activation markers. Cells were incubated for 30 min at 4°C in staining buffer (BioLegend, San Diego, CA, USA) with fluorescence-conjugated mAbs against the major histocompatibility complex cell surface activation molecules CD11c and CD206 for M1 and M2 macrophages ($CD11c^+$ and $CD206^-$, M1 macrophages; $CD11c^-$ and $CD206^+$, M2 macrophages), CD103 and CD11b for dendritic cells, and CD4 and CD25 for Tregs. After Ab staining, cells were evaluated with LSR II using FACS Diva software (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed using FlowJo software (Version 10.2).

**10. Histology and immunohistochemistry**

The kidney samples were fixed in 10% formalin. Paraffin-embedded tissues were sliced into 5 μm-thick sections for periodic acid-Schiff (PAS) and Masson's trichome staining. For immunohistochemical staining, the tissue slices were deparaffinized, hydrated in ethyl alcohol, and washed in tap water. Antigen retrieval was conducted in citrate buffer (pH 6.0) by microwaving for 15 min. The slices were blocked with donkey serum for 60 min at room temperature and incubated overnight at 4°C with anti-F4/80 (1:100), anti-CD68 (1:100), and anti-NLRP3 antibodies (1:100; all from AdipoGen Life Sciences, San Diego, CA, USA). After several washes, the antibodies were visualized using horseradish peroxidase-conjugated secondary antibodies and 3,3-diaminobenzidine with the Dako Envision Kit (Dako, Santa Clara, CA, USA).

### 11. Biochemistry

Changes in renal function were analyzed by measuring serum urea and creatinine levels using a commercial kit. Urea was measured using the blood urea nitrogen (BUN) colorimetric detection kit (Arbor Assays, Ann Arbor, MI, USA), and creatinine was measured using a creatinine assay kit (Abcam, Cambridge, UK). Serum and renal levels of inflammatory markers, such as IL-1β (R&D Systems, Minneapolis, MN, USA), IL-18 (Abcam, Cambridge, UK), IL-6, tumor necrosis factor-α (TNF-α) (Millipore, Burlington, MA, USA), the oxidative stress marker 8-hydroxy2'-deoxyguanosine (8-OHdG) (Abcam, Cambridge, UK), and 24 hr urinary albumin (Exocell, Newtown Square, PA, USA) were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits.

### 12. Statistical analysis

All values were expressed as mean ± standard error. Statistical analysis was performed using SPSS 23.0 (IBM Corp., Chicago, IL, USA). Results were analyzed using the Mann-Whitney U test or Kruskal-Wallis test. P values less than 0.05 were considered statistically significant.

## III. RESULTS

### 1. *Lactobacillus acidophilus* KBL409 decomposes p-cresol

To select probiotics that can efficiently reduce the intestinal concentration of p-

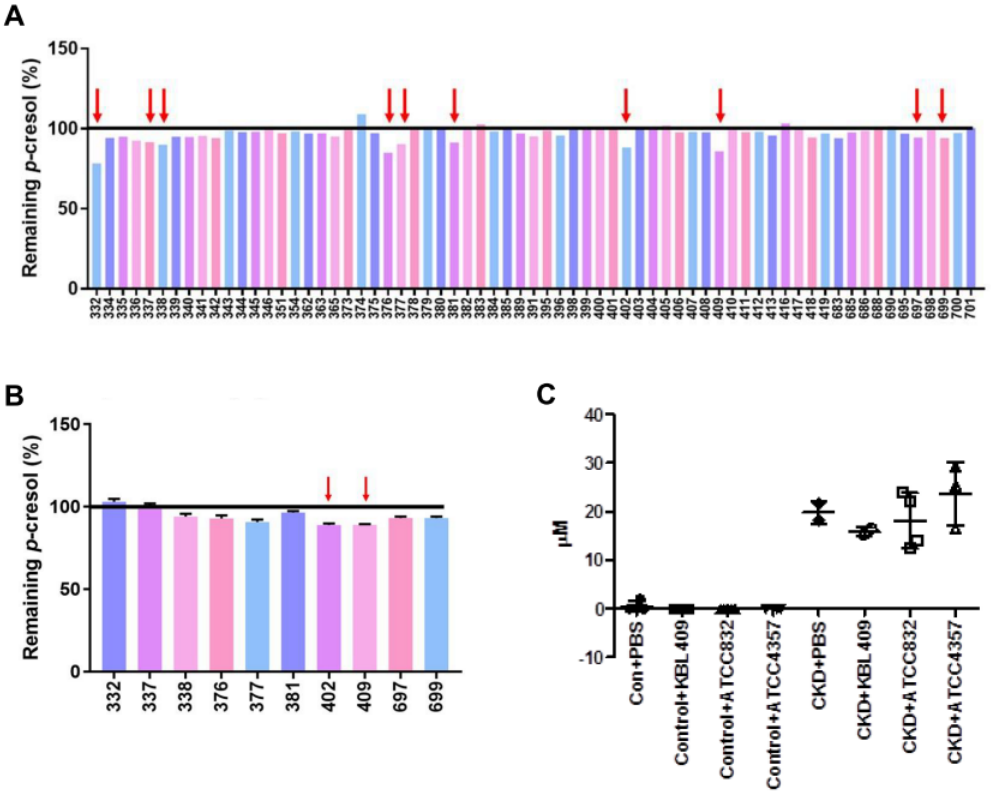


**Figure 1. *Lactobacillus acidophilus* KBL409 decomposes p-cresol.** (A) Remaining percentage of p-cresol concentrations in the cultural media in the first screening analysis. *Lactobacillus* and *Lactococcus* strains were cultured with 200 μM of p-cresol for 36 hr. (B) Remaining percentage of p-cresol concentrations in the cultural media in the second screening analysis. 10 strains that effectively decomposed p-cresol in the first screening were cultured repeatedly. (C) Serum levels of p-cresol after administration of *Lactobacillus acidophilus* to control and adenine-induced CKD mice. Lactobacillus acidophilus KBL409, ATCC832, and ATCC4357 were administered to mice at a dose of $1 \times 10^9$ CFU by oral gavage for 4 weeks.

cresol, my collaborators first screened *Lactobacillus*, *Lactococcus*, and *Bifidobacterium* strains. None of the tested *Bifidobacterium* strains reduced p-cresol levels in the culture medium. In the initial analysis, 10 *Lactobacillus* and *Lactococcus* strains reduced p-cresol levels in the medium (Figure 1A). In repeated analysis of

these 10 strains, *Lactobacillus acidophilus* KBL402 and KBL409 showed the highest levels of p-cresol decomposition (Figure 1B). Because the 16S rDNA sequences of the two strains were nearly identical, KBL409 was selected for *in vivo* analysis. Among CKD mice treated with various other *Lactobacillus acidophilus* strains, including ATCC832 and ATCC4357, mice administered KBL409 showed the lowest serum p-cresol levels (Figure 1C).

## 2. Probiotic supplementation with KBL409 attenuates renal fibrosis in CKD mice

Compared with the controls, CKD model mice fed an adenine-containing diet showed marked alterations in kidney architecture, including severe tubular degeneration, dilation, a widened interstitium, and severe interstitial fibrosis (Figure 2A). These alterations were significantly attenuated by supplementation with KBL409. Masson's trichrome staining showed consistent findings (Figure 2B). The extensive fibrotic area observed in the kidneys of adenine-fed mice was significantly decreased by KBL409 administration. In line with the observed morphologic alterations, quantitative real-time PCR analysis showed that the transcript levels of profibrotic genes, such as *Fn1*, *Col1a1*, and *Acta2*, were significantly increased in adenine-fed mice, and KBL409 supplementation reduced these increased expression levels (Figure 2C). I also confirmed the improvement in fibrosis by western blot analysis (Figure 2D).

