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Butyricoccus plays a key role in mediating the antagonism between probiotic and antibiotic on food allergy

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ABSTRACT

Food allergy is a severe food safety problem worldwide. Probiotics are effective in alleviating food allergy whereas antibiotics may exacerbate allergenic responses, but the knowledge on the interplay between them during food allergy is limited. In the present study, we investigate the activity of probiotic strain *Lactobacillus casei* (Lc) and antibiotic cocktail (Abx) on in a food allergy mouse model. The results showed that Lc relieved allergenic symptom and changed allergen-specific antibodies isotypes. Moreover, Lc upregulated chemokines in the spleen, and promoted regulatory CD103⁺ DC and IgA-producing B cell proliferation. In contrast, the application of Abx eliminated all these beneficial effects of Lc. Microbiota analysis revealed that the commensal genus *Butyricoccus* was a critical mediator in the process. These results revealed the mechanisms of Lc and Abx interaction on food allergy and provided *Butyricoccus* as a potential therapeutic target for food allergy.

Abbreviations: Abx: antibiotic; CTB: recombinant cholera toxin subunit B; FACS: fluorescence-activated cell sorting; IG: intragastric gavage; Lc: *Lactobacillus casei*; MLN: mesenteric lymph node; PCA: principal component analysis; TM: shrimp tropomyosin

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Food allergy; probiotic; antibiotic; *Lactobacillus casei*; *Butyricoccus*

1. Introduction

Food allergy is an adverse immune response triggered by usually harmless food proteins termed allergens. In the last few decades, the prevalence of allergy has been increasing especially in developed countries, which could be due to the disorder of immune system in hygienic environment, the intake of unbalance diet, the change of microbiota, the abuse of antibiotics, and so forth (Roberts et al., 2018). The most effective method to prevent from food allergy is avoiding contacting with allergens, however, due to the development of food industry and the increasing kind of allergens, it is almost unrealistic

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to totally avoid all the allergens. Therefore, relevant therapeutic approaches are in urgent need. Oral immunotherapy is a promising way to treat food allergy, but the effect is limited nowadays (Ciardiello et al., 2013). On the other hand, targeted therapeutic approaches might find another way to treat food allergy, which is based on the clarification of food allergy mechanisms and the identification of critical targets (Yu, Freeland, & Nadeau, 2016).

The intestinal microbiota has been demonstrated to be tightly involved in food allergy processing and therefore is a promising therapeutic target (Ho & Bunyavanich, 2018; Marrs & Sim, 2018). The disorder of the whole microbiota structure has been connected with the occurrence of several diseases such as diabetes, obesity and allergy (Gholizadeh et al., 2019). Moreover, particular commensal bacterial strains have been demonstrated to be required for the suppression or exacerbation of specific diseases. For instance, the commensal species *Bacteroides thetaiotaomicron* has been demonstrated to be a target of short-chain fat acid for regulatory T cell induction and inflammatory bowel diseases therapy (Furusawa et al., 2013). Besides, the relevant proportion of two dominant bacterial divisions, *Bacteroidetes* and *Firmicutes*, has been shown to be associated with obesity (Turnbaugh et al., 2006). However, similar study on food allergy is rare.

Probiotics are safe bacteria that benefit host health and have been proposed as a novel approach for treating immunological diseases including food allergies (Majamaa & Isolauri, 1997; Peltto, Isolauri, Lilius, Nuutila, & Salminen, 1998). A recent study has shown that the combination of probiotics and oral immunotherapy results in a significant therapeutic effect that can last for more than four years (Hsiao et al., 2017). Microbiota is considered as a predominant target of probiotics, the application of probiotics can regulate both the whole microbiota composition or a specific commensal bacterial strain, resulting in the subsequent modulation of host health (Fu, Song, Wang, Fu, & Wang, 2017). *Lactobacillus casei* (Lc) isolated from traditionally homemade koumiss has been intensively studied and shown various beneficial health effects such as ameliorating intestinal microflora, anti-inflammation, and stimulating metabolism (Fontenla De Petrino, Bibas Bonet, Meson, & Perdigon, 2002; Wang et al., 2013, 2016; Zhang, Wang, Guo et al., 2014; Zhang, Du, Wang, & Zhang, 2010; Zhang, Wang, Zhang et al., 2014; Zhong, Zhang, Du, Meng, & Zhang, 2012). Our recent studies showed that Lc could alleviate food allergy through the NF- κ B-dependent epithelial-dendritic-T-B cell axis. However, whether microbiota participates in the process is unknown yet.

On the contrary, antibiotic (Abx) is another common modulator of microbiota but usually results in dysbiosis and thus does harm to host health (Keeney, Yurist-Doutsch, Arrieta, & Finlay, 2014; Vangay, Ward, Gerber, & Knights, 2015). Furthermore, Abx may impair or even reverse the beneficial effect of probiotics. Although some studies showed that the application of particular probiotics (including Lc) may restore the Abx-impaired microbiota homeostasis (De Petrino, De Jorrat, De Budeguer, & Perdigon, 1997), a recent study showed that after Abx administration, the application of probiotic will impair, rather than facilitate, the Abx-distorted gut mucosal host-microbiome homeostasis, implying that Abx antagonizes probiotic function (Suez et al., 2018). However, how these antagonism and synergy comes from is not fully understood.

Overall, the objective of this work has been to assess the antagonism between Abx and Lc on modulating food allergy and investigate the underlying mechanisms. Toward this aim, a shrimp tropomyosin (TM, a representative food allergen (Mejrhith et al., 2017))

induced food allergy mouse model was established, thereafter Lc and Abx were applied to the model. Based on this approach, we found that Abx impaired Lc function through modulating host cytokines, immune cells and microbiota. By using statistical analysis, the commensal bacterial genus *Butyricoccus* was revealed as a potential mediator. These results provide *Butyricoccus* as a novel target for treating food allergy, promoting probiotics function, and relieving the adverse effect of Abx abuse.

