Allergol Immunopathol (Madr). 2020;48(2):149–157

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

ORIGINAL ARTICLE

Modulation effect of *Lactobacillus acidophilus* KLDS 1.0738 on gut microbiota and TLR4 expression in β-lactoglobulin-induced allergic mice model

W.-w. Ni, Q.-m. Zhang, X. Zhang, Y. Li, S.-s. Yu, H.-y. Wu, Z. Chen, A.-l. Li*, P. Du*, C. Li

Key Laboratory of Dairy Science, Food Science College, Northeast Agriculture University, Northeast Agriculture University, Harbin, China

Received 3 April 2019; accepted 3 June 2019
Available online 30 August 2019

KEYWORDS
Lactobacillus acidophilus;
β-lactoglobulin allergy;
Gut microbiota;
TLR4;
16S rRNA gene sequencing

Abstract
Objectives: β-lactoglobulin (β-Lg)-sensitized mice model was employed to investigate the correlation between *Lactobacillus acidophilus* KLDS 1.0738 (*Lap* KLDS 1.0738) modulating gut microbiota and inducting Toll-like receptors (TLRs) expression.
Methods: The alterations of mice fecal microbiota were analyzed by 16S rRNA gene sequencing. The serum cytokines production and TLR4/NF-κB mRNA expression in the colon tissues were measured by ELISA kit and quantitative RT-PCR, respectively.
Results: The results showed that *Lap* KLDS 1.0738 pretreatment attenuated β-Lg-induced hypersensitivity, accompanied with a diminished expression of TLR4/NF-κB signaling. Moreover, oral administration of *Lap* KLDS 1.0738 improved the richness and diversity of fecal microbiota, which was characterized by fewer *Proteobacteria* phylum and *Helicobacteraceae* family, and higher *Firmicutes* phylum and *Lachnospiraceae* family than allergic group. Notably, TLR4/NF-κB expression was positively correlated with the family of *Helicobacteraceae* in allergic group, but negatively correlated with the family of *Lachnospiraceae, Ruminococcaceae* and anti-inflammatory cytokines level. A significant positive correlation was observed between TLR4/NF-κB expression and the production of histamine, total IgE and pro-inflammatory cytokines.
Conclusions: Intake of *Lap* KLDS 1.0738 can influence the gut bacterial composition, which might result in recognizing TLRs signaling so as to inhibit allergic response.

* Corresponding authors.
E-mail addresses: aili-mail@163.com (A.-l. Li), rocgin@126.com (P. Du).

https://doi.org/10.1016/j.aller.2019.06.002
0301-0546/

Introduction

Recent epidemiologic studies suggested that IgE-mediated cow's milk allergy (CMA) has a negative impact on infants and young children's health, which is a serious public health concern.[1,2] It has been reported that the intestinal immune barrier dysfunction is partly due to food antigen-induced gastrointestinal microbial dysregulation.[3] In general, intestinal flora is considered to modulate the intestinal immune system by stimulating the Toll-like receptors (TLRs) family, and subsequently to result in activating the nuclear factor kinase (NF-κB) pathway, releasing downstream cascade cytokines and differentiating T lymphocyte.[4] For example, Shukla et al.[5] observed that the intestinal flora disorder in patients with irritable bowel syndrome was positively correlated with up-regulated TLR4 and TLR5 expression and increased IL-6, CXCL-11 and CXCR-3 secretion. Rogier et al.[6] indicated that the aberrant intestinal microbiota, induced by IL-1 receptor antagonist deficiency in mice, exacerbated inflammation via TLR4-induced release of pro-inflammatory cytokines and subsequent Th17 differentiation. Therefore, we hypothesized that intestinal microbiota flora disorder induced TLRs activation may act on the pathogenesis of CMA.

Nowadays, probiotics as a novel therapeutic perspective in the prevention of autoimmune and allergic diseases have gained considerable attention.[7] Zhang et al.[8] highlighted that *Lactobacillus rhamnosus* GG (*LGG*) alleviated allergic airway inflammation by regulating the intestinal microbiota, which induced the regulatory T cells (Treg) expression to suppress Th2 responses. Moreover, a clinical human trial also showed that the risk of asthma in infants was reduced by early-life *Lactobacillus* supplementation through modulating gut microbial diversification and promoting tolerogenic conditions characterized by Treg cell expansion.[9] In addition to the general maintaining of the intestinal flora balance, the current studies further illustrated the ability of *Lactobacillus* to trigger the innate and adaptive immune responses by binding to patterns recognition receptors, such as TLRs signaling molecular.[10,11] Kamaladevi et al.[12] indicated that *Lactobacillus casei* elicited the TLR1 mediated p38 mitogen-activated protein kinase (MAPK) pathway to resist *Klebsiella pneumoniae* infection in *Caenorhabditis elegans*. Li et al.[13] found that *Lactobacillus acidophilus* significantly inhibited the up-regulation of TLR4, NF-κB and p38 MAPK expression in enterotoxigenic *Escherichia coli*-infected piglets. However, it is not clear whether intestinal microbiota modulating the TLRs signaling participate in the pathway of *Lactobacillus* prevent the occurrence of CMA.

We previously demonstrated that heat-killed *Lap* KLDS 1.0738 induced a protective effect on CMA by improving the Treg/Th17 balance.[14] However, further studies are required to analyze its immunomodulatory mechanism. In the present study, the intestinal flora composition and TLRs mRNA expression were evaluated to clarify the anti-allergic pathway of *Lap* KLDS 1.0738 for its therapeutic effect on CMA. Our findings would provide a new insight for the anti-allergic application of *Lap* KLDS 1.0738.

Materials and methods

Preparation of lap KLDS 1.0738 and commercial probiotic capsules

The probiotics strain *Lap* KLDS 1.0738 obtained from Key Laboratory of Dairy Science (Northeast Agricultural University, Harbin, China) was anaerobically grown overnight at 37° in MRS broth (Huankai Microbial, China). The cellular pellets were harvested by centrifugation (5000×g, 10 min at 4°) and washed in sterile saline, and then determined a concentration of 10^8 or 10^9 CFU/mL and stored frozen at 80°. The eN-Lac Plus capsule (GenMont Biotec Inc., Taiwan, China) was purchased at retail outlets, which contained 2.0×10^9 CFU/g *Lactobacillus* (*L. parcheese* GM-080, *L. fermentans* GM-090 and *L. acidophilus* GMNL-185). Before being administered to mice, eN-Lac Plus capsule was suspended in sterilized saline and determined a concentration of 13 mg/mL.

