



Impacts of functional oligosaccharide on intestinal immune modulation in immunosuppressive mice

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ABSTRACT

In order to research the role of soybean oligosaccharides (SBOSs) on improvements in the microenvironment of intestinal flora and immune function of cyclophosphamide (CTX) immunosuppressive mice. Via giving intragastric administration of Soybean oligosaccharide (SBOS) at the low dose (50/(kg·BW)/d), the middle dose (200 mg/(kg·BW)/d) and the high dose (500 mg/(kg·BW)/d) partly once a day, which is also 28 days in a row. At the same time, (SBOS) mice in the drug group and (CG) mice in the positive control group were given intraabdominal injection of CTX (200 mg/kg/d). The immunosuppressive mouse model (CY) was established after 72 h in the model group and the positive control group (CG) was given intragastric administration of levamisole hydrochloric acid (LMS) for 3 days, with the data of 80ug/kg/d after injection of CTX (for actually 72 h). On the 8th, 15th and 22nd day, the number of Bifidobacterium, Lactobacillus, Enterococcus and Clostridium perfringens in the feces of mice in each dose of drug group were determined. After the test resulted, the cellular immune function, humoral immune function, monocyte/macrophage function, NK cell activity and cytokine secretion (tumor necrosis factor- α , interferon-gamma and IL-4) were measured in immunosuppressive mice each group. The results showed that 200 mg/(kg BW) soybean oligosaccharide could significantly promote the proliferation and inhibit the increase of Enterococcus in immunosuppressive mice. The soybean oligosaccharide of 500 mg/(kg BW) could dramatically promote the proliferation of both Bifidobacillus and Lactobacillus, and also inhibit the increase of both Enterobacteriaceae and Enterococcus in immunosuppressive mice. The regulatory function of SBOS on intestinal flora was positive. Soybean oligosaccharide (500 mg/(kg BW) could significantly promote the proliferation of Bifidobacillus and Lactobacillus in immunosuppressive mice and inhibit the increase of Enterococcus and Enterococcus. The proliferation of spleen lymphocytes induced by ConA, LPS in immunosuppressive mice was dose-dependent. But it was still lower than that of the normal group (CG0) ($p > 0.05$). The serum hemolysin level of immunosuppressive mice was significantly increased in each dose group ($p < 0.05$), and the level of antibody forming cells in spleen cells of each dose group was significantly increased ($P < 0.05$), and the level of antibody forming cells in spleen cells of each dose group was significantly higher than that of low dose group ($p < 0.005$), and the level of serum hemolysin in immunosuppressive mice was significantly increased in each dose group ($p < 0.05$). In the detection of immune effector cell activity in immunosuppressive mice, the phagocytic function of macrophages in high dose group and the natural killing activity of spleen NK cells in high dose drug group were significantly increased, which were not significantly different from those in positive control group ($P < 0.05$), but the expression of TNF- α , INF- γ and IL-4 cytokines in serum was increased in a dose dependent manner ($p < 0.05$). In conclusion, soybean oligosaccharide can significantly increase the diversity of intestinal microecology, increase the number of intestinal beneficial bacteria, has a correlation with the proliferation of Bifidobacterium and Lactobacillus in the intestinal tract, and inhibit the proliferation of harmful bacteria. The results showed that SBOS had a direct effect on the proliferation of intestinal flora

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under immunosuppression. Based on the improvement of intestinal microenvironment in immunosuppressive mice by soybean oligosaccharide for 25 days, the results showed that compared with the positive control group, the nonspecific and specific immunity of immunosuppressive mice in the drug group had a regulatory effect, which improved the phagocytic function of monocytes/macrophages, developed the level of antibody forming cells, enhanced the standard of the killing activity of NK cells, and promoted the expression of cytokines as well. Compared with the model group, the transformation and proliferation of spleen lymphocytes in the high and middle dose groups were remarkably increased, but all of the indexes did not reach the level of the normal blank group. By studying the improvement of intestinal microenvironment in immunosuppressive mice, to some extent, it is concluded that the proliferation of intestinal flora can improve the immunomodulatory function of the body, but it still lowers the normal immune degree, which reflects the immunomodulatory effect of the body on the stimulation of continuous external intake. The results demonstrate that the immunomodulatory ability of immunosuppressive body was insensitive to SBOS and provided a theoretical basis for the study of health care function of intestinal microenvironment improvement when SBOS acted on abnormal immune function. The results also improved the practical application value of SBOS.