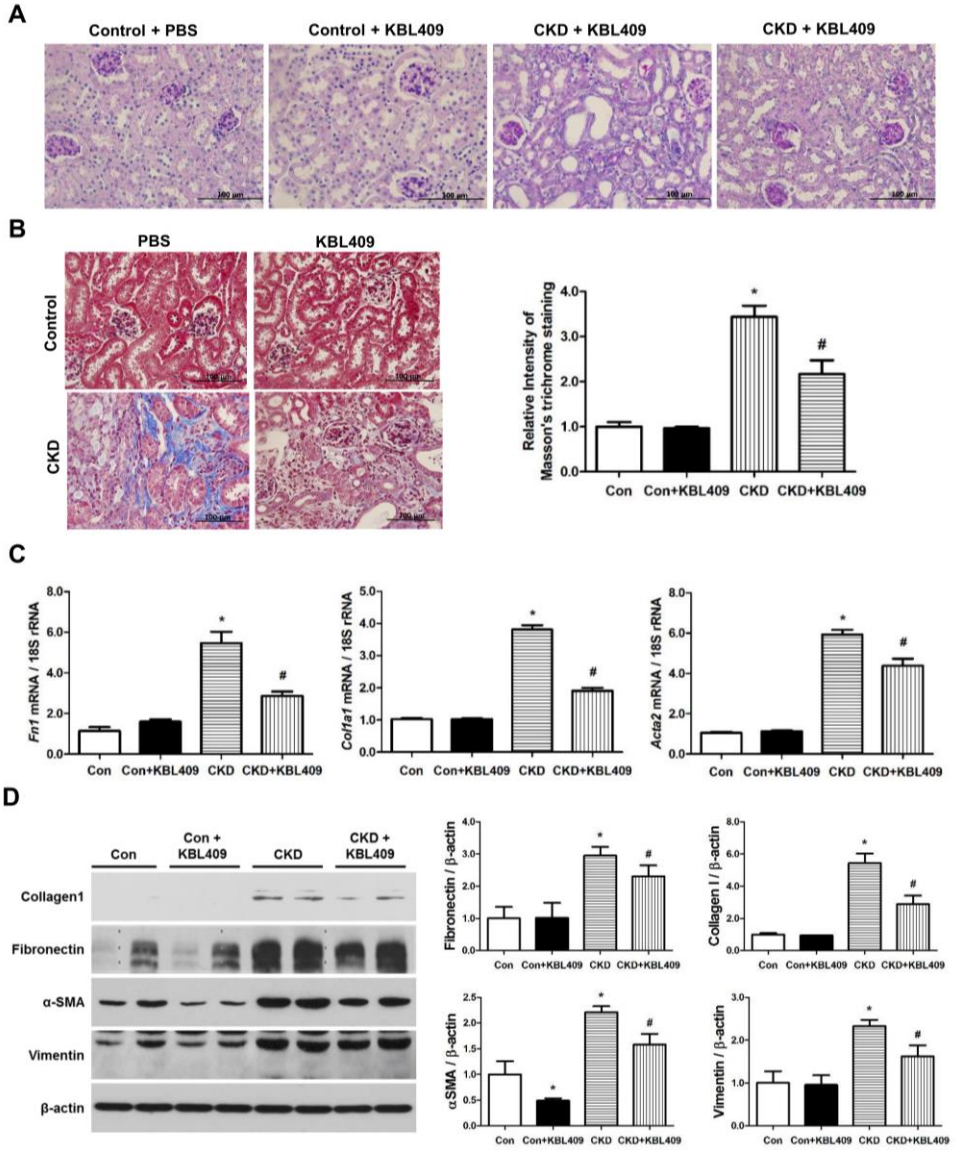


**Figure 2. Probiotic supplementation with KBL409 attenuates renal fibrosis in CKD mice.** Mice were subjected to KBL409 administration and adenine-induced CKD. (A) Representative sections of renal tissue using PAS staining. (B) Representative sections of renal tissue demonstrating renal fibrosis using Masson's trichrome staining. (C) RT-PCR analysis for mRNA expressions of profibrotic markers. (D) Western blot analysis for expressions of profibrotic proteins. *, $P<0.05$ vs. Con; #, $P<0.05$ vs. CKD.

### 3. KBL409 supplementation improves kidney function and reduces albuminuria in CKD mice

The morphological and quantitative improvements in kidney fibrosis were accompanied by functional recovery of kidney injury. In CKD mice fed an adenine-containing diet, serum concentrations of BUN and creatinine were significantly higher than those in the controls. The increases in these markers were significantly decreased by probiotic supplementation with KBL409 (Figure 3A). In addition, urinary albumin excretion was significantly higher in CKD mice than in the controls, and KBL409 administration resulted in decreased albumin excretion (Figure 3B). Based on these findings, it can be concluded that KBL409 decreased kidney fibrosis and improved kidney function in mice with adenine-induced CKD.

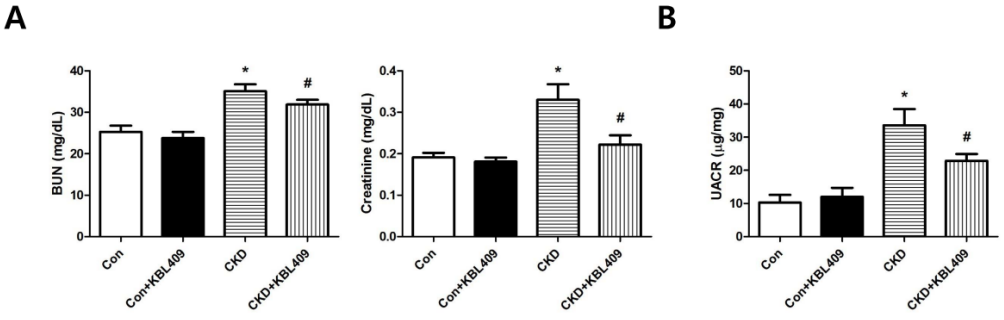


**Figure 3. KBL409 supplementation improves kidney function and reduces albuminuria in CKD mice.** Mice were subjected to KBL409 administration and adenine-induced CKD. (A) Serum BUN and creatinine levels. (B) Amount of 24 hr albuminuria. $^{*}$, $P<0.05$ vs. Con; $^{\#}$, $P<0.05$ vs. CKD.

### 4. The disrupted intestinal barrier in CKD mice is restored by KBL409 supplementation

Because the concept of a “leaky gut-inflammation axis” has been established, I

hypothesized that the beneficial effect of probiotics on kidney function might be attributed to a reduction in inflammation stemming from a leaky gut. Thus, I first examined whether KBL409 could help maintain the intestinal barrier in CKD mice. A FITC-dextran permeability assay showed that the fluorescence intensity of plasma after 4 hr of FITC-dextran oral gavage was stronger in CKD mice than in the controls (Figure 4A). This increased intensity was significantly attenuated in CKD mice administered KBL409. In line with this finding, transcript levels of tight junction genes, such as *Tjp1*, *Ocln*, and *Cldn1*, were significantly downregulated in the colon of CKD mice, and administration of KBL409 reversed the decreases in the expression of levels of these genes (Figure 4B). In contrast, the mRNA expression of Claudin-2, a pore-forming protein that is known to be upregulated in a leaky gut, was significantly increased in the colon of CKD mice, but was attenuated by KBL409. I also confirmed that the changes in the levels of these tight junction proteins were similar to the changes in corresponding gene expression levels (Figure 4C). These findings suggest that *Lactobacillus acidophilus* KBL409 could restore the disrupted intestinal barrier in the setting of kidney failure.

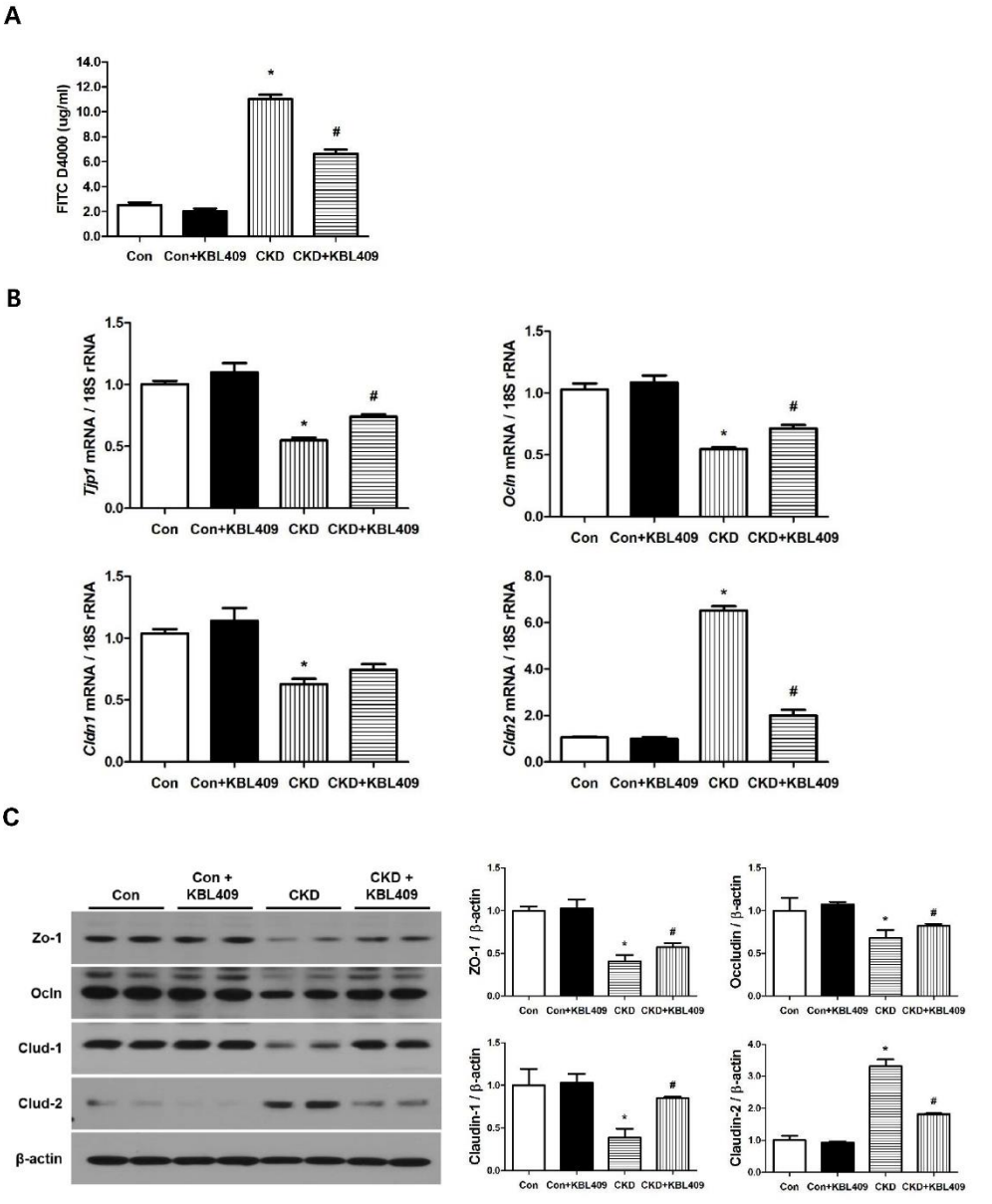


**Figure 4. The disrupted intestinal barrier in CKD mice is restored by KBL409 supplementation.** Mice were subjected to KBL409 administration and adenine-induced CKD. (A) Intestinal permeability measured by plasma fluorescein activity 4 hr after FITC-dextran oral gavage. (B) RT-PCR analysis for mRNA expressions of tight junction proteins. (C) Western blot analysis for expressions of tight junction proteins. *, P<0.05 vs. Con; #, P<0.05 vs. CKD.

### 5. Systemic inflammation and oxidative stress in CKD mice are attenuated by KBL409 supplementation

I further examined whether the protective effect of KBL409 administration on the intestinal barrier could ameliorate systemic inflammation and oxidative stress in CKD model mice. I demonstrated that circulating levels of various proinflammatory markers, including IL-6 and TNF-α, were significantly higher in CKD mice than in the controls. These changes were significantly reduced by supplementation with KBL409 (Figure 5). Moreover, the serum concentration of 8-OHdG, an oxidative stress marker, was increased in CKD mice, but was decreased by KBL409 supplementation.