2. Materials and methods

2.1. Ethics approval and consent to participate

This study was carried out in strict accordance with the recommendations of the National Guidelines for the Care and Use of Laboratory Animals of China. All the animal procedures were approved by the Zhejiang Gongshang University Laboratory Animal Welfare Ethics Review Committee.

2.2. Protein and probiotics

TM was extracted and purified as previously described from shrimp (*Litopenaeus vannamei*) (Fu, Peng et al., 2017). The protein content and purity of the fractions were determined using the BCA Protein Assay Kit (KeyGEN BioTECH, China) and SDS-PAGE, respectively.

Lactobacillus casei (strain Zhang) was kindly provided by Prof. Zhang (Inner Mongolia Agricultural University, China) as a lyophilized powder with porous dextrin at the live total bacteria concentration of 5×10^8 CFU/g. The bacterium was reconstituted in sterile PBS (5×10^8 CFU/mL) for *in vivo* oral administration. Simultaneously, dry porous dextrin reconstituted in sterile PBS (1 g/mL) was served as placebo.

2.3. In vivo mouse model

Sixty female BALB/c mice (aged 6–8 weeks, weighted 18–22 g, 10 mice each group) were purchased from and housed in the Laboratory Animal Center of Hangzhou Normal University (Hangzhou, China) and fed a basic diet (XIETONG ORGANISM, China). The mice were treated following the sensitization protocol according to a previous study (Liu et al., 2017) (Figure 1(A)). In brief, for TM sensitization, each mouse was immunized by intragastric gavage (IG) administration of purified TM (3 mg for sensitization and 9 mg for challenging each time), together with recombinant cholera toxin subunit B (CTB, 250 µg each time) as an adjuvant in a total of 200 µL PBS (TM group). The adjuvant control group (Ctrl group) received an equal dose of CTB without TM. For Lc treatment, each mouse was administered with 200 µL PBS containing 10^8 live bacteria by IG daily as indicated, and the other groups were treated with an equal volume of placebo instead. For Abx treatment, an antibiotic cocktail (1 mg/mL kanamycin, 0.09 mg/mL gentamycin, 0.56 mg/mL colistin, 0.54 mg/mL metronidazole and 0.11 mg/mL vancomycin, hereafter named Abx) was mixed into the TM and administrated by IG as indicated. All mice were sacrificed more than 6 h after the last challenge, and the serum and organ samples were collected. Fresh blood samples were incubated at 37°C for 30 min to facilitate clotting, then centrifuged at 3000 rpm for 10 min, and the upper serum was taken for further analysis.