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1. Introduction

As the non-digestible food components, prebiotics improve host health condition by optionally provoking the activity of some special bacteria belonging to the colon.¹ The increases in the amount of especial bacteria belonging to colonic microbiota are associated with decreases of prebiotics, thereby, leading to the changes of composition of the microbiota (Gibson and Roberfroid, 1995). Prebiotics are generally short-chain oligosaccharides with 2–60 polymers and can be selectively fermented by partly specific gut bacteria belonging to the large intestine, however may not be digested by ferment of the upper gastrointestinal (Flickinger and Fahey, 2002; Patterson and Burkholder, 2003). Bifidobacteria and lactobacilli are the most frequently used prebiotic oligosaccharides, thus they are the only microorganisms to be capable of improving the health of people (Mikkelsen and Jensen, 2004; Vernazza et al., 2005).

Soybean oligosaccharides (SBOs) has been regarded to be generally recognized as safe (GRAS) component, which are well-established prebiotics isolated from soybean seed (Chen et al., 2010; Kim et al., 2003). SBOs is derived from soy or other legumes and they are a kind of easily dissolved oligosaccharides mainly made of common raffinose, stachyose, and sucrose (Zhou et al., 2012; Huang et al., 2006).

Like lactobacilli and bifidobacteria, SBOs may be fermented in the intestine by particular microorganism. The dietary containing SBOs will be selectively beneficial to the existence of these microorganism in the gastrointestinal tract of the host. In fact, previous study had shown that comparing to feed fructooligosaccharide (FOS) or the control, SBOs significantly increased the level of bifidobacteria and Clostridium, but FOS had a similar effect relative to the control (Martin et al., 1998). Furthermore, soybean meal oligosaccharides (SMOs) reportedly promote competitive exclusion of pathogens (Xu et al., 2009). Hence, the amount of beneficial microorganisms of intestinal tract at the cost of consuming these prebiotics.

Additionally, as a portion of the primary line of defense in the gastrointestinal tract, SBOs irritate the activity of beneficial gut

microorganism for enhancing immune function (Boehm et al., 2005). Actually, prebiotics can gain benefits in resistance to infectious diseases by strengthening inborn immunity mechanisms (Safarpour et al., 2011). Some studies indicate that SBOs are related to immunological and hematological indexes. For instance, SBOs have been proved to raise the level of IgG as well as superoxide dismutase, raise the amount of antibody forming cells (AFCs) in mice and splenocyte proliferation, influence the indices and weights of immunity organs, mitigate negative immune influences of and S180-SAM- treated mice (Xu et al., 2005). In summary, SBOs can significantly affect the intestinal health and immune regulation, nevertheless, these specific mechanisms are still unclear. Although, such positive effects brought by some prebiotics like FOS on innate immunity, growth performance, microbial fermentation, autochthonous intestinal microbiota, hematological and serum biochemical parameters have been revealed, the study about how SBOs affect gut microbiota population and immunoregulation in a mouse model is deficient (Akrami et al., 2013; Geraylou et al., 2012; Huynh et al., 2009).

The purpose of our study is to assess the immunoreaction of mice to various levels of oligosaccharides consumption by evaluating the prebiotic influences of SBOs on gut microbiota population.