Animal model

Female BALB/C mice at six weeks of age (Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) were maintained under normal husbandry surroundings with free intake to a milk-free diet. All animal procedures were taken in conformity to the guidelines for animal care and use and approved by the Northeast Agricultural University.

All mice were randomly assigned to five experimental groups (n = 6): normal group (N); β-Lg allergic group (β-Lg); commercial probiotics group (CP); low-dose (*Lap* 1.0738-L); and high-dose (*Lap* 1.0738-H) *Lap* KLDS 1.078 group. Except for the normal group, the mice were intraperitoneally injected with 200 μL 1 mg/mL β-Lg emulsified in incomplete Freund's adjuvant (Sigma, USA) on days 7, 14, and 21. From day 1 to day 28, the mice in CP group and *Lap* KLDS 1.078-treated group were oral gavaged 200 μL 13 mg/mL commercial probiotics (Oral administration with commercial probiotics was calculated to provide approximately 0.13 mg/g body weight), 10^8 CFU/mL (*Lap* 1.0738-L) or 10^9 CFU/mL (*Lap* 1.0738-H) *Lap* KLDS 1.078 three times per week, respectively. At day 28, allergen challenge was performed with all sensitized mice by giving a gavage administration of β-Lg (20 mg/mouse) as described previously.[15] Two hours later, the blood, fecal and tissue samples from each mouse were immediately collected for further analysis.

Evaluation of allergic symptoms

Allergic symptoms were assessed by measuring inflammatory cytokines, histamine and total IgE production in serum. The serum concentrations of histamine and cytokines (IL-1β, IL-6, IL-10, TNF-α, TGF-β) were quantified using the ELISA kits, following the manufacturer's recommendations (Tiangen, China). The serum total IgE was determined by ELISA according to the previous methods.[15]

Table 1 Effects of *Lap* KLDS 1.0738 on allergic symptoms of β-Lg sensitized mice.

Item	N	β-Lg	CP	Lap 1.0738-L	Lap 1.0738-H
Colon length (cm)	8.48 ± 0.44	5.74 ± 0.25*	6.53 ± 0.47*†	7.23 ± 0.42*†	7.87 ± 0.32†
Histamine (ng/mL)	9.93 ± 1.06	18.98 ± 0.98*	16.13 ± 0.73*†	15.30 ± 0.52*†	12.58 ± 1.32†
Total IgE (ng/mL)	71.15 ± 2.45	133.78 ± 3.05*	100.47 ± 2.42*†	94.67 ± 1.53*†	74.55 ± 3.13†
IL-6 (pg/mL)	66.50 ± 10.22	120.80 ± 16.08*	74.46 ± 7.59†	94.72 ± 13.44*†	67.19 ± 8.14†
IL-1β (pg/mL)	41.04 ± 11.36	114.53 ± 19.15*	81.89 ± 3.25*†	63.56 ± 10.22*†	57.65 ± 7.11†
TNF-α (pg/mL)	320.34 ± 19.73	583.55 ± 10.45*	467.67 ± 19.30*†	359.54 ± 7.13*†	316.77 ± 26.94†
IL-10 (pg/mL)	529.31 ± 11.79	387.52 ± 16.31*	568.21 ± 2.90 *†	620.04 ± 13.65*†	627.11 ± 28.09*†
TGF-β (pg/mL)	96.72 ± 6.10	79.50 ± 8.00*	115.83 ± 9.81*†	137.43 ± 5.21*†	148.81 ± 8.81*†

Normal group (N), β-lactoglobulin group (β-Lg), commercial probiotic group (CP), low dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-L), high dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-H). The results are means ± SD (n = 3). *$P < 0.05$ versus N group. †$P < 0.05$ versus β-Lg group.

Extraction of RNA, cDNA synthesis, and real-time PCR

Total RNA was isolated from the stored colonic tissues using the Total RNA kit (Tiangen, China) and 2 μg of RNA was reverse transcribed to cDNA using FastQuant cDNA (Tiangen, China) as per the manufacturer's recommendations. Expressions of TLR-4, Myd88, NF-κB were performed by Real-MasterMix (SYBR Green) (Tiangen, China) using the ABI 7500 Fast ReaL-Time PCR (Life technologies, USA). PCRs were conducted using the following program: the initial step was 15 min at 95 °C, then followed by 40 cycles of 10 s at 95 °C and 30 s at 60 °C. Primers sequences for target genes were shown in supplementary material. The relative expression of β-actin was used for calculating the levels of target genes. The mRNA level of target gene was determined using the $2^{-\Delta\Delta CT}$ method.

16S rRNA sequencing and bioinformatical analysis

Genomic DNA was extracted from mice feces samples using the E.Z.N.A.®stool DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's recommendations. The amplification of 16S rRNA gene sequences (V3-V4 region) was performed using the TransGen AP221-02: TransStart Fastpfu DNA Polymerase. Purified amplicons were prepared for sequencing on the Illumina Miseq PE300 platform (Illumina Inc., CA, USA). The details of DNA extraction, PCR amplification, quantitative PCR, amplicons purification, sequencing and high-quality sequences criteria are those described in one study.[16]

The operational taxonomic units (OTUs) were defined as sequences with 97% similarity using UCLUST (v1.2.22) and the Silva (Release119) 16S rRNA database with a 90% confidence threshold. Alpha-diversity indices were used to investigate the bacterial diversity and richness, which were performed using QIIME (version v.1.8). Complex bacterial communities represented by column accumulation diagram were compared at different taxonomic levels, and the column accumulation diagram at different taxonomic levels was drawn by R software (version 2.15.3). Cladogram and linear discriminant analysis (LDA) produced from LEfSe analysis were utilized to identify the differentially dominant bacterial taxa at the OTU level.