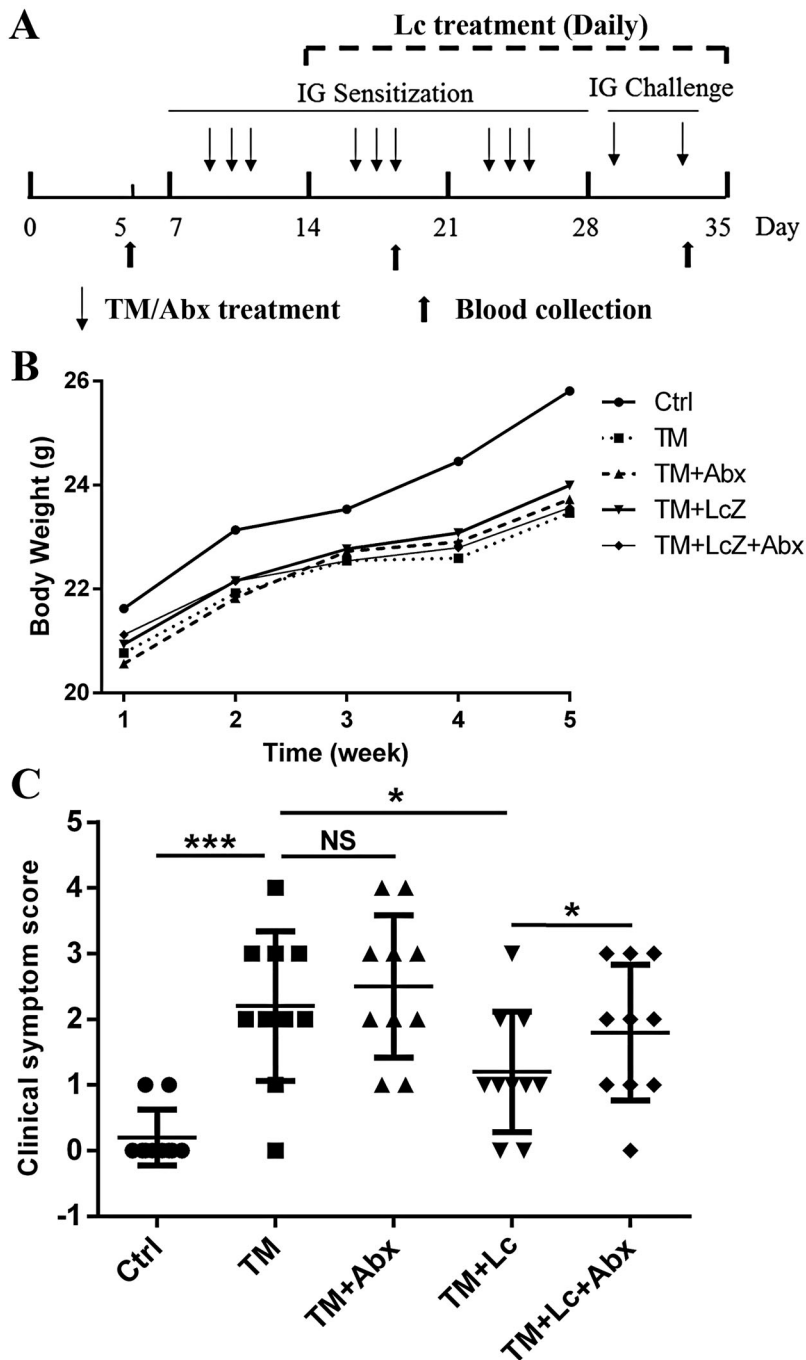


Figure 1. Antagonism between Lc and Abx on TM-induced food allergy. (A) Protocol of the in vivo mouse model. (B) Average weight changes of mice in each group. (C) The score of clinical symptoms combined with anaphylaxis and diarrhoea after the last challenge. The results are represented as the mean $\pm$ SD (* p < .05; *** p < .001; NS, not significant).

2.4. Clinical symptoms evaluation

The appearance of clinical symptoms combined with anaphylaxis and diarrhoea was observed during 30–60 min after the last challenge. Symptoms were evaluated according to a scoring system combining the systems reported by Tan et al. (2016) and Zhang et al. (2017) as follows: 0, no symptoms, normal stools; 1, repetitive mouth/ear scratching and ear canal digging with hind legs, a few wet and unformed stools; 2, decreased activity, self-isolation, and puffiness around eyes and/or mouth, wet and unformed stools with some yellow mucus; 3, periods of motionlessness for more than 1 min, lying prone on stomach, and decreased activity, more wet and unformed stools with a lot of yellow mucus; 4, no response to whisker stimuli and reduced or no response to prodding, a larger number of wet and unformed stools with moderate perianal staining of the coat; 5, tremor, convulsion, and death, severe watery stool with severe perianal staining of the coat.

2.5. Quantification of antibodies and cytokines

TM-specific serum IgE, IgG2a and IgA were measured by ELISA as previously described (Fu, Song et al., 2017). Briefly, purified TM was coated on the 96-well-plate to capture specific antibodies in serum samples, then the immobilized antibodies were recognized by horseradish peroxidase-labeled goat monoclonal anti-mouse IgE, IgG2a or IgA second antibodies (Southern Biotechnologies Associates, USA) and detected using the substrate 3,3',5,5'-tetramethylbenzidine (eBioscience, USA). The absorbance was determined with a VersaMax microplate reader (Molecular Devices, USA) at 450 nm.

The concentrations of serum cytokines and chemokines were measured using a Bio-Rad Mouse 23-plex kit (Bio-Rad, USA) and a Bio-Plex MagPix Multiplex Reader (Bio-Rad, USA) according to the manufacturer's specification. The BioPlex Manager software's five-parameter logistic curve fitting (5PL) method was used for raw data analysis and calculation of concentrations.

2.6. Fluorescence-activated cell sorting

The percentages of specific cell types were determined by fluorescence-activated cell sorting (FACS). Single cell suspension prepared from spleen or mesenteric lymph node (MLN) was stained with indicated fluorescence-labeled antibodies, and detected using FACSCalibur flow cytometry (BD, USA). Specifically, germinal centre B cells were stained with B220 (Ms CD45R/B220 APC RA3-6B2), GL7 (Ms T-B Cell ACT ATG FITC GL7) and CD95 (Ms CD95 PE Jo2) antibodies; IgA-producing B cells were stained with B220 (Ms CD45R/B220 APC RA3-6B2) and IgA (ANTI-MOUSE IGA (11-44-2) PE) antibodies; DCs were stained with MHC-II (Ms I-A/I-E FITC 2G9), CD11c (Ms CD11c APC HL3) and CD103 (Ms CD103 PE M290) antibodies. Anti-IgA antibody was purchased from eBiosciences (USA), and all the other antibodies were purchased from BD Pharmingen (USA).

2.7. Microbiota analysis

16S rRNA sequencing and bioinformatics analysis of mouse faeces were operated by Beijing Novogene Technology Co., Ltd (China). Briefly, mouse faecal samples were

collected sterilely. Total genome DNA from faecal sample was extracted using CTAB/SDS method. DNA concentration and purity were monitored on 1% agarose gels. 16S rRNA genes of distinct regions (V3-V4/16S) were amplified using specific primers and Phusion High-Fidelity PCR Master Mix (New England Biolabs, USA). Sequencing libraries were generated using Ion Plus Fragment Library Kit (Thermo Fisher Scientific, USA) following manufacturer's recommendations. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, USA). At last, the library was sequenced on an Ion S5 XL platform and 400 bp/600 bp single-end reads were generated. All data were analysed by using the online after-sales service platform provided by Beijing Novogene Technology Co., Ltd (<https://magic.novogene.com/public/customer>).

2.8. Statistical analysis

All the statistical results were analysed in at least three replicates according to a completely randomized design. Statistical differences were analysed using Student's *t* test by GraphPad Prism 7.0 (GraphPad Software, USA), and the correlations were analysed using Pearson bivariate correlation statistics by SPSS 17.0 (IBM, USA). The results are expressed as mean $\pm$ SD. Those with $p < .05$ were considered significant and indicated by asterisks in the figures, unless otherwise stated.

3. Results and discussion

3.1. Antagonism between Lc and Abx on TM-induced food allergy

To investigate whether antibiotic abuse impairs immune homeostasis and probiotic function or not, we applied an antibiotic cocktail (Abx) to a previously established mouse model, in which shrimp tropomyosin (TM) induced food allergy was alleviated by the probiotic strain *L. casei* (Lc) (Figure 1(A)). Compared with that in the control group (Ctrl), the body weight gain in the TM sensitization group (TM) was slightly decreased, demonstrating that the overall health condition was impaired. Additionally, the application of Abx (TM + Abx group), probiotic (TM + Lc group) or both of them (TM + Lc + Abx group) on TM-sensitized mouse did not further influence the body weight gain significantly, indicating that the potential effects of Abx and Lc are more specific and delicate (Figure 1(B)).