2. Experimental section

2.1. Animals and reagents

SBOs were bought from Baolingbao Biological Co. in Shandong, China. Detailed purchase information for other reagents was present in supplementary material. Kunming mice with 22.2 ± 0.6 g average weight feeding in specific pathogen-free conditions were acquired from Biotechnology Co., Ltd in Liaoning, China. The mice acquired standard rodent food and water without restraint, following weighed once a day.

2.2. Animals random allocation

Experimental mice were allocated into 6 groups randomly, as follow: control group (0.5%CMC-Na), model group (CTX), positive control group (LMS, 60 μ g/kg), high-dose group (SBOS, 500 mg/kg), middle-dose group (SBOS, 200 mg/kg) and low-dose group (SBOS, 50 mg/kg) body weight (BW)-1. There were 20 mice in each group, including 10 healthy males and 10 healthy females.

Before the study, the amount of fecal microorganism (CFU/g) of mice feces loading in sterile containers were estimated by logarithm statistics. Then mice accepted SBOs via intragastric administration, and the number of fecal bacteria was determined at 8d,

¹ SBOs were bought from Baolingbao Biological Co. in Shandong, China. Lactate dehydrogenase and chicken red blood cells (CRBCs) were acquired from Appligen Technologies, Inc in Beijing, China. Sodium dodecyl sulfate and methyl thiazolyl tetrazolium (MTT) were obtained from AMRESCO LLC in Solon, USA. Erythrocyte lysates and Concanavalin A (ConA) were achieved from Sigma-Aldrich Chemical Co in St. Louis, MO, USA. Mouse interleukin-4 (IL-4), mouse interferon- γ (IFN- γ) and mouse tumor necrosis factor- α (TNF- α) were obtained from R&D Systems, Inc in San Diego, CA, USA. Mouse IL-4, TNF- α and IFN- γ ELISA kits were achieved from Bogu Biological Technology Co in Shanghai, China. Biological stains covering vice magenta, malachite green and Giemsa dye were bought from Aojia Chemical Co in Nanjing, China.

15d, 22d, 29d. After 8d, 15d, 22d, 29d, the level of natural killer (NK) cell amount, cellular immunity, immunoglobulin and cytokine were analyzed for 10 randomly selected mice within every group (five males and five females). Phagocytic activity and humoral immunity after the chicken erythrocytes transferred into these mice were tested among the 10 residue mice in each group. During the whole study, researchers were in charge for recording mice's daily body weight, water and food and feces production.

2.3. Analysis of microbial communities in gut

Covering sterile conditions, the microbial communities of gut belonging to the mouse feces were estimated following constant intragastric administration of SBOs for 8, 15, 22, 29 d. The comparison among *E. coli*, bifidobacteria, enterococci, lactobacilli, *C. perfringens* for changes were implemented. Impacts of SBOs on the balance of the gut microbiota population were identified abidingly by the Technical Standards for Testing and Assessment Health Food (Ministry of Health of the People's Republic of China, 2003). The condition of cultivation, media as well as the method of identification is presented in Table 4.

2.4. Evaluation of immunoregulation indexes

2.4.1. Influence of SBOs on the cellular immunity of immunosuppressed mice

The evaluation of cellular immunity was depended on the percentages of lymphocytic transformation and T-lymphocytes coaxed by ConA of mice. The percentages of lymphocytic transformation and T-lymphocytes were accessed by staining and labeling enzyme of lymphocytes in the peripheral circulation. Moreover, changes in cell proliferation were obtained by MTT assays in the context of different optical densities (ΔOD_{570}).

2.4.2. Impacts of SBOs on antibody-forming cell levels of immunosuppressed mice

Splenocyte proliferation induced by SBOs in mice which was implemented as has been noted. In short, the splenocytes derived from experimental or control mice were put into 96-well microplates in triplicate with 5×10^5 cells/well, consequently injecting LPS at 5 μ g/well into the above 96-well microplates. Incubating the cells in capacity of 200 μ L/well at the context of 37 °C in 5% CO₂, pulsing it for the last 24 h with 0.6 μ Ci/well of [3H]-thymidine. The results were gained 72 h after the initial of culture. A liquid scintillation counter was used to detect the level of [3H]-thymidine incorporation.