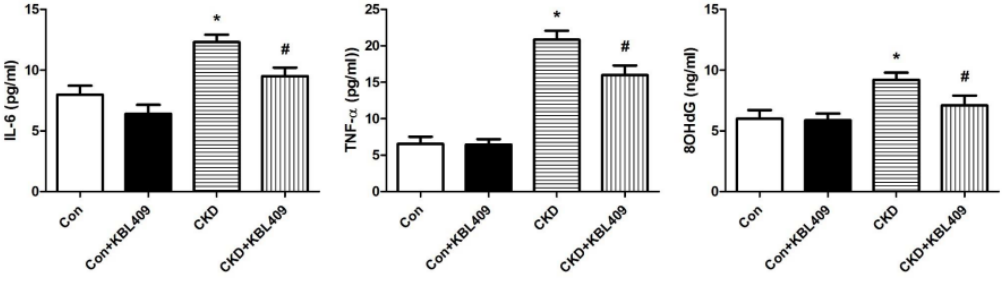


**Figure 5. Systemic inflammation and oxidative stress in CKD mice are attenuated by KBL409 supplementation.** Mice were subjected to KBL409 administration and adenine-induced CKD. ELISA analyses for serum inflammation markers (IL-6 and TNF-α) and oxidative stress marker (8-OHdG). *, $P<0.05$ vs. Con; #, $P<0.05$ vs. CKD.

### 6. KBL409 has a systemic immunomodulating effect

Then, I explored the systemic immunomodulating effect of the KBL409 probiotic. To this end, I used the spleen, because it functions as a central hub for circulating immune cells, such as macrophages, and filters most blood-borne antigens; thus, the

spleen can represent the systemic immune status.[32] Flow cytometry analysis showed that there were increased proportions of splenic dendritic cells ($CD11b^+$ $CD103^+$) and M1 macrophages ($CD11c^+$ $CD206^-$) in adenine-fed CKD mice (Figure 6). KBL409 supplementation resulted in a shift towards an anti-inflammatory state. The proportion of M2 macrophages ($CD11c^-$ $CD206^+$) was increased, while that of dendritic cells ($CD11b^+$ $CD103^+$) was decreased in the spleen by supplementation with KBL409. Moreover, KBL409 supplementation induced $CD4^+$ $CD25^+$ Tregs in the spleen, which was significantly suppressed in CKD mice.

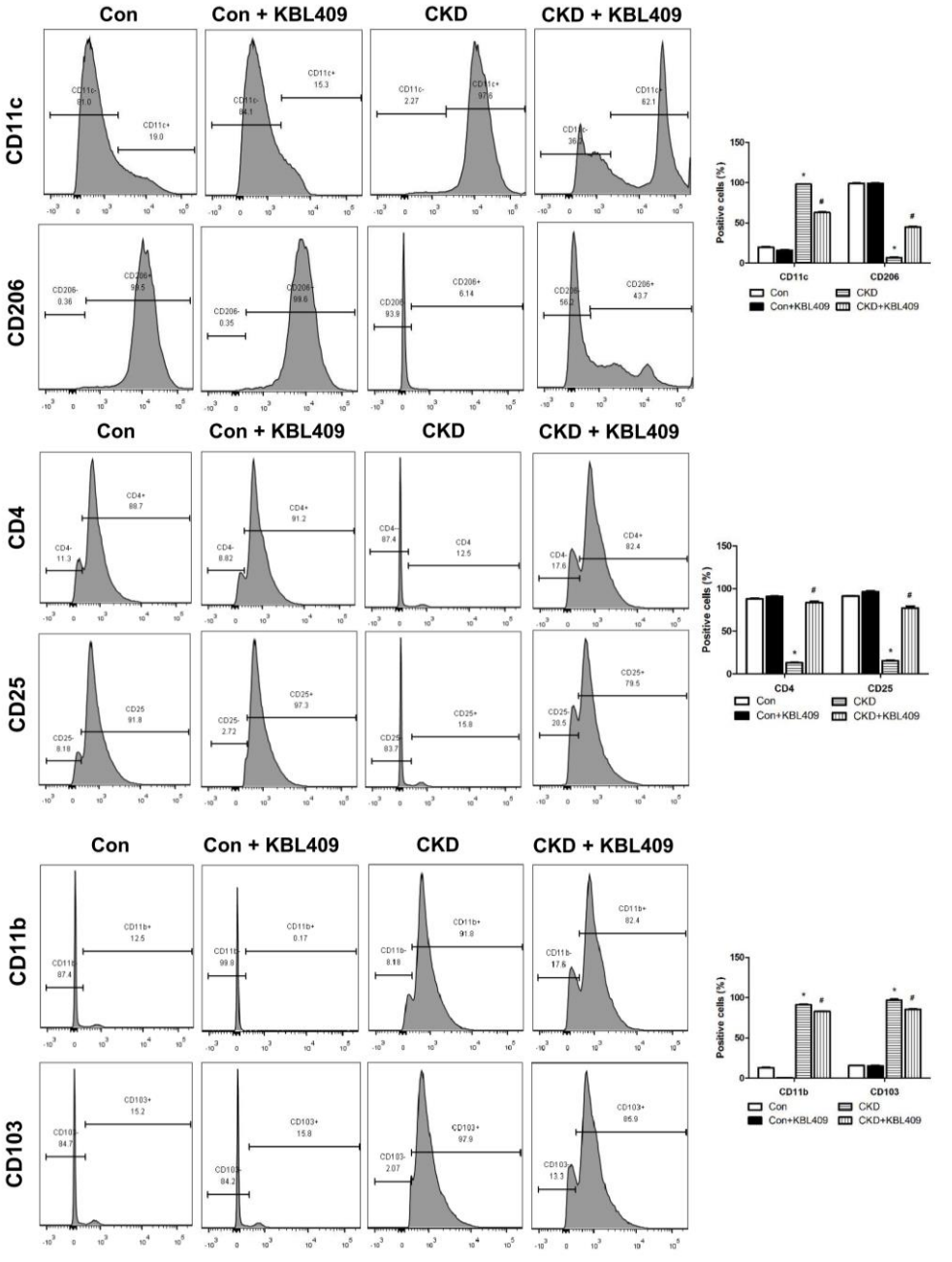

**Figure 6. KBL409 has a systemic immune modulating effect.** Mice were subjected to KBL409 administration and adenine-induced CKD. Splenic flow cytometry analysis for measurement of M1 macrophages ($CD11c^+$ $CD206^-$), M2 macrophages ($CD11c^-$ $CD206^+$), Tregs ($CD4^+$ $CD25^+$), and dendritic cells ($CD11b^+$ $CD103^+$). *, $P<0.05$ vs. Con; #, $P<0.05$ vs. CKD.

### 7. KBL409 modulates the renal immune system by reducing proinflammatory macrophages and dendritic cells and increasing Tregs in CKD mice

Next, I examined whether KBL409 could modulate the renal immune system in CKD mice. In both humans and animals with CKD, macrophages are the predominant infiltrating immune cells in the kidney.[33] In adenine-fed mice, the mRNA and protein levels of macrophage markers, including F4/80 and Cd68, were significantly increased. These expression levels were significantly reduced by KBL409 administration (Figure 7A and B). These changes in macrophage marker expression were parallel with changes in the transcript levels of MCP-1. Immunohistochemistry analysis showed markedly increased numbers of F4/80-positive and CD68-positive cells in the tubulointerstitial space of the kidney in adenine-fed mice (Figure 7C). This accumulation was significantly reduced by KBL409 administration.

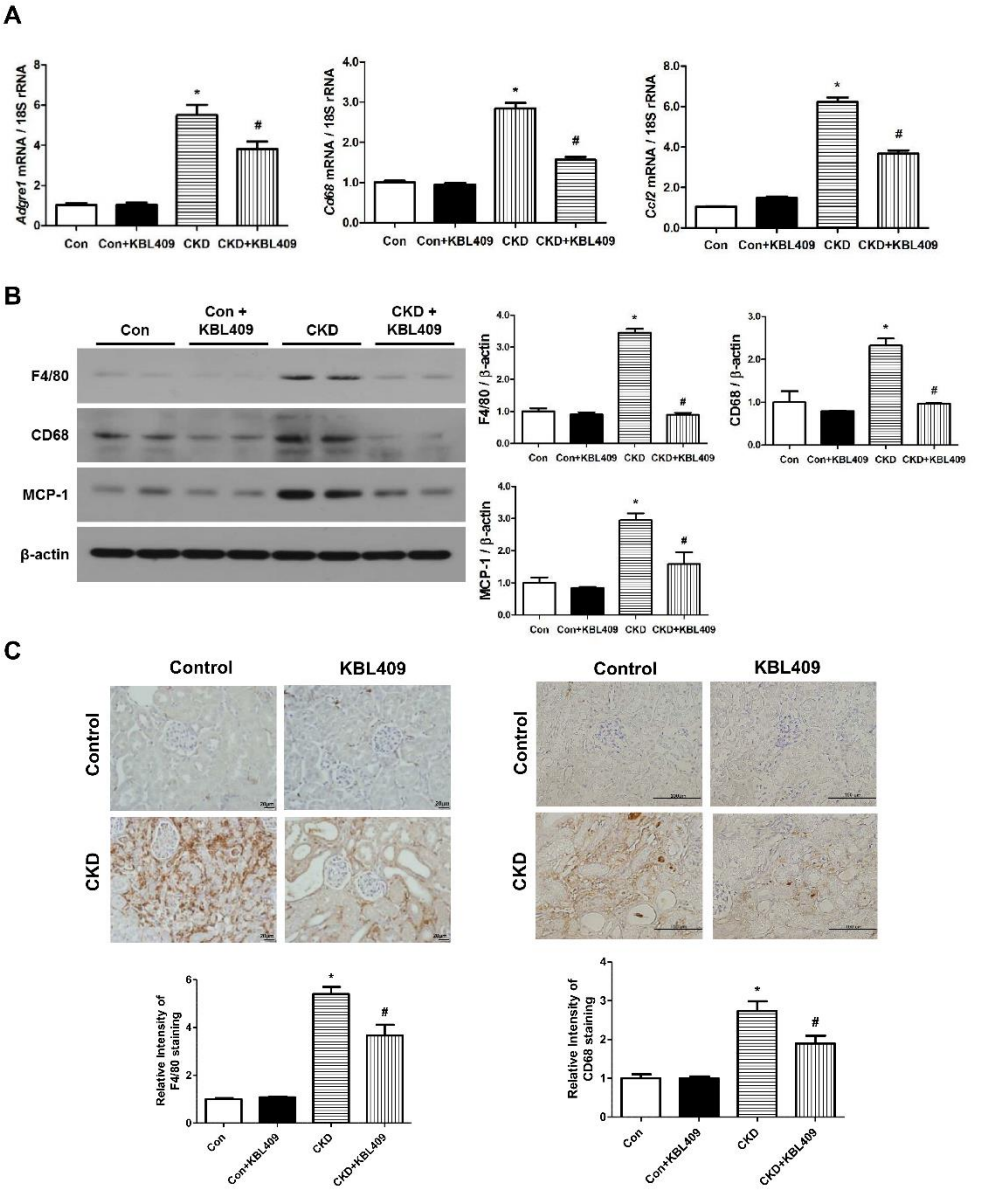


**Figure 7. KBL409 modulates renal immune system by regulating macrophages in CKD mice.** Mice were subjected to KBL409 administration and adenine-induced CKD. (A) RT-PCR analysis for mRNA expressions of macrophage markers. (B) Western blot analysis for protein expressions of macrophage markers. (C) Immunohistochemistry analysis for F4/80-positive and CD68-positive cells in kidney tissue. *, P<0.05 vs. Con; #, P<0.05 vs. CKD.