Moreover, as revealed by our previous study, while TM sensitization triggered severe allergenic responses, Lc relieved the symptoms. In contrast, the application of Abx made the responses even severer, and the combination of Lc and Abx resulted in a moderate effect between applying Lc and Abx alone (Figure 1(C)). These results indicated that Lc and Abx exhibited antagonistic effects on food allergy, however, whether these two factors acting independently or interacting with each other requires far more investigations.

3.2. Antagonism between Lc and Abx on regulating antibody class switching

Antibodies are the major mediators in adaptive immunity, specifically, IgE mediates the Th2-direct allergenic process, IgG participates in the Th1-direct general immune response,

and IgA involves in immune tolerance (Tordesillas & Berin, 2018). Consequently, in addition to determining the apparent allergic symptoms, we also evaluated the TM-specific antibody isotype variation after Abx and Lc treatment. As shown in Figure 2, the serum IgE concentration was consistent with the degree of apparent allergic symptoms, which is upregulated by Abx and downregulated by Lc (Figure 2(A)). In contrast, the application of either Lc or Abx suppressed the production of IgG2a (Figure 2(B)). These results demonstrated that while Abx promoted Th2 response and inhibited Th1 response, Lc suppressed both of them. Interestingly, although not statistically significant, the administration of Lc eliminated the suppression effect of Abx on IgG2a production, resulting in a comparative IgG2a level as that of the Lc group (Figure 2(B)), indicating a complex cross-talk between Lc and Abx. Moreover, the serum TM-specific IgA was upregulated by Lc and further suppressed by Abx application (but not significant) (Figure 2(C)), which further implied the potential interaction between Lc and Abx.

3.3. Antagonism of Abx on Lc-induced chemokines production

To investigate how Abx and Lc regulate the immune system under allergenic conditions, we measured the cytokines production pattern in the spleen from mice of different groups. Twenty-three cytokines or chemokines were evaluated synchronously by BioPlex multiplex testing, and 22 of them were quantified (except for MIP-1 α which is hardly detected) (Figure 3). Significantly, 10 of the cytokines or chemokines were greatly upregulated in the Lc group compared with TM group, which included pro-inflammatory cytokines IL-1 α /2, pro-allergic cytokines IL-4/6, and regulatory cytokine IFN- γ , indicating that the whole immune responses in spleen were upregulated. Notably, several chemokines including KC (CXCL1), RNATES (CCL5), MCP-1 (CCL2) and MIP-1 β (CCL4) were clustered together, upregulated and showed quite similar patterns after Lc treatment. Consequently, Lc may promote the production of chemokines in spleen, resulting in the accumulation of various immune cells and the subsequent production of diverse cytokines. On the other hand, the application of Abx weakened the effect of Lc on all these cytokines and chemokines, suggesting that Abx antagonized Lc function on the spleen immune niche.

3.4. Antagonism of Abx on Lc-induced immune cell accumulation

In addition to cytokines, the immune cell populations in spleen and MLN from mice of each group were also determined by FACS (Figure 4). CD103⁺ DC is a subtype of DCs that contributes to immune tolerance by several approaches such as producing TGF- β (Lukas et al., 2017). With Lc administration, the proportion of CD103⁺ DC was upregulated in the spleen. Interestingly, although Abx did not significantly regulate CD103⁺ DC when applied alone, it can abolish the effect of Lc on that cell type (Figure 4(A)). In addition to spleen, the MLN is another important peripheral immune organ, which directly participates in the allergenic responses in the intestine, and nourishes the B cells that producing food allergy-associated antibodies (Velazquez, Wei, & Braun, 2005). The FACS results showed that the germinal centre B cell (GL7⁺CD95⁺) was