2.4.3. Impacts of SBOs on the humoral immune function of immunosuppressed mice

The formation of serum hemolysin that had an immunization with sheep red blood cells (SRBC) was measured. Mice were randomized into 4 groups, containing 10 mice in every group. Physiological saline (20 ml/kg) was given to control groups by intraperitoneal injection. Mice were injected (i.p.) with 15 and 30 mg/kg of CPS-1 and 5 mg/kg of Dex. The preset injection in all groups had been once daily lasted for 7 days. All mice were immunized via using a 0.2 ml suspension of SRBC (10^9 /ml) on the third day of dose. Blood samples were acquired at 90 min after the last dose. These blood samples were centrifuged at the speed of 2000 rev./min for 10 min at one hour later. Dilute a 20 μ L sample of supernatant serum 500 times with normal saline, following reactive tubes filled with 1 ml of the diluted serum samples received 1 ml guinea pig serum (1:10 dilution) and 0.5 ml of 10% SRBC sequentially. The above reactive tubes were promptly put into an ice bath and repeated the above centrifugation steps, after incubating for 1 h at the context of 37 °C. Mix 3 ml of Drabkin's solution

with a 1 ml aliquot of the supernatant. After 10 min, the absorbency (A) was acquired at the context of 540 nm.

2.4.4. Impact of SBOs on monocyte/macrophage function of immunosuppressed mice

A carbon clearance test was carried out on two mice in each group to evaluate the function of macrophage cells. Each mouse was treated with 100 μ L/10 g diluted India ink via intravenous injection. At 2 min (t_1) and 10 min (t_2), blood samples of retinal venous plexus were obtained, then 2 ml of 0.1% Na₂CO₃ was blended with 20 μ L of blood. The absorbance at the context of 600 nm (OD₁ and OD₂) was assessed with 0.1% Na₂CO₃ as blank control, as well as OD₁ represents t_1 and OD₂ represents t_2 . Weighing the liver and spleen of experiment subjects, and the phagocytic index was estimated as follow:

$$K = (\lg OD_1 - \lg OD_2) / (t_2 - t_1)$$

$$\text{Phagocytic index } \alpha = \alpha = K^{1/3} \times C / (A + B)$$

A stands for the liver weight; B stands for spleen weight; C stands for body weight.

2.4.5. Impact of SBOs on the activity of NK cell of immunosuppressed mice

The method of the assay of NK activity was similar to the classical approach raised by Talmadge et al. in 1980. In brief, label 100 μ L of YAC-1 target cells that at 2×10^7 cells/ml with 100 μ Ci sodium[51]-Cr-chromate solution at the context of 37 °C in 5% CO₂ for 1 h as well as being washed in RPMI 1640 culture medium including 10% FBS for three times. Plating 100 μ L of 1×10^5 /ml of the target cells into 96-well microplates, and then following 0.1 ml of the splenocytes were put into the wells triplicately at effector-to-target (E:T) cell ratios of 200:1, 100:1 and 50:1. Incubating at the context of 37 °C in 5% CO₂ for 5 h later, the microplates had a centrifugation for 6 min at 1800 rpm, and 0.1 ml supernatant in every well was achieved and estimated in a γ counter. Averaging the counts each minute (cpm) for triplicate wells and the following calculation equation applied for calculating the cytotoxicity associated with NK cells:

$$\text{NK activity (\% specific lysis)} = [(\text{cpm of experimental release} - \text{cpm of spontaneous release}) / (\text{cpm of maximum release} - \text{cpm of spontaneous release})] \times 100\%$$

The cpm produced by experimental induce were estimated from the cocultures of YAC-1 cells with splenocytes, but the cpm produced by spontaneous induce were counts from the cultures of YAC-1 only, and the cpm produced by maximum release were detected by putting 0.1 ml 0.2% Triton X-100 rather than the effector cells to specific wells including YAC-1 cells. 12% of the corresponding maximum production were always more than spontaneous releases from target cells.