I further explored whether KBL409 exerts similar immunomodulating effects in the kidney to those observed in the spleen. Real-time PCR analysis showed reciprocal changes in M1/M2 macrophage markers. The mRNA expression levels of the M1 macrophage markers *Ifng* (encoding IFN-γ) and *Ccl5* were increased, whereas the levels of the M2 macrophage markers *Egr2* and *Retnla* were decreased in the kidneys of CKD mice. These changes were reversed by supplementation with KBL409 (Figure 8). Furthermore, the mRNA expression levels of the M2-related chemokines *Ccr2* and *Cx3c1* and polarization factors *Csf1* (encoding M-CSF) and *Ntn1* (encoding Netrin-1) were significantly decreased in the kidneys of CKD mice. Administration of KBL409 reversed the decreased expression of these genes. In addition, the mRNA expression levels of several proteins secreted by M2 macrophages that are related to tubular cell repair and inflammation suppression, including *Lcn2* (encoding Lipocalin-2), *Retnlb* (encoding Fizz1), *Arg1*, and *Socs3*, were also significantly decreased in the kidneys of CKD mice, and these decreases were reversed in CKD mice supplemented with KBL409.

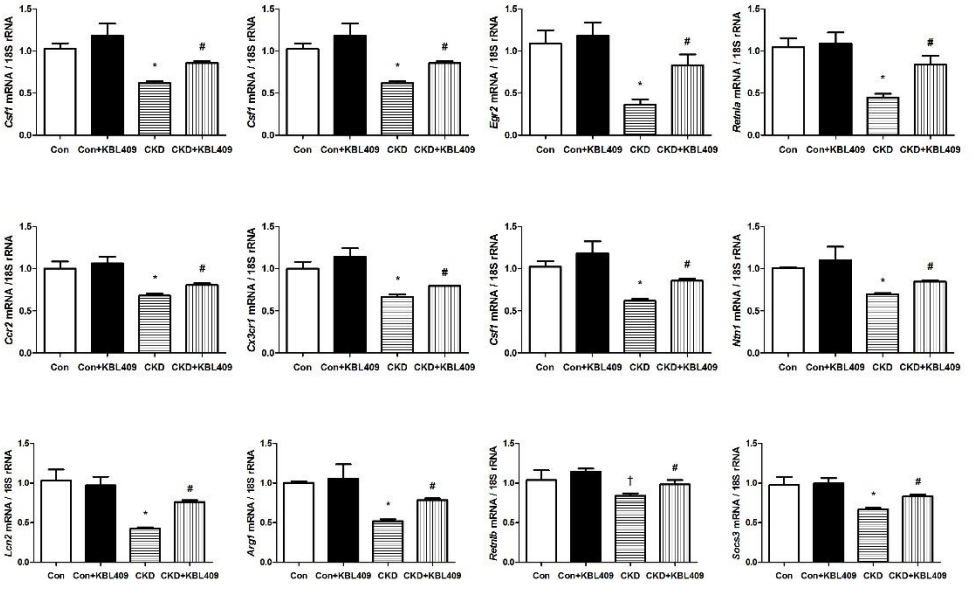


**Figure 8. KBL409 modulates renal macrophages toward M2 polarization in CKD mice.** Mice were subjected to KBL409 administration and adenine-induced CKD. RT-PCR analysis for mRNA expressions of M1 macrophage markers (IFN-γ and CCL5), M2 macrophage markers (Egr2 and Retnla), M2 macrophage chemokines (CCR2 and CX3CR1), M2 macrophage polarization factors (M-CSF and Netrin-1), proteins secreted from M2 macrophage related with tubular cell repair and inflammation suppression (Lipocalin-2, Arg1, Fizz1, SOCS3). *, $P<0.05$ vs. Con; $^{\#}$, $P<0.05$ vs. CKD; $^{\dagger}$, $P<0.05$ vs. Con+KBL409.

Flow cytometry analysis showed that the proportions of proinflammatory cells, such as dendritic cells ($CD11b^{+}$ $CD103^{+}$) and M1 macrophages ($CD11c^{+}$ $CD206^{-}$), were significantly increased in the kidneys of CKD mice (Figure 9). Administration of KBL409 decreased the proportions of these proinflammatory cells, induced macrophage polarization towards the M2 phenotype ($CD11c^{-}$ $CD206^{+}$), and increased $CD4^{+}$ $CD25^{+}$ Tregs in the kidney. Collectively, probiotic supplementation with KBL409 promoted immune modulation in CKD mice towards an anti-inflammatory state in the kidney.

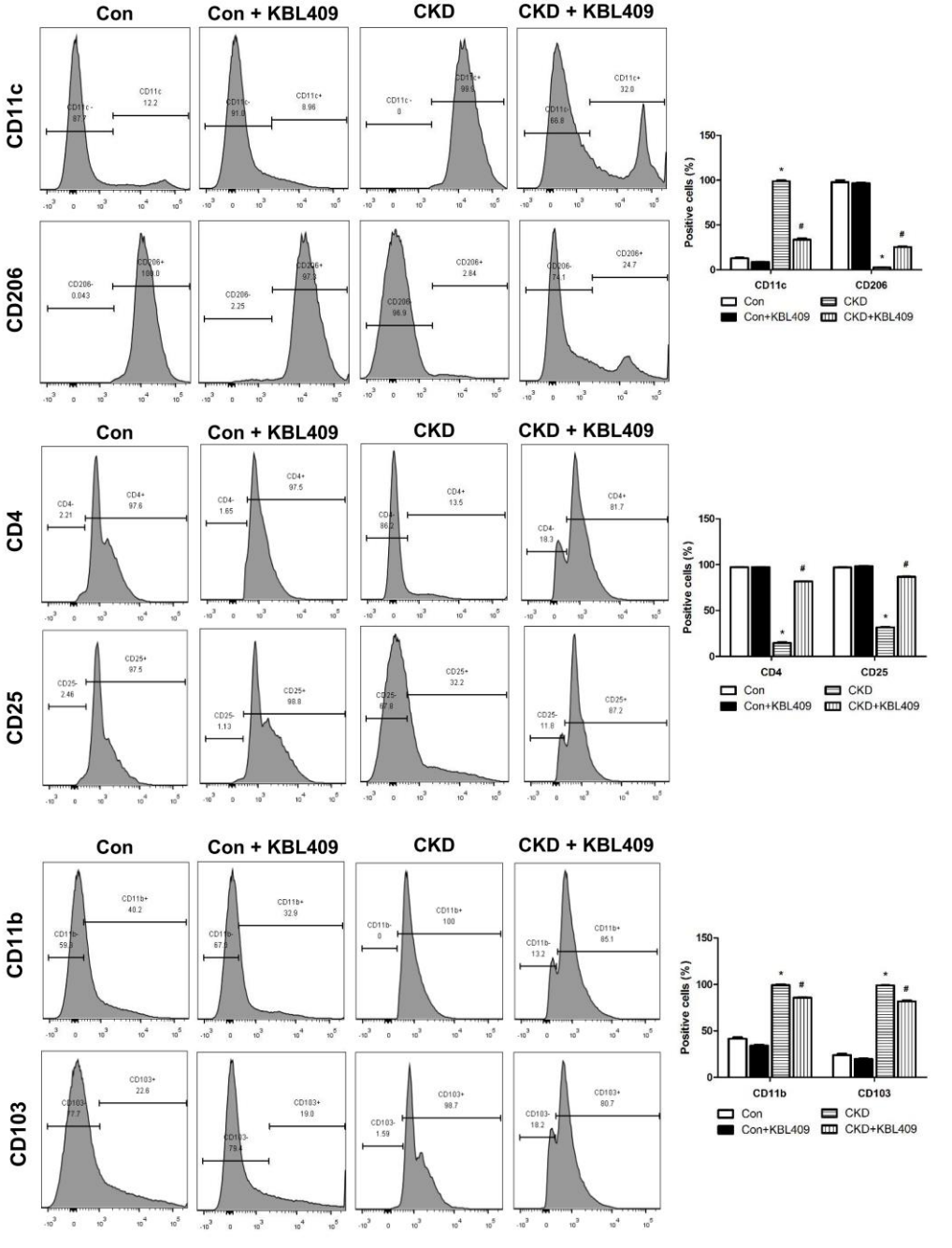

**Figure 9. KBL409 modulates the renal immune system by reducing proinflammatory macrophages and dendritic cells and increasing Tregs in CKD mice.** Mice were subjected to KBL409 administration and adenine-induced CKD. Renal flow cytometry analysis for measurement of M1 macrophages ($CD11c^+$ $CD206^-$), M2 macrophages ($CD11c^-$ $CD206^+$), Tregs ($CD4^+$ $CD25^+$), and dendritic cells ($CD11b^+$ $CD103^+$). *, $P<0.05$ vs. Con; #, $P<0.05$ vs. CKD.