Statistical analysis

Data were displayed as the mean ± SD. Statistical difference was analyzed by One-way ANOVA test using the SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The Spearman correlation was applied to reveal the relationship between dominant gut microbiota and allergic symptoms with TLR4 pathway mRNA expression. *P*-value <0.05 represents a significant difference.

Results

Lap KLDS 1.0738 showed significant inhibitory activity on CMA

First, we investigated the anti-allergic effect of *Lap* KLDS 1.0738 in the β-Lg-induced mice model (Table 1). *Lap* KLDS 1.0738 or commercial probiotics treatment exhibited lower histamine and total IgE ($P < 0.05$), accompanied with increased colon length than those of the allergic group ($P < 0.05$), especially the high dosage of *Lap* KLDS 1.0738 group. Moreover, oral supplementation *Lap* KLDS 1.0738 or commercial probiotics also down-regulated the levels of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and up-regulated the levels of anti-inflammatory cytokine (IL-10 and TGF-β) ($P < 0.05$).

Correlation between the TLR4/NF-κB pathway and allergic symptoms

Further data suggested that bovine β-Lg challenge led to aggravation of the expressions of TLR-4 and NF-κB compared with the control (Fig. 1a). In contrast, allergic mice treated with *Lap* KLDS 1.0738 dose-dependently decreased the TLR4 and NF-κB expression ($P < 0.05$). The Myd88 mRNA expression was not statistically significant in all the experimental treated groups ($P > 0.05$). The trend of commercial probiotics treatment suppressed TLR4 signaling similar to that of *Lap* KLDS 1.0738-treated groups. Notably, the expressions of TLR4/NF-κB were positively correlated with the production of histamines, total IgE, IL-6 and TNF-α ($P < 0.05$), but were negatively correlated with the secretion of anti-inflammatory cytokines TGF-β (Fig. 1b).

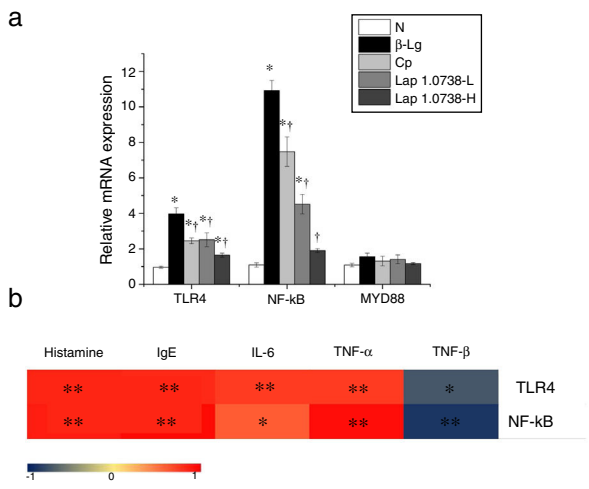

Figure 1 The expression of TLR4/NF-κB signaling (a) and the correlation between the TLR4/NF-κB expression and allergic symptoms (b). The correlation of the expression of TLR4/NF-κB signaling pathway in colonic tissues and the level of histamine, total IgE, IL-6, TNF-α, TGF-β in serum of allergic group compared to treatment groups. Normal group (N), β-lactoglobulin group (β-Lg), commercial probiotic group (CP), low dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-*L*), high dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-H). $^{*}P < 0.05$ versus N group. $^{\dagger}P < 0.05$ versus β-Lg group. $^{*}P < 0.05$, $^{**}P < 0.01$.

Effects of lap KLDS 1.0738 on the diversity of the intestinal microbiota

Next, we investigated the effect of probiotics on the fecal microbiome by 16S rRNA gene sequencing. The sequence datasets consisting of 313,132 high-quality classifiable 16SrRNA gene from 15 fecal samples were obtained and an average of 253 OTUs per group were identified. The detailed diversity estimates of fecal microbiota are listed in Table 2. Compared with the allergic group, both of microbial species richness (Chao1) and diversity (Shannon) were increased in *Lap* KLDS 1.0738-treated groups ($P < 0.05$). The difference in the Shannon and Chao1 index between the high-dose *Lap* KLDS 1.0738 group and the control was not significant ($P > 0.05$). It was worth noting that the commercial probiotics group had lower a Shannon and Chao1 index than those of the allergic group ($P < 0.05$).

Structural comparison of fecal microbiota among different groups

To further explore the microbial species and their relative abundance, the microbiota compositions were analyzed at the phylum, family, and genus levels, respectively (Fig. 2). At the phylum level, it was found that those administered with *Lap* KLDS 1.0738 reversed the alterations of fecal microbiota induced by β-lg allergen, including prompting the *Firmicutes* growth (41.62% and 61.97%) and inhibiting the overgrowth of *Proteobacteria* (0.19% and 0.16%) ($P < 0.05$). Interestingly, the ratio of *Bacteroidetes* (85.54%) in the commercial probiotics group was significantly higher than the other groups ($P < 0.05$). At the family level, *Lap* KLDS 1.0738 treatment clearly exhibited higher abundance of *Lachnospiraceae* (32.63% and 49.25% vs. 21.41%), *Bacteroidales_S24-7* (34.72% and 26.94% vs. 23.41%) and *Ruminococcaceae* (7.96% and 11.28% vs. 4.72%), but a lower proportion of *Bacteroidaceae* (3.54% and 0.81% vs. 9.86%), *Rikenellaceae* (2.52% and 1.59% vs. 9.15%) and *Helicobacteraceae* (0.02% and 0% vs. 16.65%) than those in the allergic group ($P < 0.05$). Although intake of commercial probiotics remarkably decreased *Helicobacteraceae* (0.01%), *Bacteroidaceae* (0.63%) and *Rikenellaceae* (1.52%) levels ($P < 0.05$), but failed to improve *Lachnospiraceae* (5.27%) and *Ruminococcaceae* (2.43%) levels. Consumption of *Lap* KLDS 1.0738 consistently reversed the expansions of *Helicobacter*, *Bacteroides* and *Rikenellaceae_RC9_gut_Group*, but up-regulated the *Lachnospiraceae_NK4A136_group*, *Ruminococcaceae_UCG-014* and *Ruminiclostridium_9* at the genus level.