2.4.6. Impact of SBOs on cytokine secretion in immunosuppressed mice

The level of Cytokine were assayed as previously described with slight modifications. Twenty-four hours after the last dose, the blood sample from retro-orbital blood collection was acquired. Serum derived from the above blood sample by centrifugating at the context of 2000 rpm for 10 min and was reserved at -20 °C. The level of IL-4, INF- γ , TNF- α from the serum was detected by enzyme-linked immunosorbent assay. The microtiter plate included test sample wells and standard wells. A group of 50 μ L serial diluted standard was joined into standard wells. Ten μ L of each test sample and 40 μ L of the sample diluent were added to the appropriate wells simultaneously. The blank wells were vacant.

Then, in the context of 37 °C, the wells were blocked for 1 h by using 100 µL HRP-conjugated reagent, After finishing blocking, plates were washed by using 400 µL of Wash Solution for five times. Incubating the plates for 15 min at the context of 37 °C after adding 50 µL chromogen solutions B and A. The absorbance was detected at 450 nm when the reaction was ceased after putting 50 µL Stop Solution.

2.5. Statistical analysis

Variance (ANOVA) was performed to analyze all comparison among variables. All between-group comparisons were further analyzed by Duncan's Multiple Range Test. Data were analyzed by SPSS software (version 19.0).

3. Results

3.1. Impact of SBOs on the gut microbiota population of mice

3.1.1. Impact of SBOs on bifidobacteria of mice intestines

Bifidobacteria can use iodole, hydrogen sulfide, amine and phenol produced by intestinal saprophytic bacteria as a nutrient source to yield beneficial substances, such as vitamins and amino acids (Siwicki and Anderson, 1993; Hume, 2006; Cerqueira et al., 2004). The proliferation of bifidobacteria may generate mass volatile fatty acids, which inhibits the excessive reproduction of aerobic bacteria, thus decreasing intestinal infection. Bifidobacteria promote the growth and development of immune cells and normal human metabolism (Wang et al., 2002; Kagnoff and Eckmann et al., 1997; Arnold et al., 2006).

As shown in Fig. 1A, the numbers of bifidobacteria in the control group did not vary during the trial ($p > 0.05$). Moreover, there were no significant differences between control groups and low-dose groups at the context of five kinds of bacteria respectively after 15 days of treatment. However, the number of intestinal bifidobacteria of the middle- and high-dose groups significantly raised by 13.42% ($p < 0.01$) and 16.08% ($p < 0.01$) separately. No significant differences were observed between the model group and low-dose group, as the model group and middle-dose group ($p > 0.05$), but a 7.78% increase was found in high-dose group ($p < 0.05$). The three doses of SBOs may promote the proliferation of bifidobacterial. This proliferation was positively related to the dose of SBOs. No significant increase was obtained in the three dose groups after 29 days of treatment with SBOs compared with those after 22 days of treatment, suggesting that the proliferation of LABs reached their peak regulation of intestinal flora. After 22 days of SBOs treatment, the three groups all presented to alter regulation of intestinal flora, which was positively related with the dose.

3.1.2. Impact of SBOs on LABs in mice intestines

The proliferation of LABs can produce natural antibiotics, acidophilus and lactobacillus, increase defense-barrier function of intestinal epithelia under oxidative stress, and reduce enteritis. The effect of SBOs on LABs of the different groups is shown in Fig. 1B and there was a well match between the control group and the model group ($p > 0.05$). Compared with control group, the LABs in the high-dose groups after 15 and 22 days of SBOs treatment increased by 9.31% and 11.76%, respectively, The LABs of the middle-dose group significantly raised after 15 days of treatment ($p < 0.05$), but the LABs were both well matched in the low-dose group and the control group. No significant differences were found between the LABs of the three dose groups after 29 days of treatment with SBOs relative to 22 days.