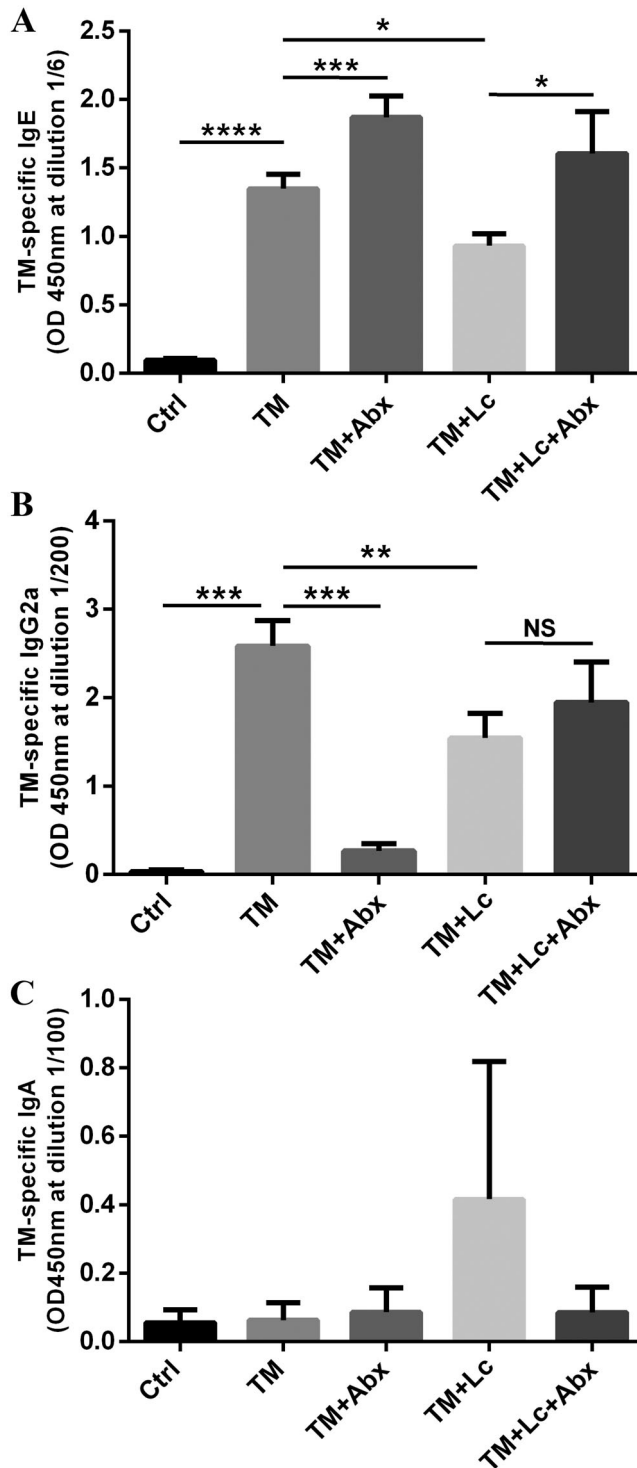


Figure 2. Antagonism between Lc and Abx on regulating antibody class switching. The concentration of TM-specific IgE (A), IgG2a (B) and IgA (C) in serum after challenge were measured by ELISA. The results are represented as the mean $\pm$ SD (* $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$; NS, not significant).

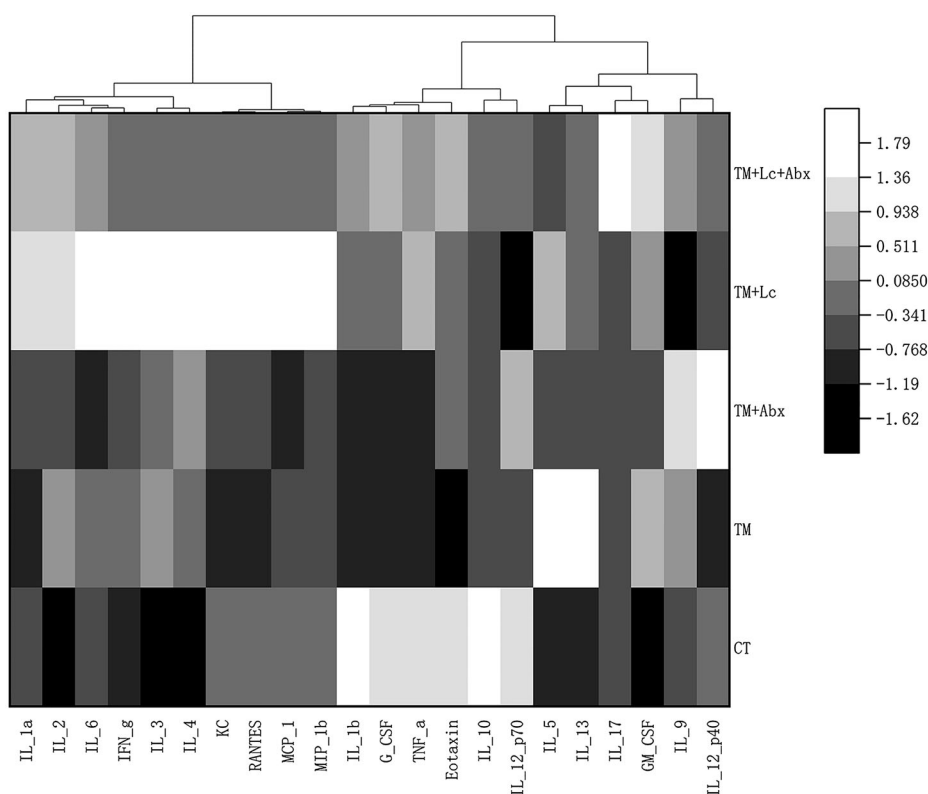


Figure 3. Antagonism of Abx on Lc-induced chemokines production. Cytokines and chemokines in serum of mouse from each group were determined by Bio-Rad Mouse 23-plex kit, the mean values of each group were presented in clustered heat map.

dramatically upregulated by Lc, indicating that B cell development, activation and differentiation were boosted (Figure 4(B)). Consistently, the proportion of subsequent IgA-producing B cell (B220+IgA+) was also increased after Lc treatment (Figure 4(C)). On the contrary, the increment of these B cells were suppressed by Abx, however Abx did not show any effect in the absence of Lc (Figure 4(B,C)). These results demonstrated that Lc promoted tolerogenic DC and IgA-producing B cell proportion, so as to established oral tolerance, and Abx showed an opposite effect by impairing Lc activity.

