J. Microbiol. Biotechnol. (2019), 29(11), 1739–1744
https://doi.org/10.4014/jmb.1812.12002

jmb

Immunomodulatory Effects by *Bifidobacterium longum* KACC 91563 in Mouse Splenocytes and Macrophages

Mijoo Choi[1], Yunjung Lee[1], Na-Kyoung Lee[2], Chun Ho Bae[3], Dae Chul Park[3], Hyun-Dong Paik[2*], and Eunju Park[1*]

[1]*Department of Food and Nutrition, Kyungnam University, Changwon 51767, Republic of Korea*
[2]*Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Republic of Korea*
[3]*Aram Co., Ltd., Gyeonggi-do 12735, Republic of Korea*

Received: December 3, 2018
Revised: August 30, 2019
Accepted: September 5, 2019

First published online:
September 9, 2019

*Corresponding authors
H.D.P.
Phone: +82-2-455-0381
Fax: +82-2-2049-6011
E-mail: hdpaik@konkuk.ac.kr
E.P.
Phone: +82-55-249-2218
Fax: +82-505-999-2104
E-mail: pej@kyungnam.ac.kr

pISSN 1017-7825, eISSN 1738-8872

The present study evaluates the immunomodulatory effect of *Bifidobacterium longum* KACC 91563 in murine primary splenocytes and macrophages. *B. longum* KACC 91563 regulated T- and B-cell proliferation and inhibited the Th1 (IL-2, IFN-γ)/Th2 (IL-4, IL-10) cytokine imbalance and immune cytokine production. Moreover, immunoglobulin E (IgE) levels were significantly lower after treatment with *B. longum* KACC 91563. These findings suggest that *B. longum* KACC 91563 could modulate the systemic immune system toward both IgE production and regulation of the Th1/Th2 balance.

Keywords: *Bifidobacterium longum* KACC 91563, cytokine, immunomodulatory effect, Th1/Th2 balance

Probiotics are now being used as prophylactic agents. *Lactobacillus* sp. and *Bifidobacterium* sp. are representative strains of probiotics [1]. Some probiotics, such as *Lactobacillus plantarum*, *Bifidobacterium breve*, and *Bifidobacterium longum W11*, can regulate the mucosal barrier, enterocytes, innate mucosal recognition (TLR), dendritic cells (DCs), T-cells, B-cells, and stem cells [2–6].

T helper (Th) cells play important roles in adaptive immune responses. Previous studies have classified Th cells as a dichotomy between Th1 and Th2 cells. However, recent studies have provided evidence that new subsets of Th cells (Th9, Th17, Th22, and Tregs) also exist, thus overturning the existing Th1-Th2 hypothesis [7, 8]. Th1 cells are responsible for cell-mediated immunity, producing IL-2, IL-12, and interferon (IFN-γ). Th2 cells regulate humoral immunity and produce IL-4, IL-6, IL-10, and TNF-α. An imbalance of Th1/Th2 can cause several immunological diseases, such as rheumatoid arthritis (Th1), allergies (Th2), and cancer (Th2). Thus, it is important to maintain a balance between Th1 and Th2 responses [9]. Recently, *Lactobacillus plantarum* CJLP133 and *Lactobacillus sakei* proBio65 were reported to help maintain Th1/Th2 balance and reduce the hypersensitivity caused by Th2 cells [10, 11].

Bifidobacterium longum KACC 91563, isolated from the feces of healthy neonates, can alleviate food allergies. It also has a therapeutic effect on skin conditions like atopic dermatitis. In addition, it has antioxidant and antihypertensive effects [12–15]. However, the immunomodulatory effects of *B. longum* KACC 91563 have not yet been reported. Therefore, the present study investigates the immunomodulatory effect of *B. longum* KACC 91563 on the Th1/Th2 balance in murine primary spleen cells and macrophages.