3.1.3. Impact of SBOs on *E. coli* in mice intestines

As seen in Fig. 1C, no significant difference was observed in the Enterobacteria number between the low-dose group and the control group, as between middle-dose group and the control group ($p > 0.05$). The Enterobacteria number belonging to the high-dose group significantly reduced ($p < 0.05$) by 1.43% relative to the control group; however, there was no diversity between the model group and the high-dose group. The amount of *E. coli* did not differ between the three dose groups after 29 days of treatment with SBOs compared to 22 days. *E. coli*, one species of *Escherichia Castellani and Chalmers*, is a commensal bacterium of the intestinal tract of human and animals. Some strains of *E. coli* are pathogenic and can lead to diarrhea and various kinds of inflammation. High-dose SBOs can promote the proliferation of *E. coli*, but pathogenicity is not determined by the number of *E. coli*.

3.1.4. Impact of SBOs on enterococcus in mice intestines

As shown in Fig. 1D, enterococcus in the intestines of the middle-dose and high-dose groups markedly reduced ($p < 0.05$) by 1.86% and 6.23%, respectively, compared to the control group. There was no diversity between the SBOs treated groups and the model group ($p < 0.05$). The spurred proliferation of bifidobacteria and LABs may restrain the growth of *E. coli*. and enterococcus by generating lactic acid and acetic acid and increasing the chance of colonization of the intestinal mucosa, which restrains contact among pathogenic bacteria and their toxins and the intestinal mucosa. This effect stops bacterial translocation and prevents pathogenic bacteria and viruses from crossing the intestinal wall to invade mesenteric lymph nodes, blood, liver and spleen, and thus protects the immune system. The amount of enterococcus was similar among the three different dose groups after 29 versus 22 days of SBOs treatment.

3.1.5. Impact of SBOs on *C. perfringens* in mice intestines

As shown in Fig. 1E, no significant differences were found in the SBOs groups relative to the control groups and the model groups except for the high-dose group after 22 days of treatment, where *C. perfringens* was significantly decreased ($p < 0.01$) by 18.65%. These results suggest SBOs are not flatulence factors and that high-dose SBOs can inhibit flatulence.

3.2. Impacts of SBOs on nonspecific immunity

3.2.1. Impacts of SBOs on the cellular immunity of immunosuppressed mice

As shown in Table 2, the stimulate index of the model group markedly reduced ($p < 0.01$) relative to the control group, which indicated proliferation from T lymphocytes activated by ConA and that proliferation from B lymphocytes activated by LPS were inhibited. Conversely, middle- and high-dose SBOs treatment promoted ConA- and LPS-induced proliferation of lymphocytes, illustrating a remarkable increase in splenic lymphocyte proliferation of immunosuppressed mice relative to the model group ($p < 0.05$). While the T and B lymphocytes did not show significant proliferation. As important lymphocytes in the specific immune response, T lymphocytes play a crucial role in the humoral immune response of TD antigen. As shown in Table 1, SBOs promoted the proliferation of T lymphocytes, which was confirmed by the significant increase in splenic lymphocyte proliferation of the high-dose group relative to the model group. High-dose SBOs may boost the cellular immune function of immunodeficient mice.