## 8. KBL409 attenuates renal activity of the NLRP3 inflammasome pathway in CKD mice

An influx of endotoxin across the intestinal barrier can activate the NLRP inflammasome.[34] In addition, oxidative stress and inflammation are the main drivers of NLRP3 inflammasome activation.[35] Interestingly, LPS-induced NLRP3 inflammasome activation is altered by M1/M2 macrophage polarization.[36] These findings led me to test whether KBL409 may affect the NLRP3 inflammasome pathway in the kidney by modulating the immune response. I first examined Toll-like receptor 4 (TLR4), which is an upstream pattern recognition receptor that initiates the innate immune response. The mRNA expression of *Tlr4* was upregulated in the kidney of CKD mice when compared with the levels in the controls, and this increased expression was significantly reduced by KBL409 supplementation (Figure 10A). There was a concomitant change in the NLRP3 inflammasome signaling pathway. In CKD mice, the mRNA and protein expression levels of inflammasome components, NLRP3 and ASC and the inflammatory cytokines IL-18 and IL-1β, which are the final products of the NLRP3 inflammasome pathway, were significantly increased in the kidneys. Administration of KBL409 notably decreased the expression levels of these components (Figure 10A-D). This finding was further confirmed by immunohistochemistry analysis. The results showed increased numbers of NLRP3-

positive cells in the kidneys of CKD mice, which were significantly attenuated by KBL409 supplementation (Figure 10E).

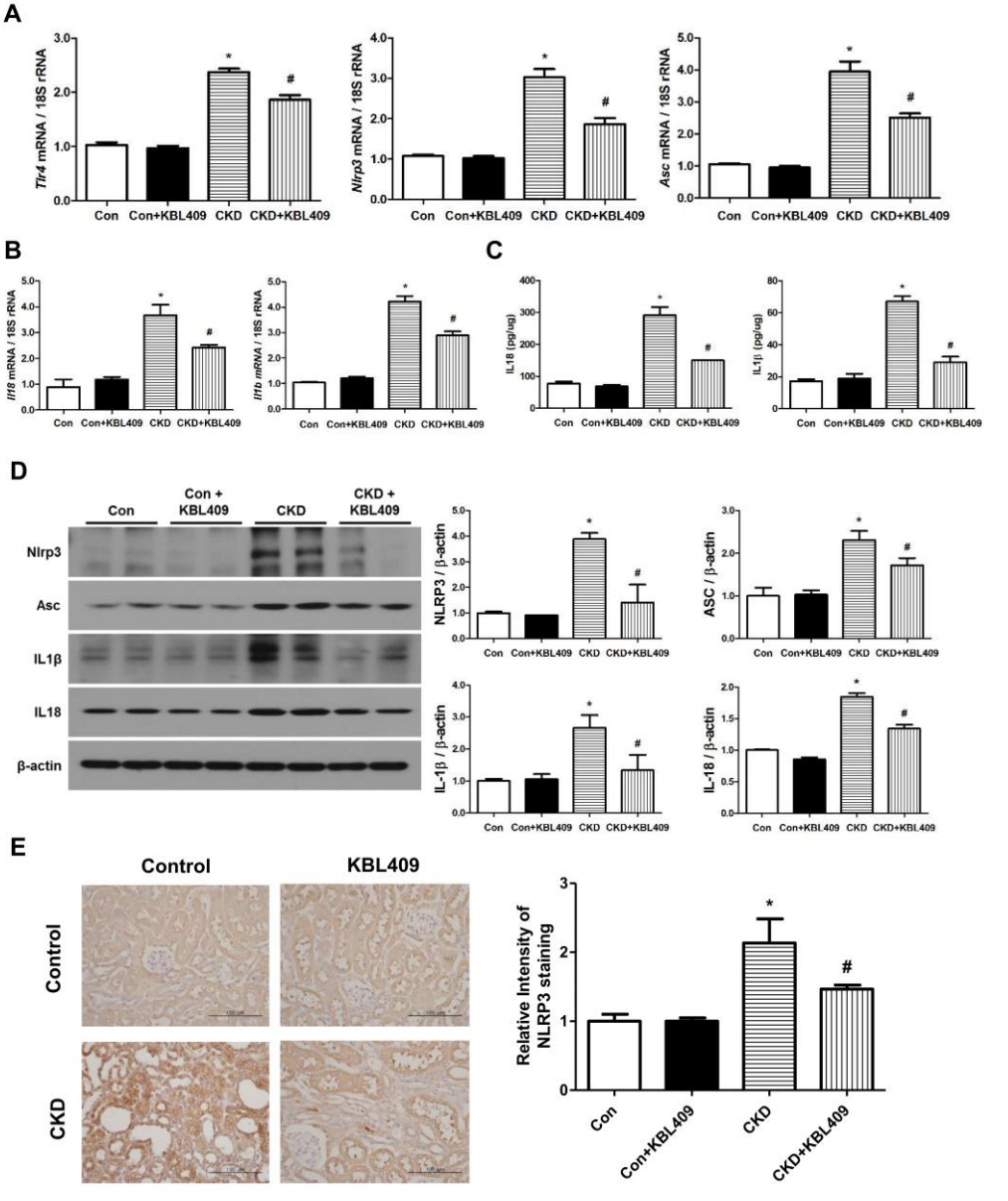

**Figure 10. KBL409 attenuates renal activity of the NLRP3 inflammasome pathway in CKD mice.** Mice were subjected to KBL409 administration and adenine-induced CKD. (A) RT-PCR analysis for mRNA expressions of TLR4 and NLRP3 inflammasome components (NLRP3 and ASC). (B and C) RT-PCR and ELISA analysis for mRNA and protein expressions of final products of NLRP3 inflammasome (IL-18 and IL-1β). (D) Western blot analysis for NLRP3 inflammasome pathway protein expressions. (E) Immunohistochemistry analysis for NLRP3-positive cells in kidney tissue. *, $P<0.05$ vs. Con; #, $P<0.05$ vs. CKD.

## 9. SCFAs have protective effects in TECs exposed to p-cresyl sulfate

It is well known that the beneficial effects of probiotics in the intestine are mediated by the production of SCFAs. Thus, I finally examined whether SCFAs had provide protective effects on TECs under uremic conditions. In primary TECs exposed to p-cresyl sulfate for 48 hr, the mRNA expression levels of two profibrotic markers, type 1 collagen and fibronectin, were significantly higher than those in the controls. However, treatment with butyrate and acetate decreased the expression levels of these markers (Figure 11A). Moreover, the mRNA expression levels of two NLRP3 inflammasome components (NLRP3 and ASC) and two downstream inflammatory cytokines (IL-18 and IL-1β) were also increased in TECs treated with p-cresyl sulfate. There was a significant reduction in these levels after incubation with butyrate and acetate (Figure 11B).

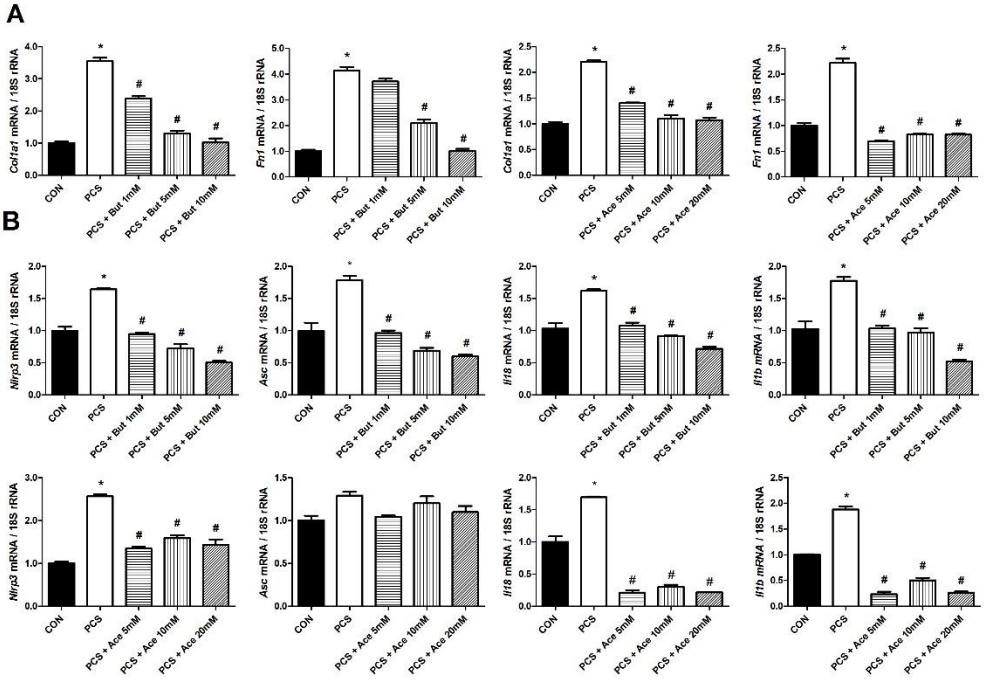


**Figure 11. SCFAs have protective effects in TECs exposed to p-cresyl sulfate.** Primary TECs were stimulated with p-cresyl sulfate (0.5 mM) in the presence of acetate (5mM, 10mM, and 20mM) or butyrate (1mM, 5mM, and 10mM) for 48 hr. (A) RT-PCR analysis for mRNA expressions of profibrotic markers. (B) RT-PCR analysis for mRNA expressions of NLRP3 inflammasome components and final products. *, $P<0.05$ vs. Con; #, $P<0.05$ vs. CKD.

In addition, the mRNA expression levels of M2 macrophage polarization factors, including *Csf1* (encoding M-CSF), *Csf2* (encoding GM-CSF), and *Ntn1* (encoding Netrin-1), were significantly decreased in TECs treated with p-cresyl sulfate (Figure 12). Treated with butyrate and acetate, significantly increased the mRNA expression levels of these polarization factors in both control and p-cresyl sulfate-treated TECs.