B. longum KACC 91563 was provided by the Rural Development Administration at the National Institute of Animal Science. Freeze-dried *B. longum* KACC 91563 was

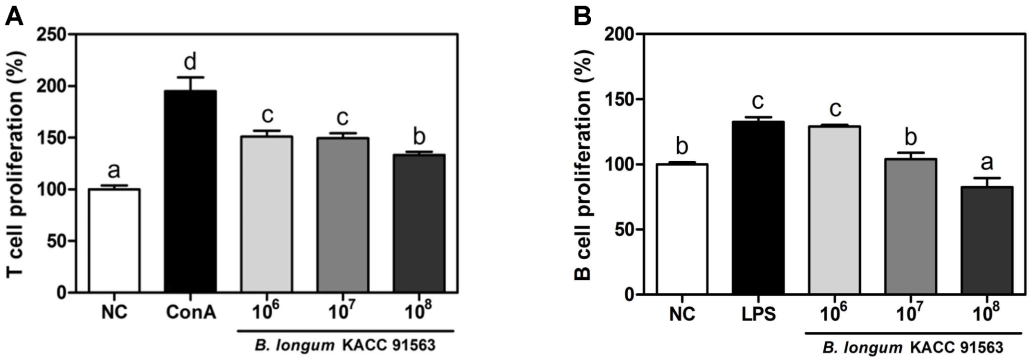

Fig. 1. Effects of *Bifidobacterium longum* KACC 91563 on T- and B-cell proliferation from splenocytes in Balb/c mice.
(**A**) Cell only, ConA; treated with concanavalin A (1 μg/ml), 10^6, 10^7, 10^8; treated with concanavalin A and different doses of *B. longum*. (**B**) Cell only, LPS; treated with lipopolysaccharide (1 μg/ml), 10^6, 10^7, 10^8; treated with lipopolysaccharide (1 μg/ml) and different doses of *B. longum*. All data are expressed as mean±standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $p < 0.05$.

manufactured by Mediogen, Inc. (Korea) and contained 100 billion colony-forming units (CFU)/g. Four-week-old male BALB/c mice (Koatech Animal Inc., Korea) were maintained in a ventilated room with a 12-h day-night cycle at a temperature of 25 ± 2°C and humidity of 50 ± 5%. The animal protocol (KUIAC-18-02) was approved by the Kyungnam University Instrumental Animal Care and Use Committee (Korea).

To obtain a sufficient number of macrophages, the mice were intraperitoneally injected with 2 ml of 4% thioglycollate medium. Three days later, the mice were sacrificed by cervical dislocation. Macrophages were harvested by peritoneal lavage with 15 ml of RPMI medium. Splenocytes were aseptically isolated from mice spleens and crushed by passage through a sterile plastic strainer. The separated splenocytes and macrophages were seeded at a concentration of 1×10^6 cells/well in 96-well plates and incubated for 12 h. Then, the splenocytes were treated with RPMI, lipopolysaccharide (LPS, 1 μg/ml), concanavalin A (Con A, 1 μg/ml), or *B. longum* KACC 91563 (10^6, 10^7, and 10^8 CFU/well) with mitogen. The macrophages were treated with zymosan, zymosan inhibitor, or *B. longum* KACC 91563 (10^6, 10^7, and 10^8 CFU/well).

Assays of T-cell and B-cell proliferation (EZ-CyTox, Daeil Lab Service, Korea) were performed using Con A or LPS as described by Park *et al*. [16]. Cytokines and IgE in the splenocytes were measured using the BD DuoSet ELISA Kit (BD Biosciences, USA) and the mouse IgE ELISA kit (LSBio, USA). The phagocytosis of peritoneal macrophages was assessed using the CytoSelect 96-well Phagocytosis Assay (zymosan substrate) Kit (Cell Biolabs, Inc., USA). Zymosan substrate contained in the kit was used to activate the macrophages. All experiments were conducted according to the manufacturers' instructions.

The experimental results were analyzed with Duncan's multiple range test after conducting a one-way analysis of variance using the SPSS statistical program (SPSS Statistics 18.0; SPSS, Inc., USA).