3.5. *Butyricoccus-mediated Lc function on immune homeostasis*

The intestinal microbiota is a direct target of both probiotics and antibiotics. Additionally, plenty of research has proved that intestinal microbiota regulates multiple immune process including food allergy. Consequently, the overall microbiota composition of mice from each group was determined by high-throughput 16s rDNA sequencing. The principal component analysis (PCA) of operational taxonomic units showed that almost all the five groups had diverse microbiota patterns (Figure 5(A)). Specifically, the microbiota was dramatically changed when challenged by TM (Ctrl vs. TM). In addition, Lc and Abx treatment changed the microbiota to discrepant directions, and

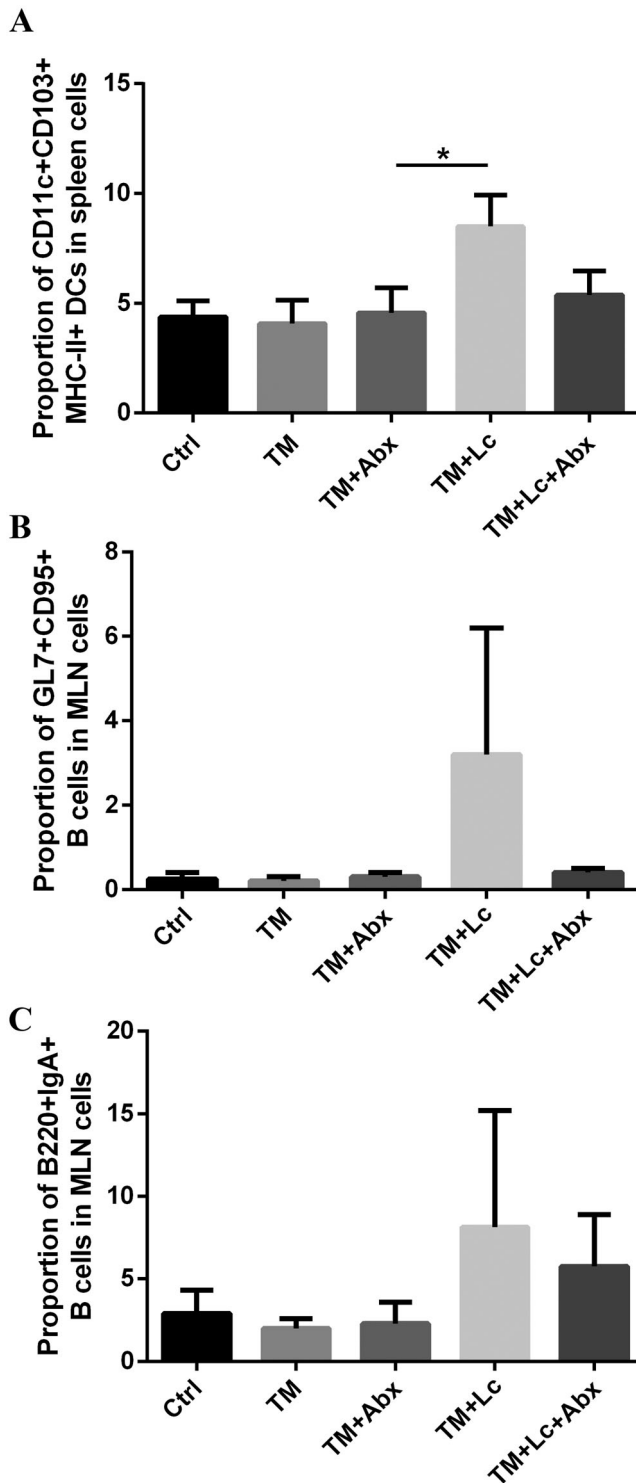


Figure 4. Antagonism of Abx on Lc-induced immune cell accumulation. The percentage of CD103+ DC (A) in spleen, GL7+CD95+ B cell (B) and B220+IgA+ B cell (C) in MLN were stained with indicated antibodies and determined by FACS.