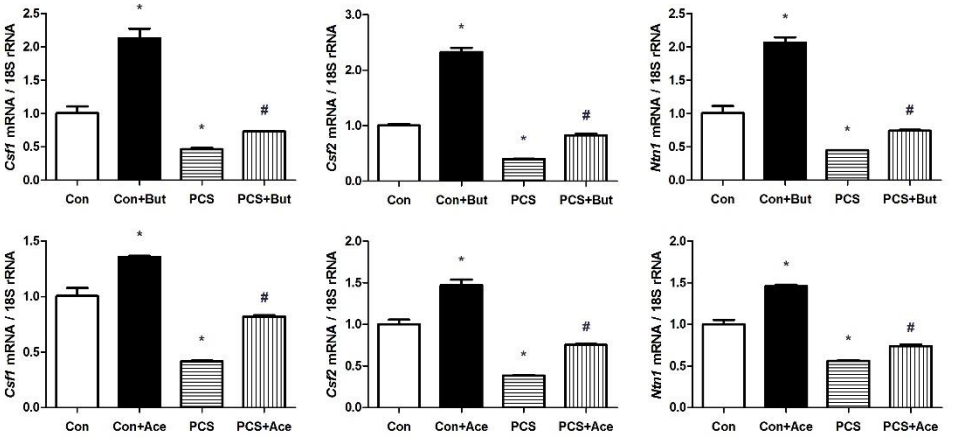


**Figure 12. SCFAs modulates M2 macrophage polarization factors in TECs exposed to p-cresyl sulfate.** Primary TECs were stimulated with p-cresyl sulfate (0.5 mM) in the presence of acetate (20mM) or butyrate (10mM) for 48 hr. RT-PCR analysis for mRNA expressions of M2 macrophage polarization factors (M-CSF, GM-CSF and Netrin-1). *, $P<0.05$ vs. Con; #, $P<0.05$ vs. CKD.

## IV. DISCUSSION

The gut microbiota has recently drawn attention for its important role in the pathogenesis of inflammatory and metabolic diseases.[17] In CKD, there are aberrant alterations in the composition and function of the gut flora, which can be a cause of systemic inflammation and the progression of CKD.[37,38] Despite recent advances in understanding the adverse effects of alterations in the gut microbiota on kidney health, there is still a lack of evidence as to whether restoration of the microbiome with probiotic supplementation can ameliorate kidney injury and delay the progression of CKD. In this study, I demonstrated that KBL409, a strain of *Lactobacilus acidophilus*, was effective for maintaining intestinal integrity and reducing systemic inflammation

and oxidative stress in a murine model of adenine-induced CKD. These favorable effects subsequently polarized immune cells towards an anti-inflammatory phenotype and reduced macrophage infiltration in the kidneys. Moreover, the reduction of inflammatory environment induced by KBL409 supplementation led to decreased NLRP3 inflammasome activity, and ultimately attenuated renal fibrosis. A summary of the potential mechanisms of the protective effects of *Lactobacillus acidophilus* KBL409, which is based on the results of this study, is presented in Figure 13.

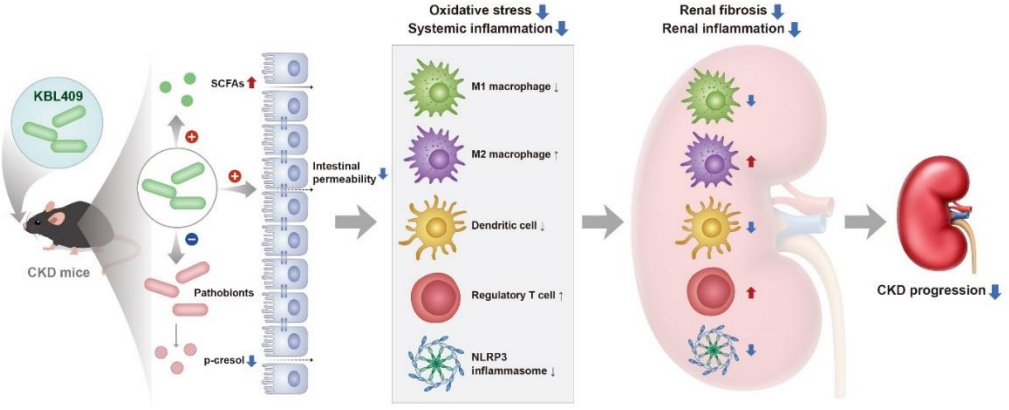


**Figure 13. Graphical presentation for molecular mechanisms responsible for the beneficial effects of KBL409.**

In CKD, homeostasis of the gut microbial populations is disrupted, and the number of healthy symbionts, such as the *Lactobacillaceae*, *Prevotellaceae*, *Ruminococcaceae*, and *Lachnospiraceae* families, are diminished.[22,37] Under these conditions, the gut microbiota is characterized by significant expansion of bacterial families that can produce uremic toxins, including indole and p-cresol.[21] These changes in the microbiome have been reported to be correlated with the presence of altered metabolites

in the serum and feces of patients and animals with CKD.[22] Thus, a substantial proportion of the uremic toxins that accumulated in the body during kidney failure can be explained by dysbiosis. Interestingly, literature reviews of previous clinical studies showed that increased serum levels of uremic toxins are significantly associated with the progression of CKD as well as the development of cardiovascular disease and death in patients with CKD.[39,40] This significant link between a dysregulated gut microbiome and systemic disease provide a rationale for the therapeutic potential of probiotics, with an aim of reducing uremic toxins. Based on the results from my collaborators, I tested the effects of KBL409 because it decomposed p-cresol *in vitro* and reduced p-cresol levels in an adenine-fed CKD model. Under gut dysbiosis, P-cresol is produced by the degradation of tyrosine.[41] It is then absorbed by the intestine and conjugated into p-cresyl sulfate, which induces various adverse effects. In fact, p-cresyl sulfate increases oxidative stress in leukocytes and endothelial cells, which negatively affects vascular remodeling and function.[42,43] In addition, increased production of reactive oxygen species and inflammatory cytokines induced by p-cresyl sulfate can also damage renal tubular epithelial cells.[44] In this study, I clearly demonstrated that increased levels of systemic inflammation and oxidative stress in CKD mice were significantly reduced by supplementation with KBL409. Disruption of the intestinal barrier in the setting of kidney failure can also contribute to the translocation of uremic toxins into the bloodstream and an increase in circulating levels. I also showed that administration of KBL409 restored the expression levels of colonic tight junction proteins and intestinal permeability in CKD mice. In line with the results of the present research, earlier studies also demonstrated that supplementation with *Lactobacillus* species effectively

attenuated the increased intestinal permeability in CKD.[28,45] Together, these findings indicate that the ability of *Lactobacillus acidophilus* KBL409 to decrease the production and influx of uremic toxins from a leaky gut could be responsible for its beneficial effects in CKD mice.

It should be noted that *Lactobacillus acidophilus* KBL409 has immunomodulatory effects, specifically anti-inflammatory properties. Through flow cytometry analysis, I showed that supplementation with KBL409 significantly decreased the proportion of M1 macrophages and dendritic cells and increased the number of M2 macrophages and Tregs in the spleen and kidneys of adenine-fed CKD mice. The beneficial effects of probiotics on the immune system have gained increased attention.[46,47] In fact, some probiotic strains have been shown to induce macrophage phenotype switching from M1 to M2 in various experimental models. In particular, *Lactobacillus acidophilus* LA1 attenuated the severity of colitis by inducing M2 macrophages from peritoneal cavity cells.[48] Other *Lactobacillus* species have also been reported to promote M2 macrophages under different conditions.[49,50] In addition, there is evidence that several *Lactobacillus* strains can increase the activity of Tregs.[47,51] In my study, *Lactobacillus acidophilus* KBL409 had similar systemic effects on the immune system in an animal model of CKD. Notably, I demonstrated that these beneficial effects occurred in the kidney. In the kidneys of adenine-fed CKD mice supplemented with *Lactobacillus acidophilus* KBL409, macrophage infiltration decreased, along with M2 polarization and an increase in the population of Tregs. This finding is particularly intriguing because it suggests that the immunomodulatory effects of this probiotic strain occur in the target organs. More interestingly, renal TECs are important sources of M2

polarization factors, including M-CSF, GM-CSF, and Netrin-1.[52] I clearly showed significant increases in the expression levels of these factors in CKD mice supplemented with KBL409. This finding was further substantiated by my *in vitro* study using renal TECs. Collectively, these results indicate that *Lactobacillus acidophilus* KBL409 may have therapeutic potential for reducing systemic and local inflammation. However, anti-inflammatory M2 macrophages are also known to promote fibrosis by secreting profibrotic factors, such as Galectin-3 and transforming growth factor β.[53] Interestingly, the role of macrophages in renal fibrosis may vary depending on the phase of injury. A previous study reported that systemic macrophage depletion decreased tubular cell apoptosis and fibrosis in the early phase after unilateral ureteral obstruction (UUO).[54] However, in the recovery phase after UUO, depletion of renal macrophages resulted in more severe interstitial fibrosis.[55,56] Thus, there is a phase-dependent balance between the profibrotic and antifibrotic roles of macrophages in the UUO model. In this study, a decrease in renal interstitial fibrosis was accompanied by a decrease in interstitial macrophage infiltration and increased M2 macrophage polarization following administration of KBL409. Therefore, it can be presumed that the macrophages remaining in the recovery phase after supplementation with KBL409 might have an antifibrotic effect. Further studies are needed to determine the effect of M1/M2 macrophage polarization on fibrosis during the various phases of disease the adenine-induced CKD murine model.

Disruption of the intestinal barrier can also promote intestinal translocation of uremic toxins as well as bacterial endotoxins. This endotoxin influx can worsen systemic inflammation in CKD. A previous study by McIntyre et al. reported that circulating

levels of bacterial endotoxins increased as renal function declined and was associated with mortality risk.[57] Endotoxin is transported to the intestinal capillaries and systemic circulation through a TLR4-dependent mechanism.[58] TLR4 is expressed in most cell types, and the TLR4-myeloid differentiation primary-response protein 88 (MyD88)-dependent signaling pathway triggers activation of the innate immune system.[59] TLR4-mediated inflammation also activates the NLRP3 inflammasome pathway. In monocytes, endotoxin directly activates the TLR4-MyD88 signaling pathway and increases the production of IL-1β and IL-18.[34] Endotoxin delivered into the cytosol can activate NLRP3 inflammasome through a caspase-11-mediated pathway.[34] As mentioned above, there is inter-connectivity among inflammation, oxidative stress, macrophage polarization, and the NLRP3 inflammasome.[35,36] Furthermore, previous studies have consistently shown reductions in renal fibrosis and cell death with suppression of the NLRP3 inflammasome in mouse models of kidney disease.[60] Thus, given the significant association of leaky gut and inflammation with the activation of the NLRP3 inflammasome pathway and its contribution to kidney injury,[61] it was tempting to test whether probiotic supplementation could attenuate kidney injury by modulating the NLRP3 inflammasome. In this study, I demonstrated that the expression of renal TLR4, two components of the inflammasome (NLRP3 and ASC), and the final products of the inflammasome (IL-1β and IL-18) were significantly downregulated after administration of KBL409 in CKD mice. Therefore, the renal protective effect of KBL409 was likely partially mediated by suppression of renal NLRP3 inflammasome activation.