T-cell proliferation was increased in the splenocytes of the group treated with *B. longum* KACC 91563 compared to the ConA-treated splenocytes. B-cell proliferation in the splenocytes was significantly decreased (104.0 ± 4.8% and 82.6 ± 67.0%) in the group treated with *B. longum* KACC 91563 (10^7 and 10^8 CFU/well) compared to the LPS-treated splenocytes (Fig. 1).

The IL-2, IFN-γ, IL-4, IL-6, IL-10, and TNF-α production in cells treated with *B. longum* KACC 91563 was significantly lower than that in cells treated only with mitogen (Figs. 2A–2F).

IgE concentrations in the NC and groups treated with *B. longum* KACC 91563 were significantly lower than those of the LPS-treated group (Fig. 3).

The effects of *B. longum* KACC 91563 on macrophage phagocytosis are shown in Fig. 4. The phagocytic activities in the groups treated with *B. longum* KACC 91563 at 10^6-10^7 CFU/well were comparable to those in the NC+Zymosan group. However, treatment with *B. longum* KACC 91563 at 10^8 CFU/well had no significant effect on phagocytosis compared to the NC group.

The spleen works with the immune system and attacks external antibodies and infectious agents. Along with the lymph nodes, it plays a very important role in immune system function [17–19].

Con A is a mannose/glucose-binding plant lectin isolated

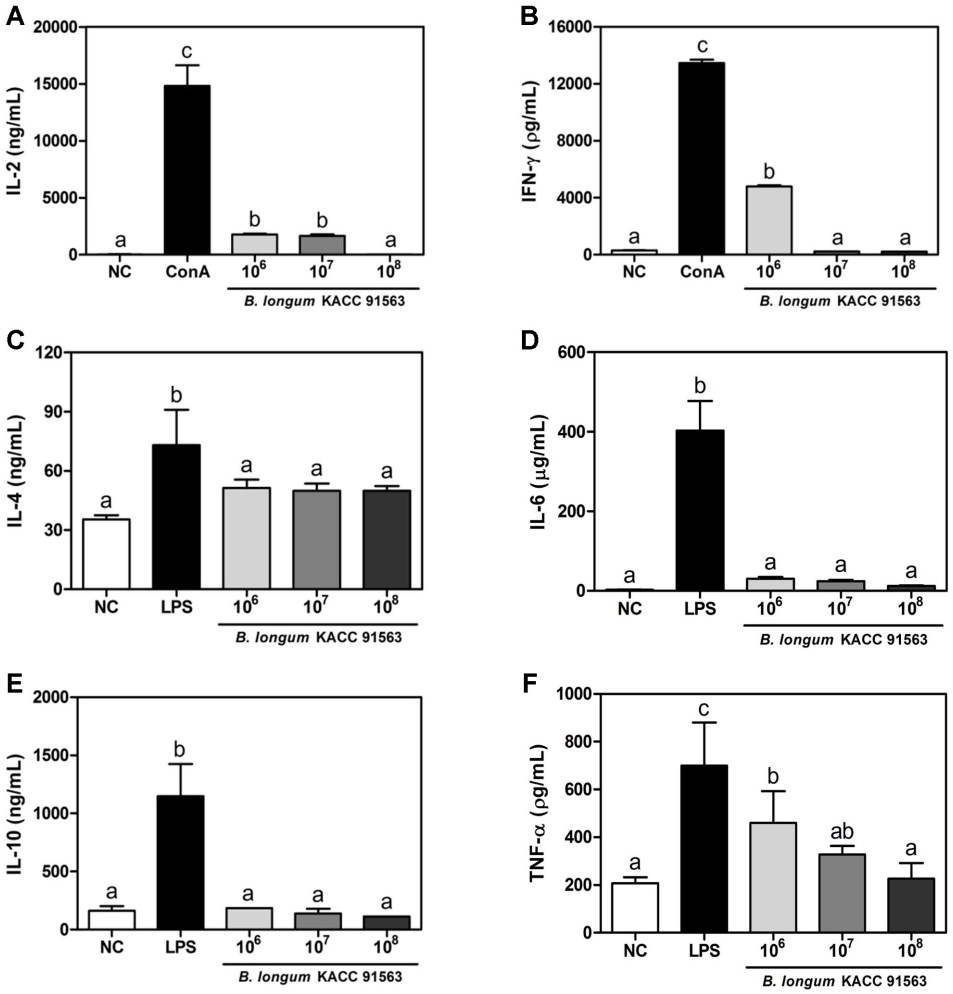

Fig. 2. Effects of *Bifidobacterium longum* KACC 91563 on Th1-type cytokine (IL-2 and IFN-γ) and Th2-type cytokine (IL-4, IL-6, IL-10, and TNF-α) production from splenocytes in Balb/c mice.

NC; cell only, ConA; treated with concanavalin A (1 μg/ml), LPS; treated with lipopolysaccharide (1 μg/ml), 10^6, 10^7, 10^8; treated with concanavalin A or lipopolysaccharide (1 μg/ml) and different doses of *B. longum*. All data are expressed as mean ± standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $p < 0.05$.