Subsequently, LEfSe analysis was performed to investigate the biomarker species of feces microbiota between the allergic group and the high-dose *Lap* KLDS 1.0738 group (Fig. 3). The cladogram and linear discriminant analysis scores (LDA, values >4) exhibited 22 dominant taxa (from phylum to genus level), including 15 dominant taxa in allergic group and seven dominant taxa in high-dose *Lap* KLDS 1.0738 group. The family of *Lachnospiraceae* and *Ruminococcaceae*, which belong to *Firmicutes* phylum (*Clostridia* class, *Clostridiales* order), were mainly taxa enriched in the high-dose *Lap* KLDS 1.0738 group, while the family of *Helicobacteraceae* belonging to *Proteobacteria* phylum (*Epsilonproteobacteria* class, *Campylobacterales* order) and family of *Bacteroidaceae* and *Rikenellaceae* belonging to *Bacteroidetes* phylum were mainly taxa enriched in the allergic group. Similarly to the family level, allergic mice displayed a significant

Table 2 Illumina sequencing data summary.

Group	Sequences produced	OTU numbers	Shannon	Chao1
N	21982 ± 6141.327	260.333 ± 7.023	6.073 ± 0.084	261.731 ± 1.086
β-Lg	11409.5 ± 54.447*	240.667 ± 3.511*	5.391 ± 0.017*	269.177 ± 4.858
CP	21318 ± 3470.626†	199.333 ± 7.234*†	4.660 ± 0.034 *†	213.133 ± 5.146*†
Lap 1.0738-L	24990.667 ± 4455.059†	290.333 ± 3.786 *†	5.517 ± 0.430*	289.467 ± 3.576*†
Lap 1.0738-H	24677 ± 4370.144†	272.333 ± 7.506 †	5.682 ± 0.220	276.552 ± 18.468

Normal group (N), β-lactoglobulin group (β-Lg), commercial probiotic group (CP), low dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-L), high dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-H). The results are means ± SD (n = 3). $^{*}P < 0.05$ versus N group. $^{\dagger}P < 0.05$ versus β-Lg group.

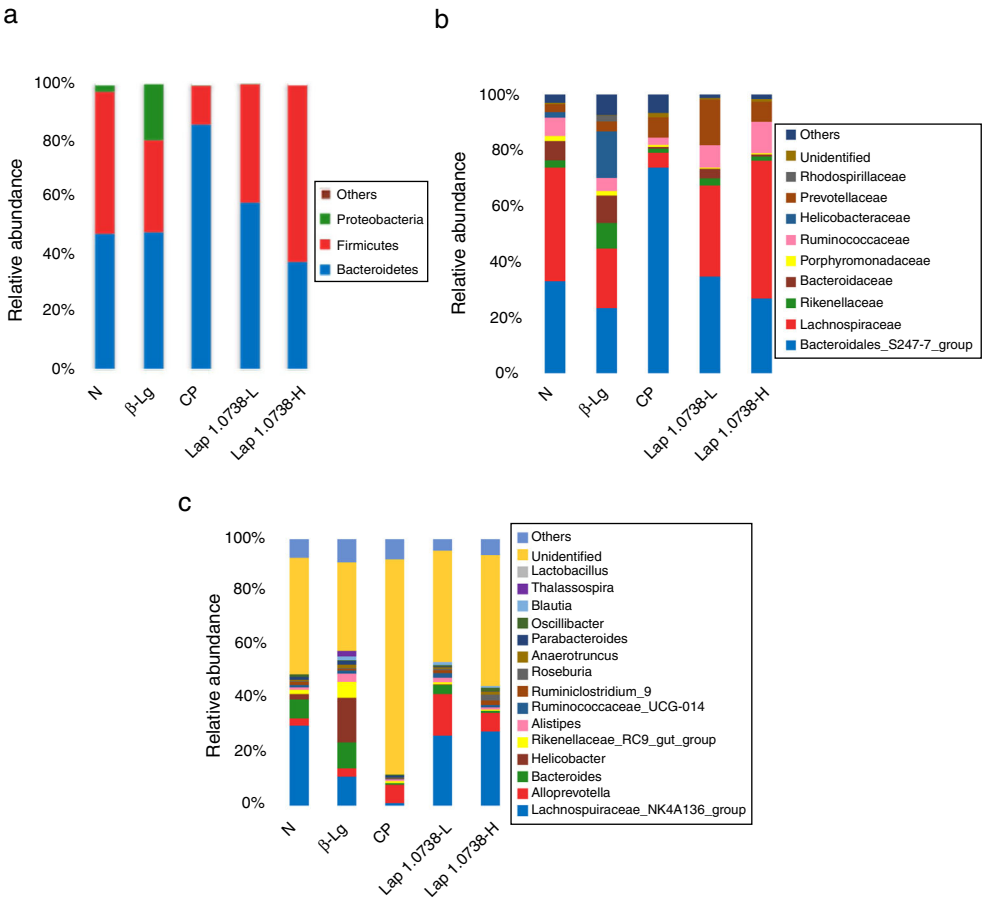

Figure 2 Comparisons of fecal bacterial communities at phylum (a), family (b), and genus (c) levels in all groups. Normal group (N); β-lactoglobulin group (β-Lg); commercial probiotic group (CP); low dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-L); high dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-H).

increase in *Helicobacter* and *Bacteroides* at the genus level.

Correlation between dominant gut microbiota and TLR4/NF-κB pathway

To determine which specific bacteria modulated the colonic mucosal TLRs and thereby attenuated allergic symptoms, the Spearman correlation was performed to compare the dominant bacteria at the family level with TLR4 expression and inflammatory markers. Fig. 4 shows that the abundance of *Rikenellaceae*, *Helicobacteraceae*, and *Bacteroidaceae* were positively correlated with the expression of TRL4 and TRL4-linked NF-κB, especially the *Helicobacteraceae* family belonging to *Proteobacteria* phylum ($P < 0.05$). Moreover, a significant positive correlation was observed between the family of *Helicobacteraceae* and *Bacteroidaceae* and pro-inflammatory cytokines IL-6 ($P < 0.05$). On the other hand, the abundance of *Lachnospiraceae* and *Ruminococcaceae* were significantly negatively related to the expressions of TLR4/NF-κB and the production of pro-inflammatory cytokines TNF-α ($P < 0.05$), but were positively correlated with the anti-inflammatory cytokines TGF-β level ($P < 0.05$). There was no significant relationship between *Bacteroidales_S24-7* and TLR4 pathway ($P > 0.05$).