Another mechanism responsible for the beneficial effects of probiotics involves the

production of SCFAs, such as butyrate and acetate. As mentioned above, depletion of SCFA-producing bacteria is a key feature of gut dysbiosis in CKD.[21,22] Andrade-Oliveira et al. reported that SCFAs improved kidney dysfunction in an animal model of acute kidney injury by decreasing dendritic cell maturation and inhibiting T cell proliferation.[62] Moreover, increased production of SCFAs through dietary fiber intake protected against kidney damage by reducing inflammatory cytokines and renal fibrosis in a diabetic kidney model.[63] In line with these studies, my *in vitro* study showed that administration of butyrate and acetate significantly decreased the mRNA expression levels of profibrotic markers and NLRP3 inflammasome markers under p-cresyl sulfate stimulation. SCFAs have been reported to stimulate formation of the intestinal barrier following its lipopolysaccharide-induced disruption by inhibiting the NLRP3 inflammasome.[26] Therefore, it can be presumed that the renal protective effects of KBL409 observed in this study could by mediated, at least in part, by the increased production of SCFAs. However, contradictory results have been reported. Macia et al. reported that SCFAs activated the NLRP3 inflammasome to maintain the integrity of the healthy gut epithelium.[64] Yao et al. also showed that hyperactivity of NLRP3 enhanced the anti-inflammatory capacity of the gut by increasing the induction of Tregs in a murine model.[65] This effect was mainly mediated through remodeling of the gut microbiota, including an increased proportion of *Lactobacillus* species. It is possible that SCFAs function differently, depending on the conditions, i.e., healthy or unhealthy. Thus, further studies are needed to clarify the relationships among probiotic supplementation, intestinal integrity, and the NLRP3 inflammasome in CKD models.

Although preclinical studies showed that pre- and probiotic supplementation

recovered dysbiosis and ameliorated systemic and renal inflammation,[66] the results of human clinical trials have been disappointing because they failed to show significant reductions in plasma uremic toxins and inflammatory markers in patients with CKD.[67,68] Therefore, more studies are needed to find optimal species that are specifically helpful under uremic conditions and to clarify the underlying mechanisms by which probiotics exert protective effects in CKD. KBL409 showed significant renal protective effects in our experimental study and could be a candidate probiotic for future clinical trials in CKD patients.

## V. CONCLUSION

Supplementation with *Lactobacillus acidophilus* KBL409 reduced kidney fibrosis and preserved kidney function in an animal model of CKD. These beneficial effects were mediated by maintaining the integrity of the intestinal barrier, reducing systemic inflammation and oxidative stress, modulating immune cells towards an anti-inflammatory phenotype, and attenuating the NLRP3 inflammasome pathway. These findings suggest the therapeutic potential of KBL409 for treating kidney disease, which should be further investigated in clinical trials involving patients with CKD.

## REFERENCES

1. Murphy D, McCulloch CE, Lin F, Banerjee T, Bragg-Gresham JL, Eberhardt MS, et al. Trends in Prevalence of Chronic Kidney Disease in the United States. Ann Intern Med 2016;165:473-81.
2. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis. PLoS One 2016;11:e0158765.
3. Park JI, Baek H, Jung HH. Prevalence of Chronic Kidney Disease in Korea: the Korean National Health and Nutritional Examination Survey 2011-2013. J Korean Med Sci 2016;31:915-23.
4. Jin DC, Han JS. Renal replacement therapy in Korea, 2012. Kidney Res Clin Pract 2014;33:9-18.
5. Mann JF, Gerstein HC, Pogue J, Bosch J, Yusuf S. Renal insufficiency as a predictor of cardiovascular outcomes and the impact of ramipril: the HOPE randomized trial. Ann Intern Med 2001;134:629-36.
6. Ruggenenti P, Perna A, Gherardi G, Garini G, Zoccali C, Salvadori M, et al. Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet 1999;354:359-64.
7. Neuen BL, Young T, Heerspink HJL, Neal B, Perkovic V, Billot L, et al. SGLT2 inhibitors for the prevention of kidney failure in patients with type 2 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol 2019;7:845-54.
8. Cachofeiro V, Goicochea M, de Vinuesa SG, Oubina P, Lahera V, Luno J. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. Kidney Int Suppl 2008:S4-9.
9. Wu J, Xia S, Kalionis B, Wan W, Sun T. The role of oxidative stress and inflammation in cardiovascular aging. Biomed Res Int 2014;2014:615312.

10. Lee SH, Nam BY, Kang EW, Han SH, Li JJ, Kim DH, et al. Effects of an oral adsorbent on oxidative stress and fibronectin expression in experimental diabetic nephropathy. Nephrol Dial Transplant 2010;25:2134-41.
11. Yoshifuji A, Wakino S, Irie J, Matsui A, Hasegawa K, Tokuyama H, et al. Oral adsorbent AST-120 ameliorates gut environment and protects against the progression of renal impairment in CKD rats. Clin Exp Nephrol 2018;22:1069-78.
12. Cha RH, Kang SW, Park CW, Cha DR, Na KY, Kim SG, et al. A Randomized, Controlled Trial of Oral Intestinal Sorbent AST-120 on Renal Function Deterioration in Patients with Advanced Renal Dysfunction. Clin J Am Soc Nephrol 2016;11:559-67.
13. Schulman G, Berl T, Beck GJ, Remuzzi G, Ritz E, Arita K, et al. Randomized Placebo-Controlled EPPIC Trials of AST-120 in CKD. J Am Soc Nephrol 2015;26:1732-46.
14. Voelker J, Berg PH, Sheetz M, Duffin K, Shen T, Moser B, et al. Anti-TGF-β1 Antibody Therapy in Patients with Diabetic Nephropathy. J Am Soc Nephrol 2017;28:953-62.
15. Sharma K, Ix JH, Mathew AV, Cho M, Pflueger A, Dunn SR, et al. Pirfenidone for diabetic nephropathy. J Am Soc Nephrol 2011;22:1144-51.
16. Pergola PE, Raskin P, Toto RD, Meyer CJ, Huff JW, Grossman EB, et al. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. N Engl J Med 2011;365:327-36.
17. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 2015;26:26191.
18. Simenhoff ML, Dunn SR, Zollner GP, Fitzpatrick ME, Emery SM, Sandine WE, et al. Biomodulation of the toxic and nutritional effects of small bowel bacterial overgrowth in end-stage kidney disease using freeze-dried Lactobacillus acidophilus. Miner Electrolyte Metab 1996;22:92-6.

19. Hida M, Aiba Y, Sawamura S, Suzuki N, Satoh T, Koga Y. Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. Nephron 1996;74:349-55.
20. Meijers B, Evenepoel P, Anders HJ. Intestinal microbiome and fitness in kidney disease. Nat Rev Nephrol 2019;15:531-45.
21. Wong J, Piceno YM, DeSantis TZ, Pahl M, Andersen GL, Vaziri ND. Expansion of urease- and uricase-containing, indole- and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. Am J Nephrol 2014;39:230-7.
22. Wang X, Yang S, Li S, Zhao L, Hao Y, Qin J, et al. Aberrant gut microbiota alters host metabolome and impacts renal failure in humans and rodents. Gut 2020;69:2131-42.
23. Vaziri ND, Yuan J, Nazertehrani S, Ni Z, Liu S. Chronic kidney disease causes disruption of gastric and small intestinal epithelial tight junction. Am J Nephrol 2013;38:99-103.
24. Felizardo RJ, Castoldi A, Andrade-Oliveira V, Camara NO. The microbiota and chronic kidney diseases: a double-edged sword. Clin Transl Immunology 2016;5:e86.
25. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients 2011;3:858-76.
26. Feng Y, Wang Y, Wang P, Huang Y, Wang F. Short-Chain Fatty Acids Manifest Stimulative and Protective Effects on Intestinal Barrier Function Through the Inhibition of NLRP3 Inflammasome and Autophagy. Cell Physiol Biochem 2018;49:190-205.
27. Kim MH, Kang SG, Park JH, Yanagisawa M, Kim CH. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. Gastroenterology 2013;145:396-406.

28. Yang J, Lim SY, Ko YS, Lee HY, Oh SW, Kim MG, et al. Intestinal barrier disruption and dysregulated mucosal immunity contribute to kidney fibrosis in chronic kidney disease. Nephrol Dial Transplant 2019;34:419-28.
29. Wei M, Wang Z, Liu H, Jiang H, Wang M, Liang S, et al. Probiotic Bifidobacterium animalis subsp. lactis Bi-07 alleviates bacterial translocation and ameliorates microinflammation in experimental uraemia. Nephrology (Carlton) 2014;19:500-6.
30. Mu Q, Zhang H, Liao X, Lin K, Liu H, Edwards MR, et al. Control of lupus nephritis by changes of gut microbiota. Microbiome 2017;5:73.
31. Jia T, Olauson H, Lindberg K, Amin R, Edvardsson K, Lindholm B, et al. A novel model of adenine-induced tubulointerstitial nephropathy in mice. BMC Nephrol 2013;14:116.
32. Bronte V, Pittet MJ. The spleen in local and systemic regulation of immunity. Immunity 2013;39:806-18.
33. Duffield JS. Macrophages and immunologic inflammation of the kidney. Semin Nephrol 2010;30:234-54.
34. He Y, Hara H, Núñez G. Mechanism and Regulation of NLRP3 Inflammasome Activation. Trends Biochem Sci 2016;41:1012-21.
35. Sharma A, Tate M, Mathew G, Vince JE, Ritchie RH, de Haan JB. Oxidative Stress and NLRP3-Inflammasome Activity as Significant Drivers of Diabetic Cardiovascular Complications: Therapeutic Implications. Front Physiol 2018;9:114.
36. Awad F, Assrawi E, Jumeau C, Georgin-Lavialle S, Cobret L, Duquesnoy P, et al. Impact of human monocyte and macrophage polarization on NLR expression and NLRP3 inflammasome activation. PLoS One 2017;12:e0175336.
37. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, DeSantis TZ, et al. Chronic kidney disease alters intestinal microbial flora. Kidney Int 2013;83:308-15.