from Jack bean (*Canavalia ensiformis*) seed. It is well known as a selective T-cell mitogen that can activate the immune system, recruit T-lymphocytes, and regulate cytokine production [20]. LPS is a mitogen stimulant for B-lymphocytes. LPS induces the proliferation and differentiation of mature B-cells. It also induces the secretion of IL-4, IL-6, and IL-10 from B-cells [21–25].

T-cells in acquired immunity are divided into CD4+ T-cells and CD8+ killer T-cells. CD4+ T-cells have different functions determined by the presence of Th1 and Th2. CD8+ T-cells are cytotoxic T-cells that can directly attack natural killer cells, virus-infected cells, tumor cells, and abnormal cells [26, 27]. Th1-type cytokines stimulate phagocytosis mainly by increasing macrophage activity, while Th2-type cytokines stimulate B-cell activity and increase antibody production. The balance of immunity is maintained by the complementary regulation of Th1- and Th2-type cytokines [28–30].

Suppression of the Th2 lineage development decreases the level of antigen-specific IgE and total IgE [31]. Macrophages play an important role in the defense mechanism against host infection and the killing of tumor cells. Modulation of the antitumor properties of macrophages by various biological response modifiers is an area that is attracting

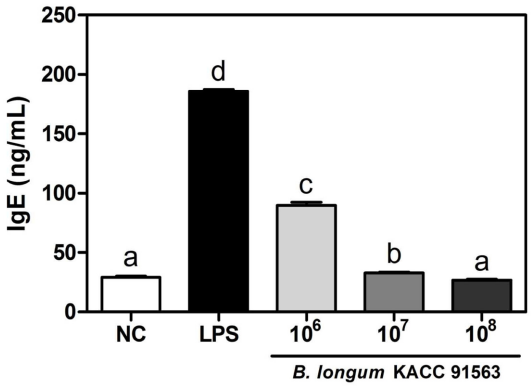

Fig. 3. Effects of *Bifidobacterium longum* KACC 91563 on immunoglobulin E production from splenocytes in Balb/c mice.

NC; cell only, LPS; treated with lipopolysaccharide (1 μg/ml), 10^6, 10^7, 10^8; treated with lipopolysaccharide (1 μg/ml) and different doses of *B. longum*. All data are expressed as mean ± standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $p < 0.05$.

much interest in the area of cancer chemotherapy, which can involve immunomodulation [32]. TNF-α secreted by activated macrophages is also an important cytokine that can regulate immune cell activity because it induces inflammation and apoptosis [33]. Macrophages can be classically activated (M1 macrophages) and alternatively

