Molecular Immunology 103 (2018) 71–77

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Probiotics SOD inhibited food allergy via downregulation of STAT6-TIM4 signaling on DCs

Bo Yang[a,b], Yan Luo[b], Zhigang Liu[b], Pingchang Yang[b,*], Yaoting Gui[a,*]

[a] Guangdong and Shenzhen Key Laboratory of Male Reproductive Medicine and Genetics, Institute of Urology, Peking University Shenzhen Hospital, Shenzhen PKU-HKUST Medical Center, Shenzhen, 518036, China

[b] Institute of Allergy and Immunology, School of Medicine, Shen Zhen University, Shen Zhen 518060, China

ARTICLE INFO

Keywords:
Food allergy
Probiotics
SOD
STAT6
TIM4

ABSTRACT

Bacterial probiotics are of increasing use against intestinal disorders such as food allergy. However, the detailed molecular mechanism underlying probiotics-mediated anti-allergic effect remains unknown. In the present study, we orally treated OVA-sensitized mice with *Bifidobacterium infantis* (BB) for two weeks. It was found that OVA specific-IgE and-IgG levels in serum were significantly decreased after BB administration. BB treatment also significantly reduced the release of IL-4, -5, -13 in splenocytes. Besides, after challenge with OVA, the occurrence of temperature drop and diarrhea was dramatically reduced in BB group. Moreover, the protective effect of BB on allergic mice was correlated with its antioxidative enzyme, superoxide dismutase (SOD). The antioxidative effect of BB on Dendritic cells (DCs0 was further demonstrated to be mediated by cAMP/PKA signaling. We also found that the mRNA and protein expression levels of TIM4 were attenuated in BB group. Finally, ChIP-qPCR assay studies indicate that BB reduced the binding of STAT6 to its response elements in the TIM4 promoter. In conclusion, orally administration of BB protected allergic mice via attenuation of oxidative stress, which further reduced TIM4 expression by inhibiting its transcription factor STAT6.

1. Introduction

Food allergy is a major public health problem in the world with dramatically increasing prevalence (Tang and Mullins, 2017; Leung et al., 2018). In current, avoidance of dietary allergens is the only proven remedy available for food allergic suffers. Thus, it is indeed in need to develop safe and effective therapies. Recently, both clinical and laboratory researches have highlighted that probiotics treatment is a promising nonspecific method for food allergy. For example, a meta-analysis of 17 trials (2947 infants) indicated that a combined pre- and postnatal probiotics treatment reduced the risk of food sensitization (Zhang et al., 2016). Probiotics supplementation in lactating women was demonstrated to induce TGF-β and IL-10 elevation in breast milk (Prescott et al., 2008). In murine models, probiotics could ameliorate food allergy by reducing Th2 polarization and inducing $Foxp3^+$ Treg (Barletta et al., 2013; Lyons et al., 2010). Besides, probiotics were also demonstrated to induce mast cell apoptosis via extracellular vesicle-derived protein (Kim et al., 2015). In this way, the World Allergy Organization (WAO) suggests to use probiotics in pregnant and lactating women and infants (Fiocchi et al., 2015). However, specific advice on choice of strains, dose, timing, mode of administration and duration is not possible to give due to the great heterogeneity between studies performed so far. Therefore, further mechanistic studies of probiotics are required to translate the WAO recommendation into practice guidelines.

Accumulating studies showed that oxidative stress mediated a crucial effect in the pathogenesis of allergic diseases, including asthma, rhinitis and food allergy. Either intracellular (Koike et al., 2007) or extracellular (Lee et al., 2010) antioxidant could ameliorate asthma by suppressing IgE, Th2 cytokines, eosinophilia, and mucus hypersecretion in a mouse model. Furthermore, reducing agents were demonstrated to decrease ROS production and attenuated airway inflammation via modulation of NFκB activity and HIF-1α level (Lee et al., 2004, 2006).

Early in 2002, some strains of probiotics have been identified to be antioxidative, as they contained a remarkable level of glutathione and Mn-SOD (Kullisaar et al., 2002). Probiotics consumption could significantly improve Th1/Th2 immune homeostasis, and intracellular levels of antioxidant redox enzymes, including glutathione peroxidase (GPx), catalase (CAT) and SOD (Sharma et al., 2014). Another investigation suggested that oral administration of *Bifidobacterium breve*

* Corresponding author at: Guangdong and Shenzhen Key Laboratory of Male Reproductive Medicine and Genetics, Institute of Urology, Peking University Shenzhen Hospital, Shenzhen PKU-HKUST Medical Center.

E-mail addresses: pcyang2356@163.com (P. Yang), guiyaoting2007@163.com (Y. Gui).

https://doi.org/10.1016/j.molimm.2018.09.001
Received 6 November 2017; Received in revised form 3 September 2018; Accepted 4 September 2018

strain Yakult prevented the UV-induced increase in hydrogen peroxide levels, oxidation of proteins and lipids, and xanthine oxidase activity in the skin (Ishii et al., 2014). However, whether the antioxidative capacity of probiotics plays a role in the attenuation of food allergy is completely unknown.

DCs are key antigen presenting cells strategically localize at tissue niches. Of note, ROS have been proposed to act as second messengers in DCs functionality and generation (Sheng et al., 2010). Ebselen, a specific inhibitor of ROS, have been identified to disrupt bidirectional DC-T cell activation processes duiring Ag presentation (Matsue et al., 2003). Based on literatures, environmental factors and viral infections (e.g. LPS) are known to cause ROS production and exacerbate allergic inflammation (Qin et al., 2015). In the other hand, cholera toxin (CT), commonly used as an adjuvant in allergic model, was shown to modulate DC differentiation by cAMP-mediated pathways (la Sala et al., 2009). Moreover, it was found that cAMP analog could stimulate ROS formation (Tai and Ascoli, 2011). Thus, we hypothesized that CT might also facilitate ROS production in DCs via cAMP pathway.