3.2.2. Impacts of SBOs on the antibody-forming cell level of immunosuppressed mice

As shown in Table 3, the amount of antibody-forming cells in the spleens from the model group significantly decreased relative

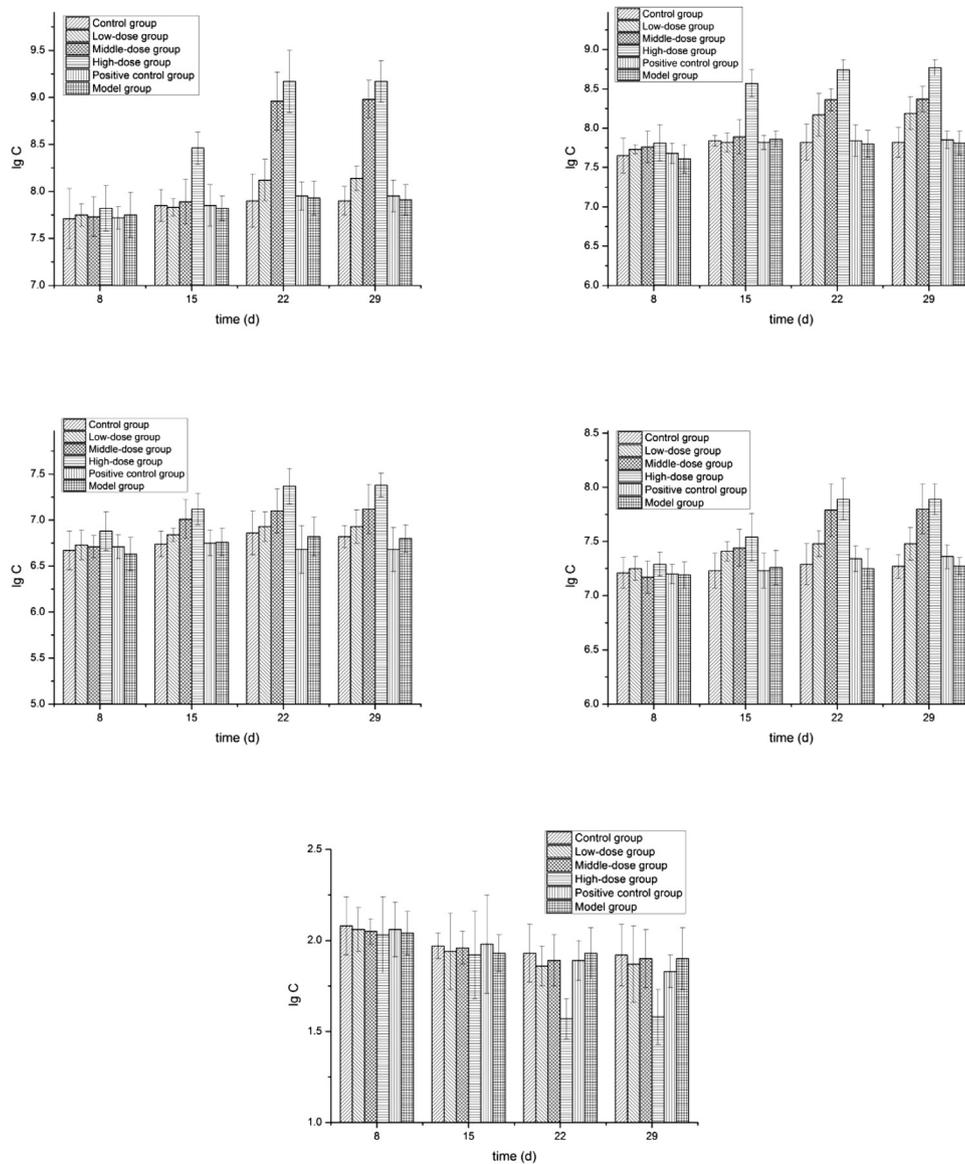


Fig. 1. Effects of different doses of SBOS on intestinal microbes. Bifidobacteria (A), LAB (B), *E. coli* (C), enterococci (D), and *C. perfringens* (E) (mean \pm SD, n = 20) were analyzed. Statistical significance is indicated versus the control group. **p* < 0.05, ***p* < 0.01. C: colonies.

to the control group (*p* < 0.01). SBOSs can regulate the immunosuppression induced by cyclophosphamide and improve the antibody-forming cell level, which can be verified by the

Table 1
Media, culture conditions, and identification methods of intestinal microbes.