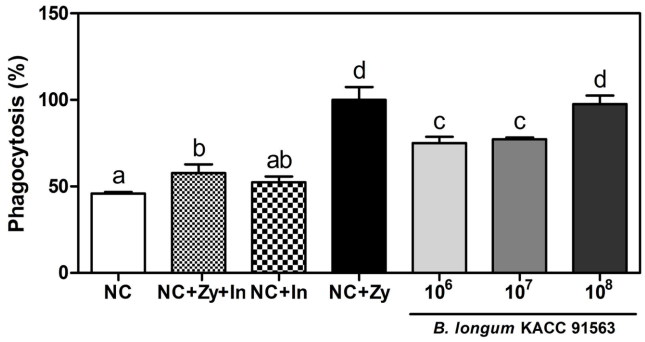

Fig. 4. Effects of *Bifidobacterium longum* KACC 91563 on phagocytosis activity from peritoneal macrophage in Balb/c mice.

NC; cell only, NC+Zy+In; NC+zymosan+zymosan inhibitor, NC+in; NC+zymosan inhibitor, NC+zy; NC+zymosan, 10^6, 10^7, 10^8; treated with different doses of *B. longum*. All data are expressed as mean ± standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $p < 0.05$.

activated (M2 macrophages) [34, 35]. M1 macrophages promote the Th1 immune system and M2 macrophages promote the Th2 immune response [36–38].

Several probiotic strains, such as *B. longum* and *L. johnsonii* have been reported to possess immunostimulatory properties, although most of them have been isolated from dairy or animal products [39, 40]. Hui *et al.* [41] found that B-cells stimulated with 100–10,000 ng/ml LPS produced significant levels of IL-4 and IL-10. Ren *et al.* [42] reported that treatment with *Bifidobacterium breve* decreased the production of ovalbumin-specific IgE and relieved type I allergy disease in mice. Our findings showed that *B. longum* KACC 91563 had modulatory effects on the Th1/Th2 balance in spleen cells and macrophages in vitro. It also modulated IgE levels. These results suggest that *B. longum* KACC 91563 has the potential to be used as an immunostimulant in immunodeficient subjects.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

Reference

1. Reid G. 2016. Probiotics: definition, scope and mechanisms of action. *Best Pract. Res. Clin. Gastroenterol.* **30:** 17-25.
2. Lee HA, Kim H, Lee KW, Park KY. 2016. Dead *Lactobacillus plantarum stimulates* and skews immune responsestoward T helper 1 and 17 polarizations in RAW 264.7 cells and mouse splenocytes. *J. Microbiol. Biotechnol.* **26:** 469-476.
3. Ren J, Zhao Y, Huang S, Lv D, Yang F, Lou L, *et al.* 2018. Immunomodulatory effect of *Bifidobacterium breve* on experimental allergic rhinitis in BALB/c mice. *Exp. Ther. Med.* **16:** 3996-4004.
4. Inturri R, Mangano K, Santagati M, Intrieri M, Di Marco R, Blandino G. 2017. Immunomodulatory effects of *Bifidobacterium longum* W11 produced exopolysaccharide on cytokine production. *Curr. Pharm. Biotechnol.* **18:** 883-889.
5. Choi HJ, Lee NK, Paik HD. 2015. Health benefits of lactic acid bacteria isolated from kimchi, with respect to immunomodulatory effects. *Food Sci. Biotechnol.* **24:** 783-789.
6. Prescott SL, Björkstén B. 2007. Probiotics for the prevention or treatment of allergic diseases. *J. Allergy Clin. Immunol.* **120:** 255-262.
7. Araujo-Pires AC, Francisconi CF, Biguetti CC, Cavalla F, Aranha AM, Letra A, *et al.* 2014. Simultaneous analysis of T helper subsets (Th1, Th2, Th9, Th17, Th22, Tfh, Tr1, and Tregs) markers expression in periapical lesions reveals multiple cytokine clusters accountable for lesions activity and inactivity status. *J. Appl. Oral. Sci.* **22:** 336-346.

8. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. 2006. Th17: An Effector CD4 T cell lineage with regulatory T cell ties. *Immunity* **24:** 677-688.
9. Kidd P. 2003. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern. Med. Rev.* **8**: 223-246.
10. Lim JH, Seo BJ, Kim JE, Chae CS, Im SH, Hahn YS, *et al.* 2011. Characteristics of immunomodulation by a *Lactobacillus sakei* proBio65 isolated from Kimchi. *Korean J. Microbiol. Biotechnol.* **39:** 313-316.
11. Won TJ, Kim B, Song DS, Lim YT, Oh ES, Lee DI, *et al*. 2011. Modulation of Th1/Th2 balance by *Lactobacillus* strain isolated from kimchi via stimulation of macrophage cell line J774A.1 in vitro. *J. Food. Sci*. **76:** H55-H61.
12. Bae CH, Lee JW, Park HJ, Nam KI, Kim JH, Park JS, *et al*. 2018. Therapeutic effects of *Bifidobacterium longum* KACC 91563 on Dncb-induced atopic dermatitis-like skin lesions in Nc / Nga mice. *Int. J. Food Nutr. Sci.* **5:** 30-37.
13. Kim JH, Jeun EJ, Hong CP, Kim SH, Jang MS, Lee EJ, *et al.* 2016. Extracellular vesicle derived protein from *Bifidobacterium longum* alleviates food allergy through mast cell suppression. *J. Allergy Clin. Immunol.* **137:** 507-516.
14. Kim HW, Jeong SG, Ham JS. 2016. Functional properties *Bifidobacterium longum* and their incorporation into cheese making process. *J. Milk Sci. Biotechnol.* **34:** 75-82.
15. Song M, Park WS, Yoo J, Han GS, Kim BM, Song PN, *et al.* 2017. Characteristics of Kwark cheese supplemented with *Bifidobacterium longum* KACC 91563. *Korean J. Food Sci. An.* **37:** 773-779.
16. Park SJ, Lee D, Lee M, Kwon HO, Kim H, Park J, *et al*. 2018. The Effects of curcuma longa l., purple sweet potato, and mixtures of the two on immunomodulation in C57BL/6J mice infected with LP-BM5 murine leukemia retrovirus. *J. Med. Food*. **21:** 689-700.
17. Iida R, Saito K, Yamada K, Basile AS, Sekikawa K, Takemura M, *et al.* 2000. Suppression of neurocognitive damage in LP-BM5-infected mice with a targeted deletion of the TNF-alpha gene. *FASEB J*. **14:** 1023-1031.
18. Liang B, Wang JY, Watson RR. 1996. Murine AIDS, a key to understanding retrovirus-induced immunodeficiency. *Viral. Immunol*. **9:** 225-239.
19. Odeleye OE, Eskelson CD, Watson RR. 1992. Change in hepatic lipid composition after infection by LP-BM5 murine leukemia virus causing murine AIDS. *Life Sci*. **51:** 129-134.
20. Lei HY, Chang CP. 2009. Lectin of concanavalin A as an anti-hepatoma therapeutic agent. *J. Biomed. Sci.* **16:** 10. doi: 10.1186/1423-0127-16-10.
21. Sweet MJ, Hume DA. 1996. Endotoxin signal transduction in macrophages. *J. Leukoc. Biol.* **60:** 8-26.
22. Mosier DE, Subbarao B. 1982. Thymus-independent antigens: complexity of B-lymphocyte activation revealed. *Immunol. Today.* **3:** 217-222.
23. Hobbs MV, McEvilly RJ, Koch RJ, Cardenas GJ, Noonan DJ. 1991. Interleukin-6 Production by Murine 6 Cells and B Cell Lines. *Cell. Immunol.* **132:** 442-450.
24. O'Garra A, Chang R, Go N, Hastings R, Haughton G, Howard M. 1992. Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. *Eur. J. Immunol.* **22:** 711-717.
25. Burger C, Vitetta ES. 1991. The response of B cells in spleen, Peyer's patches, and lymph nodes to LPS and IL-4. *Cell. Immunol.* **138:** 35-43.
26. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, *et al*. 2009. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat. Immunol.* **10:** 29-37.
27. Furtado GC, Curotto de Lafaille MA, Kutchukhidze N, Lafaille JJ. 2002. Interleukin 2 signaling is required for CD4 (+) regulatory T cell function. *J. Exp. Med.* **196:** 851-857.
28. Cher DJ, Mosmann TR. 1987. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by Th1 clones. *J. Immunol.* **138:** 3688-3694.
29. Teh HS, Kisielow P, Scott B, Kishi H, Uematsu Y, Blüthmann H, *et al.* 1988. Thymic major histocompatibility complex antigens and the αβ T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature* **335:** 229-233.
30. Powrie F, Coffman RL. Cytokine regulation of T-cell function: Potential for therapeutic intervention. 1993. *Immunol. Today* **14:** 270-274.
31. Park MB, Ko E, Ahn C, Choi H, Rho S, Shin MK, *et al.* 2004. Suppression of IgE production and modulation of Th1/Th2 cell response by electroacupuncture in DNP-KLH immunized mice. *J. Neuroimmunol.* **151:** 40-44.
32. Kang NS, Park SY, Lee KR, Lee SM, Lee BG, Shin DH, *et al*. 2002. Modulation of macrophage function activity by ethanolic extract of larvae of Holotrichia diomphalia. *J. Ethnopharmacol*. **79:** 89-94.
33. Fernández-Ortega C, Dubed M, Ramos Y, Navea L, Alvarez G, Lobaina L, *et al*. 2004. Non-induced leukocyte extract reduces HIV replication and TNF secretion. *Biochem. Biophys. Res. Commun*. **325:** 1075-1081.
34. Wynn TA, Chawla A, Pollard JW. 2013. Macrophage biology in development, homeostasis and disease. *Nature* **496:** 445-455.
35. XQ W, Dai Y, Yang Y, Huang C, Meng XM, BM W, *et al*. 2016. Emerging role of microRNAs in regulating macrophage activation and polarization in immune response and inflammation. *Immunology* **148:** 237-248.
36. Tan HY, Wang N, Li S, Hong M, Wang X, Feng Y. 2016. The reactive oxygen species in macrophage polarization: Reflecting its dual role in progression and treatment of human diseases. *Oxid. Med. Cell. Longev.* **2016:** 2795090.
37. Gordon S, Martinez FO. 2010. Alternative activation of macrophages: Mechanism and functions. *Immunity* **32:** 593-604.