In the present study, we demonstrated that administration of a single strain of human probiotic bacteria, BB, suppressed OVA-induced food allergy in mice via its anti-oxidative effect. Furthermore, we used DCs to dissect the underlying molecular mechanism mediated by BB. It was found that the STAT6/TIM4 pathways were involved in the anti-allergenesity of BB.

2. Materials and methods

2.1. Animals

All the animal experimental procedures were approved by the ethics committee of Shenzhen University, and conducted according to the guidelines published by the US National Institutes of Health (NIH publication no.85-23, revised 1996). 6–8 weeks old female Balb/c mice were housed in a SPF animal facility with a 12 h-12 h light-dark cycle and were free to access OVA-free diet and water.

Balb/c mice were randomly divided into seven groups: 1) *Naïve group*, the mice were treated with normal saline; 2) *CT group*, the mice were treated with CT; 3) *CTPro group*, the mice were treated with CT and BB; 4) *FA group*, the mice were treated with OVA and CT; 5) *FAPro group*, the mice were treated with OVA, CT and BB; 6) DDC_{low} *group*, the mice were i.p. injected with 400 mg/kg DDC (diethyldithiocarbamate, an inhibitor of SOD) before BB treatment; 7) DDC_{high} *group*, the mice were i.p. injected with 800 mg/kg DDC before BB treatment.

2.2. Food allergy animal model

OVA-sensitized food allergic model was established with published procedures in our lab (Yang et al., 2014). Briefly, mice were orally administered 100 μg OVA mixed with 20 μg CT once a week for 4 consecutive weeks. When the period of sensitization was finished, the mice were orally challenged with 5 mg OVA. After 24 h, mice were killed, the serum was collected and splenocytes were harvested for the following immunoglobulin or cytokines analysis. The rectal temperature was measured by digital thermometer every 5 min during the first hour post challenge. The occurrence of diarrhea was observed within the first hour post challenge.

2.3. BB and DDC supplementation

BB powder (SQC013-2, Kexing Biotech Company Limited, Shenzhen, China) was cultured in TPY medium for 16 h at 37 °C on the day before use. The probiotics were centrifuged at 1400 rpm for 5 min and 10^9 organisms were resuspended in 500ul normal saline. From Day 15 to Day 28, the mice were orally administered with 10^8 cfu of living BB every other day. The control mice were given same volume of normal saline.

For inhibitor treatment, low (400 mg/kg b.w., s.c.) or high dose (800 mg/kg b.w., s.c.) of DDC was administered daily for 14 days (Day 15 to Day 28) (Gaafa et al., 2011). On day 29, all the mice were challenged and subjected to experiments as described above.

2.4. Histological analysis

A segment of the intestinal was excised and fixed with 4% paraformaldehyde overnight. The tissue was processed for paraffin sections. The sections were stained with hematoxylin and eosin. To identify mast cell infiltration in intestinal mucosa, toluidine blue staining was performed as previously described (Yang et al., 2013a). Briefly, several drops of staining solution (toluidine blue stain dissolved in 70% ethanol) were applied on intestinal section for 90 s and then washed away quickly with running tap water. The tissue structure and inflammation of intestinal mucosa were observed under a light microscope. To avoid the observer bias, the sections were coded; the observers were not aware of the code. The number of eosinphils cells per mm^2 was quantified and at least 8 fields per mouse were counted.

2.5. Splenocytes culture for cytokines measurement

After antigen challenge, the mice were sacrificed and splenocytes suspension were prepared as described previously (Castillo-Courtade et al., 2015). 5×10^6 cells/well were cultured in RPMI1640 medium and stimulated with or without OVA (100 ug/ml) for 72 h. The supernatants were then collected and stored in −80 °C for the following cytokines measurement.

2.6. ELISA

Total IgE, OVA-specific IgE, and IL-4, IL-5, IL-13 was detected by commercial ELISA kit (Biolegend, USA) according to the manufacturer's instructions.

2.7. Bone marrow-derived dendritic cells culture

Bone marrow-derived dendritic cells (BMDCs) were isolated and cultured as previously described (Yang et al., 2016). Briefly, after the mice were anaesthetized, the whole femurs and tibiae were removed and the attached muscle tissues were scraped. The ends of the bones were gently cut off, and the bone marrow was flushed out with medium using a 27.5-gauge needle and a 5-ml syringe. The resulting cells suspension was subjected to drip through a 70 μm cell strainer, and the red cells were removed by ACK buffer. After the cells were washed with medium twice, the cells were seeded in 6-well plates and stimulated with 10 ng/ml IL-4 and GM-CSF. On day 3 and day 6, the medium was removed and replaced with fresh medium containing 10 ng/ml IL-4 and GM-CSF. On day 8, $CD11c^+$ DCs were isolated with MACS and stimulated with CT (range from 0.002 to 20 ug/ml) in the presence or absence of BB lysates or conditioned medium for 24 h.

2.8. Thiobarbituric acid reactive substance (TBARS) assay

In order to determine the oxidative stress level in the intestinal tissues from allergic mice or BMDCs treated with CT, the commercial TBARS assay kit (Cayman Chemical Company, MI, USA) was used to measure the concentrations of the lipid peroxidation product, malondialdehyde (MDA), according to the instructions from manufacturer. The results were calculated against the total protein contents in each sample.

2.9. Western blot

Total proteins from BMDCs were prepared and separated by SDS-PAGE as previously described (Yang et al., 2016). The proteins were