Discussion

Numerous studies have very recently pointed out that the interaction among probiotics and gut microbiota ecology is a critical determinant in maintaining immune system maturation and tolerance acquisition.[17,18] In this study, oral *Lap* KLDS 1.0738 administration displayed inhibitory effects on β-Lg-induced inflammation in an allergic mice model. Furthermore, we observed that the alterations of the gut microbiota mediated by probiotics were associated with reducing the risk of allergic sensitization.

First, *Lap* KLDS 1.0738-treated restored enteric microbial richness and diversity, which were characterized by lower numbers of *Proteobacteria* phylum and higher numbers of *Firmicutes* phylum than the allergic group. The enormous growth of *Proteobacteria* phylum was considered to be a potential diagnostic signature for gut microbial dysbiosis, which has been observed in various inflammatory disease states.[19] For instance, one study of fecal microbiota in patients with IBD exhibited higher proportion of *Proteobacteria* (particularly *Enterobacteriaceae*) and the lower level of *Firmicutes* than those of the healthy controls.[20] Similar animal experiments demonstrated that, after probiotics *LGG* therapy, the changes in phylum-level shift from *Firmicutes* to *Proteobacteria* caused by homotypic viru-

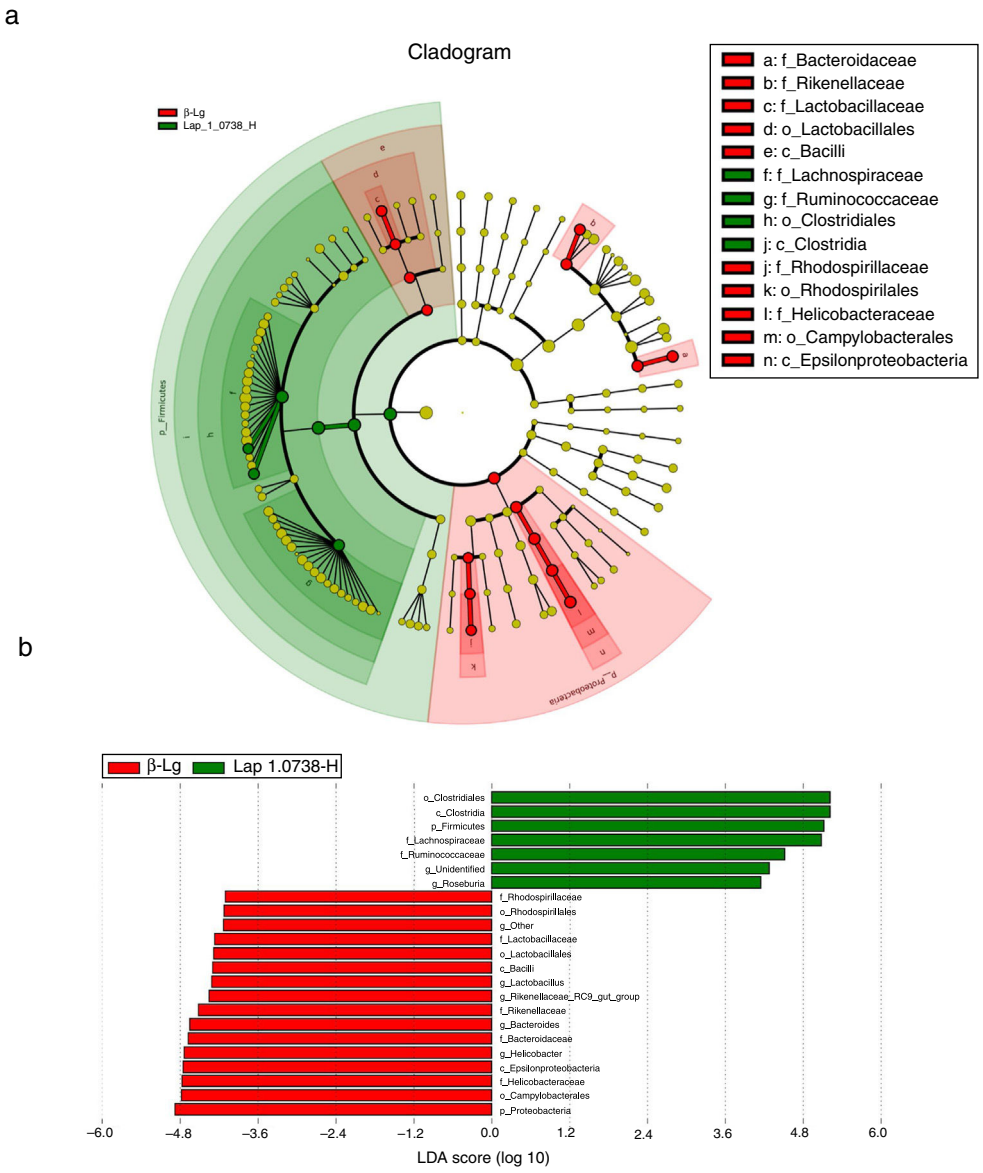

Figure 3 Cladogram (a) and linear discriminant analysis (LDA) (b) produced from LEfSe analysis. LEfSe identifies the most differentially abundant bacterial taxa among groups. Only taxa meeting an LDA significant threshold of >4 are shown. Normal group (N); β-lactoglobulin group (β-Lg); commercial probiotic group (CP); low dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-L); high dose of *Lap* KLDS 1.0738 group (*Lap* 1.0738-H).

lent human rotavirus in pig were effectively prevented and managed.[21]