38. Gordon S, Plüddemann A, Martinez Estrada F. 2014. Macrophage heterogeneity in tissues: Phenotypic diversity and functions. *Immunol. Rev.* **262:** 36-55.
39. Kaburagi T, Yamano T, Fukushima Y, Yoshino H, Mito N, Sato K. 2007. Effect of Lactobacillus johnsonii La1 on immune function and serum albumin in aged and malnourished aged mice. *Nutrition* **23:** 342-350.
40. Mu llié C, Yazourh A, Thibault H, Odou MF, Singer E, Kalach N, *et al.* 2004. Increased poliovirus-specific intestinal antibody response coincides with promotion of *Bifidobacterium longum*-infantis and Bifidobacterium breve in infants: a randomized, double-blind, placebo-controlled trial. *Pediatr. Res*. **56:** 791-795.
41. Hui Xu, Lip Nyin Liew, I Chun Kuo, Chiung Hui Huang, Denise Li-Meng Goh, Kaw Yan Chua. 2008. The modulatory effects of lipopolysaccharide-stimulated B cells on differential T-cell polarization. *Immunology* **125:** 218-228.
42. Ren J, Zhao Y, Huang S, Lv D, Yang F, Lou L, *et al.* 2018. Immunomodulatory effect of *Bifidobacterium* breve on experimental allergic rhinitis in BALB/c mice. *Exp. Ther. Med*. **16:** 3996-4004.