38. Jiang S, Xie S, Lv D, Wang P, He H, Zhang T, et al. Alteration of the gut microbiota in Chinese population with chronic kidney disease. Sci Rep 2017;7:2870.
39. Vanholder R, Schepers E, Pletinck A, Nagler EV, Glorieux G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. J Am Soc Nephrol 2014;25:1897-907.
40. Vanholder R, Baurmeister U, Brunet P, Cohen G, Glorieux G, Jankowski J. A bench to bedside view of uremic toxins. J Am Soc Nephrol 2008;19:863-70.
41. Gryp T, Vanholder R, Vaneechoutte M, Glorieux G. p-Cresyl Sulfate. Toxins (Basel) 2017;9:52.
42. Schepers E, Meert N, Glorieux G, Goeman J, Van der Eycken J, Vanholder R. P-cresylsulphate, the main in vivo metabolite of p-cresol, activates leucocyte free radical production. Nephrol Dial Transplant 2007;22:592-6.
43. Gross P, Massy ZA, Henaut L, Boudot C, Cagnard J, March C, et al. Para-cresyl sulfate acutely impairs vascular reactivity and induces vascular remodeling. J Cell Physiol 2015;230:2927-35.
44. Watanabe H, Miyamoto Y, Honda D, Tanaka H, Wu Q, Endo M, et al. p-Cresyl sulfate causes renal tubular cell damage by inducing oxidative stress by activation of NADPH oxidase. Kidney Int 2013;83:582-92.
45. Yoshifuji A, Wakino S, Irie J, Tajima T, Hasegawa K, Kanda T, et al. Gut Lactobacillus protects against the progression of renal damage by modulating the gut environment in rats. Nephrol Dial Transplant 2016;31:401-12.
46. Wang Y, Liu H, Zhao J. Macrophage Polarization Induced by Probiotic Bacteria: a Concise Review. Probiotics Antimicrob Proteins 2020;12:798-808.
47. Kwon HK, Lee CG, So JS, Chae CS, Hwang JS, Sahoo A, et al. Generation of regulatory dendritic cells and CD4+Foxp3+ T cells by probiotics administration suppresses immune disorders. Proc Natl Acad Sci U S A 2010;107:2159-64.

48. Kim DH, Kim S, Lee JH, Kim JH, Che X, Ma HW, et al. Lactobacillus acidophilus suppresses intestinal inflammation by inhibiting endoplasmic reticulum stress. J Gastroenterol Hepatol 2019;34:178-85.
49. Sohn W, Jun DW, Lee KN, Lee HL, Lee OY, Choi HS, et al. Lactobacillus paracasei Induces M2-Dominant Kupffer Cell Polarization in a Mouse Model of Nonalcoholic Steatohepatitis. Dig Dis Sci 2015;60:3340-50.
50. Woo JY, Gu W, Kim KA, Jang SE, Han MJ, Kim DH. Lactobacillus pentosus var. plantarum C29 ameliorates memory impairment and inflammaging in a D-galactose-induced accelerated aging mouse model. Anaerobe 2014;27:22-6.
51. Kim HJ, Kim YJ, Lee SH, Yu J, Jeong SK, Hong SJ. Effects of Lactobacillus rhamnosus on allergic march model by suppressing Th2, Th17, and TSLP responses via CD4(+)CD25(+)Foxp3(+) Tregs. Clin Immunol 2014;153:178-86.
52. Chen T, Cao Q, Wang Y, Harris DCH. M2 macrophages in kidney disease: biology, therapies, and perspectives. Kidney Int 2019;95:760-73.
53. Cao Q, Harris DC, Wang Y. Macrophages in kidney injury, inflammation, and fibrosis. Physiology (Bethesda) 2015;30:183-94.
54. Sung SA, Jo SK, Cho WY, Won NH, Kim HK. Reduction of renal fibrosis as a result of liposome encapsulated clodronate induced macrophage depletion after unilateral ureteral obstruction in rats. Nephron Exp Nephrol 2007;105:e1-9.
55. Nishida M, Okumura Y, Fujimoto S, Shiraishi I, Itoi T, Hamaoka K. Adoptive transfer of macrophages ameliorates renal fibrosis in mice. Biochem Biophys Res Commun 2005;332:11-6.
56. Nishida M, Fujinaka H, Matsusaka T, Price J, Kon V, Fogo AB, et al. Absence of angiotensin II type 1 receptor in bone marrow-derived cells is detrimental in the evolution of renal fibrosis. J Clin Invest 2002;110:1859-68.

57. McIntyre CW, Harrison LE, Eldehni MT, Jefferies HJ, Szeto CC, John SG, et al. Circulating endotoxemia: a novel factor in systemic inflammation and cardiovascular disease in chronic kidney disease. Clin J Am Soc Nephrol 2011;6:133-41.
58. Neal MD, Leaphart C, Levy R, Prince J, Billiar TR, Watkins S, et al. Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. J Immunol 2006;176:3070-9.
59. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004;4:499-511.
60. Komada T, Muruve DA. The role of inflammasomes in kidney disease. Nat Rev Nephrol 2019;15:501-20.
61. Anders HJ, Andersen K, Stecher B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. Kidney Int 2013;83:1010-6.
62. Andrade-Oliveira V, Amano MT, Correa-Costa M, Castoldi A, Felizardo RJ, de Almeida DC, et al. Gut Bacteria Products Prevent AKI Induced by Ischemia-Reperfusion. J Am Soc Nephrol 2015;26:1877-88.
63. Li YJ, Chen X, Kwan TK, Loh YW, Singer J, Liu Y, et al. Dietary Fiber Protects against Diabetic Nephropathy through Short-Chain Fatty Acid-Mediated Activation of G Protein-Coupled Receptors GPR43 and GPR109A. J Am Soc Nephrol 2020;31:1267-81.
64. Macia L, Tan J, Vieira AT, Leach K, Stanley D, Luong S, et al. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. Nat Commun 2015;6:6734.
65. Yao X, Zhang C, Xing Y, Xue G, Zhang Q, Pan F, et al. Remodelling of the gut microbiota by hyperactive NLRP3 induces regulatory T cells to maintain homeostasis. Nat Commun 2017;8:1896.
66. Koppe L, Mafra D, Fouque D. Probiotics and chronic kidney disease. Kidney

Int 2015;88:958-66.

67. McFarlane C, Ramos CI, Johnson DW, Campbell KL. Prebiotic, Probiotic, and Synbiotic Supplementation in Chronic Kidney Disease: A Systematic Review and Meta-analysis. J Ren Nutr 2019;29:209-20.

68. Jia L, Jia Q, Yang J, Jia R, Zhang H. Efficacy of Probiotics Supplementation On Chronic Kidney Disease: a Systematic Review and Meta-Analysis. Kidney Blood Press Res 2018;43:1623-35.

ABSTRACT (IN KOREAN)

# 만성콩팥병 동물 모델에서 *Lactobacillus acidophilus* KBL409의 면역 조절에 의한 신장 보호 효과

<지도교수 한승혁>

연세대학교 대학원 의학과

김형래

**배경:** 장내 미생물 불균형은 최근 만성콩팥병을 비롯한 각종 질병의 진행에 중요한 역할을 하는 것으로 알려졌다. 이 연구는 만성콩팥병 동물 모델에서 프로바이오틱스의 신장 손상에 대한 보호 효과를 평가하고자 하였다.

**방법:** 후보 균주 중 p-cresol 분해 능력이 확인된 *Lactobacillus Acidophilus* KBL409를 선정하였다. 만성콩팥병 동물 모델은 0.2% 아데닌이 포함된 사료를 C57BL/6 쥐에게 먹임으로써 유도하였다. 이 쥐에게 KBL409를 4주 동안 매일 $1 \times 10^9$ CFU의 용량으로 경구 투여하였다. 체외 실험으로 KBL409의 신장 보호 기전을 확인하기 위해 일차 배양한 쥐의 세뇨관 상피 세포를 p-cresyl sulfate로 자극하고 단쇄지방산(butyrate와 acetate)을 함께 투여하였다.

**결과:** 만성콩팥병이 유도된 쥐는 대조군에 비해 신장 섬유화가 의미 있게 증가하였으며, 혈청 크레아티닌과 단백뇨도 증가하였다. 그러나 KBL409 투여로 신장 내 섬유화 관련 인자들의 발현이 감소하고 단백뇨도 감소하였다. 또한 만성콩팥병이 유도된 쥐는 장내 밀착 연접이 손상이 증가하고 혈청 내 전신 염증 지표(TNF-α와 IL-6)와 산화 스트레스 지표(8-hydroxy-2'-deoxyguanosine)의 증가 소견을 보였다. 이러한 변화들은 KBL409 투여에 의해 의미 있게

감소되었다. 나아가 KBL409 투여는 만성콩팥병이 유도된 쥐의 신장 내 대식세포 침투를 의미 있게 감소시키고, M2 표현형 대식세포와 조절 T세포의 발현을 증가시켜 항염증 상태로의 면역 유도 효과를 보였다. 또한, 만성콩팥병 쥐에서 KBL409 투여에 의해 NLRP3, ASC, IL-18 및 IL-1β의 신장 내 발현이 의미 있게 감소하여 NLRP3 inflammasome 활성도가 감소됨을 확인하였다. 체외 실험에서 p-cresyl sulfate로 자극한 세뇨관 상피세포에서 NLRP3 inflammasome과 섬유화 지표들의 발현이 증가함을 확인하였다. 이러한 변화는 단쇄지방산의 투여에 의해 감소하였다.

**결론:** 이상의 결과를 통해 프로바이오틱스 KBL409 투여가 만성콩팥병에서 면역 조절 효과를 통해 신장 손상의 진행을 억제시킬 수 있다는 것을 확인하였다.

---

핵심되는 말: 프로바이오틱스, KBL409, 섬유화, 대식세포, 만성콩팥병