Second, the observed differences in family and genus levels revealed that the CMA mice gut microbiota comprised predominantly by the family of *Helicobacteraceae*, belonging to *Proteobacteria* phylum, and the family of *Bacteroidaceae* and *Rikenellaceae*, belonging to the *Bacteroidetes* phylum. Similar to family-level analysis, studies at genus level also showed significant enrichment in β-lg-sensitized mice samples of *Bacteroides* and *Helicobacter*. The gram-negative *Helicobacteraceae* was considered a determinant pathogenic role in the intestinal immune system, and has been detected in several enteritis and infection disease models.[22,23] Although members of the *Bacteroidaceae* family represented a potential anti-inflammatory effect in host health,[24] the specific taxa exacerbated enteric infection when the gut microbiota was imbalanced. For instance, Curtis et al.[25] reported that the increase of *Bacteroides* could alter intestinal permeability, lead to excessive antigen transfer and hence contribute to perpetuating the inflammatory reaction. Notably, a recent clinical trial reported that CMA children maintained a greater average number of *Bacteroides* than non-allergic children.[26] The *Rikenellaceae* family was found more often in the intestinal microbiota of disease models than in healthy controls, while its potential risk for inflammation and allergy development remained uncertain. Those observations demonstrated that the damage of the gut immunologic barrier modified the abundance of resident bacteria, leading to exacerbation of the inflammation in β-Lg allergic mice.

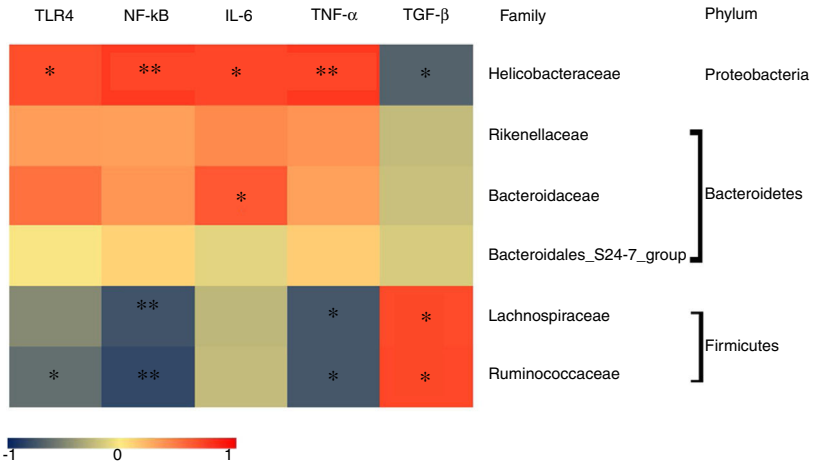

Figure 4 The correlation between key genera, TLR4/NF-κB expression and cytokines. The correlation of key genera at family level in fecal samples, TLR4/NF-κB signaling expression in colonic tissues and cytokines in serum of allergic group compared to treatment groups. $^{*}P < 0.05$, $^{**}P < 0.01$.

Conversely, supplementation with *Lap* KLDS 1.0738 not only reversed the expansion of potentially pathogenic bacteria, but also remarkably up-regulated the proportion of *Lachnospiraceae* and *Ruminococcaceae* (phylum *Firmicutes*, class *Clostridia*), as well as *Bacteroidales_S24-7* (phylum *Bacteroidetes*). The family of *Lachnospiraceae* and *Ruminococcaceae* are prevalent in the digestive tract of mammals, and have a protective effect on the intestinal immune system because of their ability to produce short-chain fatty acids, especially butyric acid.[27,28] Early research focused on their functions link butyric acid to suppress colon cancer and obesity levels.[29] Currently, Wang et al.[30] found that treatment of colitis mice with *B.bifidum* increased the abundance of *Lachnospiracea*-bacterium, which resulted in regulating oxidative stress and inflammatory mediators. Sagheddu et al.[31] demonstrated the beneficial role of *Ruminococcaceae* in promoting protein synthesis and preventing infant malnutrition. Interestingly, consumption of commercial probiotics statistically increased the relative abundance of genus *Bacteroidales_*S24-7, which was consistent with the increase in the *Bacteroidetes* phylum. New insights indicated that the presence of *Bacteroidales_*S24-7 induced accumulation of bacterial extracellular DNA to down-regulate TNF-α, IL-6 levels and up-regulate IL-10. These results were supported by Zhao et al.[32] and Qi et al.,[33] who demonstrated that the ability of Bacteroidales_S24-7 to maintain immune homeostasis was closely accompanied with inhibiting inflammatory cytokines, including IL-6. Therefore, the regulatory function of *Bacteroidales_*S24-7 in β-Lg allergy remains in need of further validation.

In general, pathogenic bacteria activate TLR4, resulting in aggravating the inflammatory response in the intestine by influencing downstream NF-κB pathway and cytokines release.[34] In this study, the up-regulated TLR4 expression and increased serum IL-6, TNF-α levels were observed in β-lg-induced mice. Moreover, *Helicobacteraceae*, *Bacteroidaceae* and *Rikenellaceae* were found to be involved in the β-lg allergy's pathogenesis and positively related to TRL4/NF-κB pathway and IL-6. In agreement with our studies, Loganathan et al.[35] showed that genetic polymorphisms in TLR4 and TLR9 were associated with chronic *Helicobacter pylori*-related infection. While dysbiotic *Bacteroides* species were accompanied by defective innate immunity and ineffective regulatory T cells, which were considered as important factors of IBD pathogenesis. Kim et al.[36] indicated that TLR4 was required for *Bacteroides fragilis* induced NF-κB activation and proinflammatory IL-8 release. In contrast, after the treatment of allergic mice with *Lap* KLDS 1.0738, TLR4 overexpression and inflammatory cytokines production both were declined, with increasing tendencies in the number of protective gut commensal strains. It is worth noting that increased *Lachnospiraceae* and *Ruminococcaceae* in *Lap* KLDS 1.0738-treated group was positively associated with TGF-β, but a negative association was recognized with TLR4, NF-κB, and TNF-α. In agreement with our studies, the genera *Faecalibacterium* and *Ruminococcus* belonging to the family *Ruminococcaceae* were reported to be inversely correlated with TLR4 expression in Chinese patients with functional gastrointestinal disorders.[37] Similarly, the severity of colon inflammation in Nlrp12$^{-/-}$mice was attenuated by promoting beneficial *Lachnospiraceae* strains, accompanied by suppressing excessive immune signaling, such as NF-κB, ERK and STAT3.[38] Moreover, we found the correlation between MyD88 and dominant gut microbiota showed no significant difference in all groups. Though MyD88 as an adapter molecule was commonly responsible for the TLR-induced inflammatory cytokines, MyD88-independent pathways could be triggered by TLRs ligands to activate NF-κB pathway.[39,40] Our previous studies also provided evidence that *L. acildophilus* peptidoglycan treatment had no effect on Myd88 mRNA level in β-Lg allergic mice.[41] Therefore, we speculated that *Lap* 1.0738's might protect against β-Lg-induced hypersensitivity through MyD88-independent signaling.