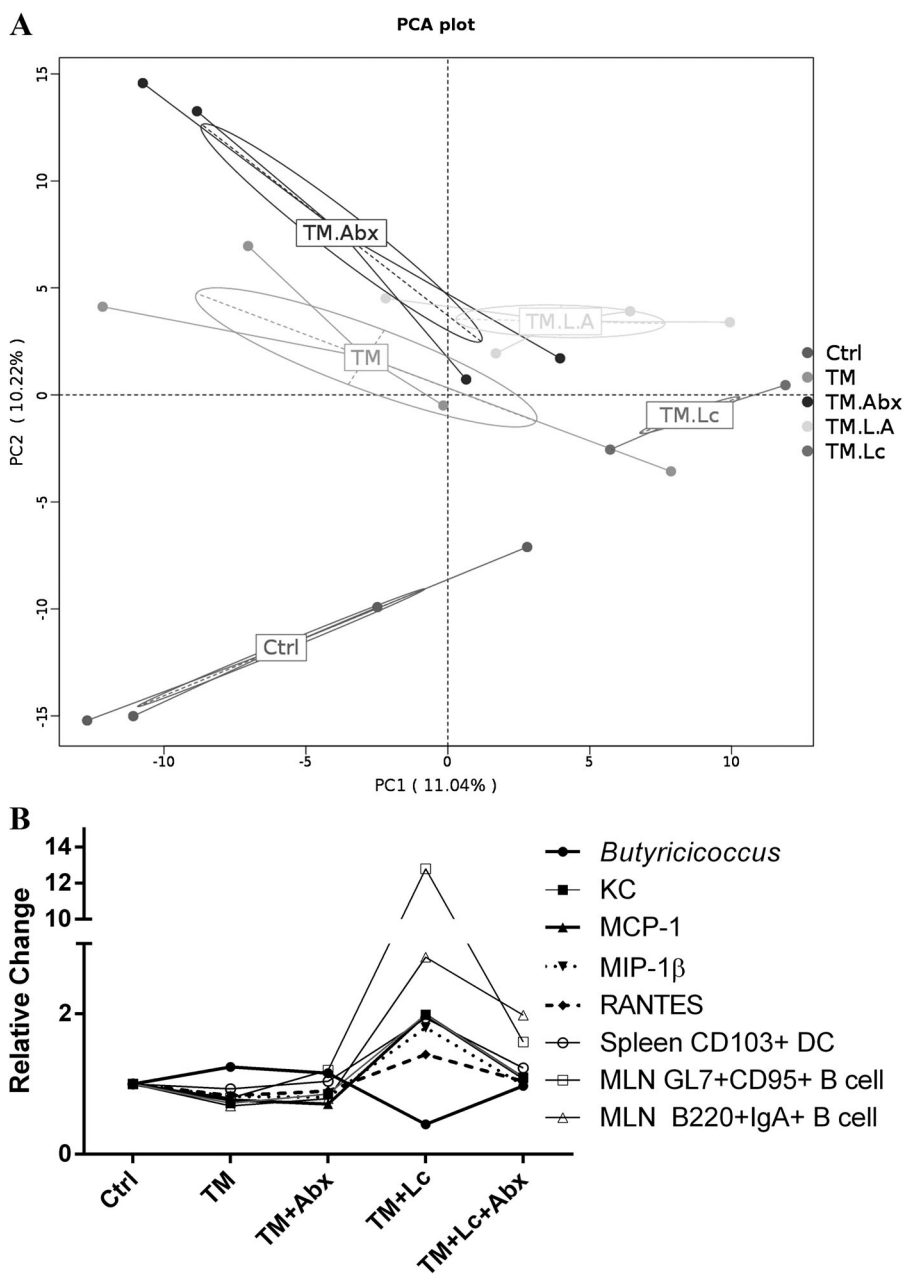


Figure 5. *Butyricococcus*-mediated Lc function on immune homeostasis. (A) The faeces microbiota of each mouse was determined by 16S rRNA sequencing and analysed by PCA. (B) The relative change folds of indicated chemokines concentrations, cell types proportions and *Butyricococcus* percentage in each group were shown in the same coordinate system.

the combination of Lc and Abx resulted in a moderate microbiota pattern between that of TM+Lc and TM+Abx groups. These results demonstrated that the application of Abx and Lc regulated the intestinal microbiota composition in antagonistic ways.

In addition to determining the overall microbiota composition, the detailed differential bacteria were also investigated by statistical analysis. The correlation between bacterial genera and cytokines or cell subtypes were calculated by Pearson correlation analysis. The results showed that *Butyricicoccus* was a critical genus under Lc or Abx treatment, whose proportion was tightly negatively correlated with several chemokines and cell types, including KC, MCP-1, MIP-1 β , RANTES, spleen CD103+ DC, MLN GL7+CD95 + and B220+IgA+ B cells (Table 1 and Figure 5(B)). As mentioned in Figures 3 and 4, these chemokines and cells were upregulated by Lc but suppressed by further application of Abx. Consequently, it can be implied that the upregulation of these factors by Lc was mediated by the reduction of *Butyricicoccus*, which can be reversed by Abx.

4. Discussion and conclusion

Food allergy is a global food safety problem with increasing prevalence but effective treatment approaches are limited. Probiotics have been considered as a promising way to prevent and treat food allergy, and our previous studies have also shown that several probiotic strains, including Lc, can alleviate food allergy (Fu, Peng et al., 2017; Fu, Song et al., 2017). On the other hand, antibiotic abuse has been proved to be a pro-allergenic factor, which disturbs the intestinal microbiota and thus impairs the immune system homeostasis (Keeney et al., 2014; Suez et al., 2018; Vangay et al., 2015). In the present study, we also found that the administration of Abx exacerbated food allergy responses and changed allergen-specific antibody isotype pattern. Interestingly, we found that Abx inhibited the anti-allergenic activity of Lc in some aspects, indicating that probiotic abuse may impair the function of probiotics.

Some previous studies showed that particular probiotics may restore microbiota homeostasis after Abx treatment (De Petrino et al., 1997), whereas a recent study demonstrated that the application of multi-strain probiotics impaired the reconstitution of antibiotic-damaged gut mucosal microbiome (Suez et al., 2018). In the present study, the function of Lc and Abx on intestinal microbiota were antagonized rather than synergized. In the PCA, Lc and Abx changed the microbiome to opposite directions, and the combination of them resulted in a moderate microbiome. Therefore, we suggested that the effect of probiotics and antibiotics are dependent on the exact type used and the way they applied, which has been proved by plenty of previous research (Fu, Wang, & Wang, 2018). Moreover, it is also suggested that the application of probiotics and antibiotics should be more careful especially when applied synchronously.

Table 1. Pearson correlation analysis between *Butyricicoccus* and relevant cytokines and cell types.

Group		<i>Butyricicoccus</i>	KC	MCP-1	MIP-1b	RANTES	CD103+	GL7+ CD95+	B220+ IgA+
Relative change fold	Ctrl	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	TM	1.247	0.727	0.774	0.825	0.836	0.932	0.800	0.690
	TM+Abx	1.155	0.861	0.716	0.812	0.900	1.042	1.200	0.793
	TM+Lc	0.427	1.992	1.986	1.807	1.421	1.939	12.800	2.810
	TM+Lc+Abx	0.975	1.096	1.087	0.985	1.039	1.228	1.600	1.983
	Pearson	–	–.996	–.988	–.984	–.999	–.969	–.948	–.927
Correlation									
Significance									
(p Value)		–	.000	.002	.002	.000	.006	.014	.023