Moreover, some studies found that the TLRs activation plays an important role in the development of allergies.[42] The antigen-specific inflammatory markers disorders were mediated by TLR4 and NF-κB signaling mechanism in gliadin and gluten sensitized mice.[43] Complete Freund's adjuvant-induced arthritis mice exhibited higher relative expression

of TLR4 signaling, accompanied with increased inflammatory biomarkers (IL-6, IL-1β and TNF-α) levels.[44] Our results showed that TLR4/NF-κB signaling was positively associated with the level of histamines, total IgE, IL-6 and TNF-α, but negatively correlated with the production of anti-inflammatory cytokines TGF-β. These correlations could explain that the aberrant intestinal microbiota may be related to TLR signaling overexpression that aggravates immunodysregulation.

Conclusion

In summary, we speculated that the increased number of opportunistic bacterial pathogens might cause TLR4/NF-κB pathway activation, whereas correcting the dysbiosis and altering gut microbiota by administration of *Lap* KLDS 1.0738 may be contributing to weakening TLR4 transmit signals, which in turn inhibited β-Lg-induced hypersensitivity. Our study potentially provides an important link between *Lap* KLDS 1.0738 intervention and TLR4 signaling expression through gut microbiota modification in alleviating the β-Lg-induced inflammatory response. In addition, the consumption of *Lap* KLDS 1.0738 against β-Lg-induced allergy needs to be investigated in further clinical studies.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by the National key R&D Program of China [grant numbers 2017YFD0400304]; the National key R&D Program of China [grant numbers 2018YFD0502404]; and the Heilongjiang province technology fund project [grant numbers C2018022].

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aller.2019.06.002.

References

1. Pouessel G, Turner PJ, Worm M, Cardona V, Deschildre A, Beaudouin E, et al. Food-induced fatal anaphylaxis: From epidemiological data to general prevention strategies. Clin Exp Allergy. 2018;48:1584–93.
2. Wood RA, Sicherer SH, Vickery BP, Jones SM, Liu AH, Fleischer DM, et al. The natural history of milk allergy in an observational cohort. J Allergy Clin Immunol. 2013;131:805–12.
3. Zimmermann P, Messina N, Mohn WW, Finlay BB, Curtis N. Association between the intestinal microbiota and allergic sensitization, eczema, and asthma: A systematic review. J Allergy Clin Immunol. 2019;143:467–85.
4. Kasselman LJ, Vernice NA, DeLeon J, Reiss AB. The gut microbiome and elevated cardiovascular risk in obesity and autoimmunity. Atherosclerosis. 2018;271:203–13.
5. Shukla R, Ghoshal U, Ranjan P, Ghoshal UC. Expression of toll-like receptors, pro-, and anti-inflammatory cytokines in relation to gut microbiota in Irritable Bowel syndrome: The evidence for its micro-organic basis. J Neurogastroenterol Motil. 2018;24:628–42.
6. Rogier R, Ederveen THA, Boekhorst J, Wopereis H, Scher JU, Manasson J, et al. Aberrant intestinal microbiota due to IL-1 receptor antagonist deficiency promotes IL-17- and TLR4-dependent arthritis. Microbiome. 2017;5:63.
7. Sharma G, Im SH. Probiotics as a potential immunomodulating pharmabiotics in allergic diseases: Current status and future prospects. Allergy Asthma Immunol Res. 2018;10:575–90.
8. Zhang J, Ma JY, Li QH, Su H, Sun X. Lactobacillus rhamnosus GG induced protective effect on allergic airway inflammation is associated with gut microbiota. Cell Immunol. 2018;332:77–84.
9. Durack J, Kimes NE, Lin DL, Rauch M, McKean M, McCauley K, et al. Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by Lactobacillus supplementation. Nat Commun. 2018;9:707.
10. Jeffrey MP, Strap JL, Taggart HJ, Green-Johnson JM. Suppression of intestinal epithelial cell chemokine production by Lactobacillus rhamnosus R0011 and Lactobacillus helveticus R0389 is mediated by secreted bioactive molecules. Front Immunol. 2018;9:13.
11. Martens K, Pugin B, De Boeck I, Spacova I, Steelant B, Seys SF, et al. Probiotics for the airways: Potential to improve epithelial and immune homeostasis. Allergy. 2018;73:1954–63.
12. Kamaladevi A, Balamurugan K. Lactobacillus casei triggers a TLR mediated RACK-1 dependent p38 MAPK pathway in Caenorhabditis elegans to resist Klebsiella pneumoniae infection. Food Funct. 2016;7:3211–23.
13. Li HH, Zhang L, Chen LB, Zhu Q, Wang WJ, Qiao JY. Lactobacillus acidophilus alleviates the inflammatory response to enterotoxigenic Escherichia coli K88 via inhibition of the NF-kappa B and p38 mitogen-activated protein kinase signaling pathways in piglets. BMC Microbiol. 2016;16:8.
14. Li AL, Meng XC, Duan CC, Huo GC, Zheng QL, Li D. Suppressive effects of oral administration of heat-killed Lactobacillus acidophilus on T Helper-17 immune responses in a bovine beta-Lactoglobulin-sensitized mice model. Biol Pharm Bull. 2013;36:202–7.
15. Wang JJ, Li SH, Li AL, Zhang QM, Ni WW, Li MN, et al. Effect of Lactobacillus acidophilus KLDS 1.0738 on miRNA expression in in vitro and in vivo models of beta-lactoglobulin allergy. Biosci Biotechnol Biochem. 2018;82:1955–63.
16. Zheng JP, Cheng G, Li QY, Jiao SM, Feng C, Zhao XM, et al. Chitin oligosaccharide modulates gut microbiota and attenuates high-fat-diet-induced metabolic syndrome in mice. Mar Drugs. 2018;16.
17. Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, Velez E, Perdigon G. Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab. 2019;74:115–24.
18. Tsai YL, Lin TL, Chang CJ, Wu TR, Lai WF, Lu CC, et al. Probiotics, prebiotics and amelioration of diseases. J Biomed Sci. 2019;26.
19. Shin NR, Whon TW, Bae JW. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33:496–503.
20. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 2012;13.
21. Zhang HS, Wang HF, Shepherd M, Wen K, Li GH, Yang XD, et al. Probiotics and virulent human rotavirus modulate the transplanted human gut microbiota in gnotobiotic pigs. Gut Pathog. 2014;6.
22. Papamichael K, Konstantopoulos P, Mantzaris GJ. Helicobacter pylori infection and inflammatory bowel disease: is there a link? World J Gastroenterol. 2014;20:6374–85.
23. Yu Q, Zhang S, Li L, Xiong L, Chao K, Zhong B, et al. Enterohepatic helicobacter species as a potential causative factor in