Chemokines are signaling proteins that induce directed chemotaxis in nearby specific responsive cells. We found that four chemokines, KC, RNATES, MCP-1 and MIP-1 β , were upregulated by Lc and suppressed by further Abx application, which was quite similar with the change of allergenic symptoms. The common target cell types of these chemokines includes neutrophil, macrophage, T cell and DC (Palomino & Marti, 2015), therefore, the Lc-induced chemokines can attract various kinds of immune cells to the spleen and thus lead to complex immune reactions. However, how these chemokines take action to modulate host immunity requires far more investigations. In addition to chemokines, the tolerogenic CD103⁺ DC was also promoted by Lc, which is consistent with the fact that CD103⁺ DC is a kind of regulatory DCs that contribute to oral tolerance and suppress food allergy (Coombes et al., 2007). Spleen is an important organ for oral tolerance and IgA production (Weiberg et al., 2018), whereas MLN is considered as another important immune organ in food allergy process, which provides a draining secondary lymphoid compartment for the intestinal environment for B cells residence (Velazquez et al., 2005). FACS results showed that IgA-producing B cells in MLN were upregulated by Lc. IgA is the most produced antibody that normally secreted in to the intestinal lumen, which binds to bacteria and antigens in the lumen and contributes to oral tolerance in multiple ways (Singh, Chang, & Gershwin, 2014). The results indicated that Lc promoted the production of IgA and thus facilitated oral tolerance, which is consistent with the ELISA result of serum IgA concentration, however further investigations are required to verify the suggestion. On the other hand, the Lc-induced B cell activation was also blocked by Abx. Overall, all these chemokines and cells showed similar responses under Lc and Abx treatment, indicating the presence of a connection between all these immune activities.

T cells are the crucial immune cells that regulate immune responses. The antibody pattern (Figure 2) indirectly reflected that Abx promoted Th2 response and inhibited Th1 response, whereas Lc suppressed both of them. However, the cytokine pattern (Figure 3) and FACS results (data not shown) did not show significant tendency of T cell responses. We suggested that the discrepancy was due to the improper time or position that the samples were collected for cytokine or FACS analysis. Therefore, T cell response might be a relatively early event before antibody production in the whole TM-sensitization process under Lc or Abx regulation, and far more investigations are required to elucidate the direct T cell response to Lc or Abx treatment.

Plenty of research has demonstrated the indispensable role of microbiota in regulating host immunity, and the high diversity of gut microbiota has been shown to be critical for maintaining immune homeostasis and suppressing food allergy (Gholizadeh et al., 2019). More specifically, several recent studies have shown that particular bacterial strain in the microbiota mediated the function of some immune modulators (Furusawa et al., 2013; Turnbaugh et al., 2006). However, food allergy-related strains are rarely reported. Both probiotics and antibiotics can significantly regulate microbiota composition, and our results showed that the change of microbiota and particular cytokines and cells were quite similar under Lc and Abx treatment. Consequently, we presumed that microbiota mediated the function of Lc and Abx on the immune system, which might result from a specific bacterial group. To reveal the potential critical bacterial group, we analysed all the obtained data by Pearson correlation analysis. The result showed that the bacterial genus *Butyricicoccus* was tightly negatively correlated with all the target cytokines and cell types and was thus a potential pro-allergenic factor. On

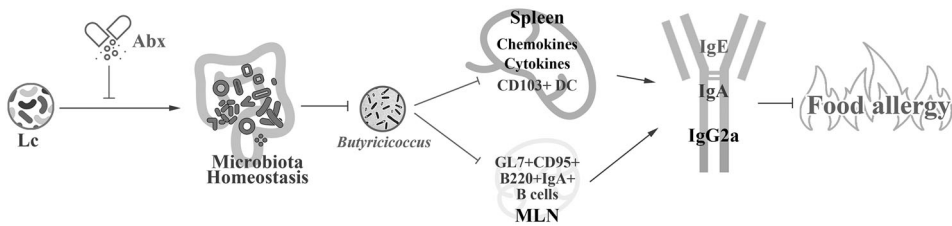


Figure 6. Scheme representing the mechanism of *Butyricoccus*-mediated antagonism between Lc and Abx on food allergy. *Butyricoccus* is an adverse bacterial genus in the gut microbiota that inhibits the accumulation of chemokines, CD103+ DC and IgA-producing B cell, therefore disturbing the immune homeostasis and exacerbating food allergy responses. The administration of Lc suppresses *Butyricoccus*, whereas the application of Abx reverses Lc function and leads to dysbiosis, resulting in the boost of *Butyricoccus*, finally aggravates food allergy responses.

the other hand, *Butyricoccus* has been reported to be a beneficial bacteria that suppress inflammatory bowel diseases (Devriese et al., 2017; Eeckhaut et al., 2013). Consequently, we suggested that *Butyricoccus* mediates the function of Lc and Abx on food allergy, but the exact role of *Butyricoccus* depended on specific situations. Therefore, more direct investigations, such as the clarification of whether the butyrate-producing activity of *Butyricoccus* is required in the process, the identification of the critical species and strain of *Butyricoccus*, the depletion or transplant of that strain, and the physiological significant in human bodies, are required to verify the present conclusions. Moreover, it should be noted that *Butyricoccus* can only partially explain the effect of Abx on food allergy, since the application of Abx alone also exacerbated food allergy responses, which was not dependent on the blockage of Lc-regulated *Butyricoccus* composition variation.

Overall, all these data suggest a potential mechanism for the antagonistic activity of Lc and Abx on food allergy mediated by *Butyricoccus*, as shown in Figure 6. *Butyricoccus* is an adverse bacterial genus in the gut microbiota that inhibits the production of chemokines as well as the expansion of tolerogenic CD103+ DC and IgA-producing B cell populations, disturbing the immune homeostasis and exacerbating food allergy responses. The administration of Lc suppresses *Butyricoccus*, so as to relieve the *Butyricoccus*-mediated food allergy. Furthermore, the application of Abx reverses Lc function and leads to dysbiosis, resulting in the boost of *Butyricoccus* and subsequent chemokines and immune cells disorders, finally aggravates food allergy responses. These results reveal the mechanisms of Lc and Abx on food allergy, provid Lc as a potential approach and *Butyricoccus* as a potential therapeutic target for treating food allergy, and indicate probiotics and microbiota as major victims of antibiotic abuse.

Disclosure statement

No potential conflict of interest was reported by the authors.

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