inflammatory bowel disease: A meta-analysis. Medicine (Baltimore). 2015;94:e1773.
24. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012;336:1268–73.
25. Curtis MM, Hu Z, Klimko C, Narayanan S, Deberardinis R, Sperandio V. The gut commensal Bacteroides thetaiotaomicron exacerbates enteric infection through modification of the metabolic landscape. Cell Host Microbe. 2014;16:759–69.
26. Berni Canani R, De Filippis F, Nocerino R, Paparo L, Di Scala C, Cosenza L, et al. Gut microbiota composition and butyrate production in children affected by non-IgE-mediated cow's milk allergy. Sci Rep. 2018;8:12500.
27. Meehan CJ, Beiko RG. A phylogenomic view of ecological specialization in the Lachnospiraceae, a family of digestive tract-associated bacteria. Genome Biol Evol. 2014;6:703–13.
28. Esquivel-Elizondo S, Ilhan ZE, Garcia-Pena EI, Krajmalnik-Brown R. Insights into butyrate production in a controlled fermentation system via gene predictions. Msystems. 2017;2.
29. Sossai P. Butyric acid: What is the future for this old substance? Swiss Med Wkly. 2012;142:w13596.
30. Wang YY, Guo YL, Chen H, Wei H, Wan CX. Potential of Lactobacillus plantarum ZDY2013 and Bifidobacterium bifidum WBIN03 in relieving colitis by gut microbiota, immune, and antioxidative stress. Can J Microbiol. 2018;64:327–37.
31. Sagheddu V, Patrone V, Miragoli F, Puglisi E, Morelli L. Infant early gut colonization by lachnospiraceae: High frequency of Ruminococcus gnavus. Front Pediatr. 2016;4:57.
32. Zhao L, Zhang Q, Ma WN, Tian F, Shen HY, Zhou MM. A combination of quercetin and resveratrol reduces obesity in high-fat diet-fed rats by modulation of gut microbiota. Food Funct. 2017;8:4644–56.
33. Qi C, Li Y, Yu RQ, Zhou SL, Wang XG, Le GW, et al. Composition and immuno-stimulatory properties of extracellular DNA from mouse gut flora. World J Gastroenterol. 2017;23:7830–9.
34. Chen CY, Kao CL, Liu CM. The cancer prevention, anti-inflammatory and anti-oxidation of bioactive phytochemicals targeting the TLR4 signaling pathway. Int J Mol Sci. 2018;19.
35. Loganathan R, Nazeer M, Goda V, Devaraju P, Ali M, Karunakaran P, et al. Genetic variants of TLR4 and TLR9 are risk factors for chronic Helicobacter pylori infection in South Indian Tamils. Hum Immunol. 2017;78:216–20.
36. Kim JM, Oh YK, Kim YJ, Oh HB, Cho YJ. Polarized secretion of CXC chemokines by human intestinal epithelial cells in response to Bacteroides fragilis enterotoxin: NF-kappa B plays a major role in the regulation of IL-8 expression. Clin Exp Immunol. 2010;123:421–7.
37. Dong LN, Wang JP, Liu P, Yang YF, Feng J, Han Y. Faecal and mucosal microbiota in patients with functional gastrointestinal disorders: Correlation with toll-like receptor 2/toll-like receptor 4 expression. World J Gastroenterol. 2017;23:6665–73.
38. Chen L, Wilson JE, Koenigsknecht MJ, Chou WC, Montgomery SA, Truax AD, et al. NLRP12 attenuates colon inflammation by maintaining colonic microbial diversity and promoting protective commensal bacterial growth. Nat Immunol. 2017;18:541–51.
39. Lu CC, Kuo HC, Wang FS, Jou MH, Lee KC, Chuang JH. Upregulation of TLRs and IL-6 as a marker in human colorectal cancer. Int J Mol Sci. 2015;16:159–77.
40. Takeda K, Akira S. TLR signaling pathways. Semin Immunol. 2004;16:3–9.
41. Li AL, Sun YQ, Du P, Meng XC, Guo L, Li S, et al. The effect of Lactobacillus actobacillus peptidoglycan on bovine beta-Lactoglobulin-sensitized mice via TLR2/NF-kappa B pathway. Iran J Allergy Asthma Immunol. 2017;16:147–58.
42. Michels KR, Lukacs NW, Fonseca W. TLR activation and allergic disease: Early life microbiome and treatment. Curr Allergy Asthma Rep. 2018;18.
43. Vijaykrishnaraj M, Mohan Kumar BV, Muthukumar SP, Kurrey NK, Prabhasankar P. Antigen-specific gut inflammation and systemic immune responses induced by prolonging wheat gluten sensitization in BALB/c murine model. J Proteome Res. 2017;16, acs.jproteome.7b00199.
44. Saad MA, El-Sahhar AE, Arab HH, Al-Shorbagy MY. Nicorandil abates arthritic perturbations induced by complete Freund's adjuvant in rats via conquering TLR4-MyD88-TRAF6 signaling pathway. Life Sci. 2019;218:284–91.