Species name	Medium	Culture conditions	Identification method
Bifidobacteria	TYG (tryptone yeast glucose extract agar)	37 °C, 48 h, anaerobic	Gram-positive staining of all non-bacillus
LAB	EMB (eosin methylene blue medium)	37 °C, 24 h	Gram-negative staining of all bacilli
<i>E. coli</i>	MRS (<i>Lactobacillus</i> bacteria-selective medium)	37 °C, 48 h, anaerobic	GB/T.4789.34-2003
Enterococci	SSM (<i>Streptococcus</i> -selective medium)	37 °C, 24 h	Gram-positive staining of all cocci and obvious brown circle
<i>C. perfringens</i>	TSC (tryptone-sulfite cycloserine medium)	37 °C, 48 h, anaerobic	All fluorescent black colonies under UV

Table 2
Effect of SBOS on splenic lymphocyte proliferation of immunosuppressed mice (mean \pm SD, n = 10).

Group	Dosage (mg/kg)	Stimulate Index	
		ConA	LPS
Normal control	0	2.011 \pm 0.413	2.453 \pm 0.486
Model control	0	1.169 \pm 0.154##	1.213 \pm 0.211##
Positive control	80	1.623 \pm 0.332**	1.577 \pm 0.321*##
High dosage	500	1.489 \pm 0.406**	1.397 \pm 0.357*
Middle dosage	200	1.436 \pm 0.255*	1.408 \pm 0.300*
Low dosage	50	1.193 \pm 0.189*	1.383 \pm 0.62*

Compared With normal control #*P* < 0.05, ##*P* < 0.01; Compared With model control, **p* < 0.05, ***p* < 0.01.

marked raise in the antibody-forming cell level of the middle-dose and high-dose groups (*p* < 0.05) when comparing to the model group. The antibody-forming cell level of the low-dose group displayed a more significant increase (*p* < 0.01), which suggested that SBOSs boosted the humoral immune function of B lymphocytes. No significant difference in the antibody-forming cell level between the low-dose and the control group, as between

Table 3Effect of SBOS on the antibody-forming cell level of immunosuppressed mice (mean \pm SD, n = 10).

Group	Dosage (mg/kg)	Antibody-forming cell (OD ₄₁₃)
Normal control	0	0.341 \pm 0.101
Model control	0	0.158 \pm 0.046 ^{##}
Positive control	80	0.269 \pm 0.125*
High dosage	500	0.232 \pm 0.227*
Middle dosage	200	0.226 \pm 0.034*
Low dosage	50	0.237 \pm 0.030*

Compared With normal control [#]*P* < 0.05, ^{##}*P* < 0.01; Compared With model control, **p* < 0.05, ** *p* < 0.01

the high-dose groups and the control group was detected, which may be due to cytokine secretion or a boost in T lymphocyte function.

3.2.3. Impact of SBOSs on the serum hemolysin (SRBC antibody) level of immunosuppressed mice

As depicted in Table 4, after an intramuscular injection of CTX, the serum hemolysin level markedly reduced (*p* < 0.01) in immunosuppressed mice compared with the control group. However, the serum hemolysin level of the three dose SBOSs groups all markedly raised (*p* < 0.05), which suggested that SBOSs are able to promote the division, proliferation, differentiation and maturation of B lymphocytes to antibody-forming cells to secrete SRBC antibodies. The antibody secretion level of the SBOSs groups was lower than the control group.

3.2.4. Impact of SBOSs on monocyte/macrophage function of immunosuppressed mice

The monocyte/macrophage system, an important defense system of the body, can remove allograft particles and soluble foreign matter and harmful substances produced by human body. These cells also present antigens and bring their superiority into each step of immune adjustment and the immune response. The determination of monocyte/macrophage phagocytosis is used to study the strength of the nonspecific immunological function and the killing activity of target tumor cells and viruses.

As Table 5 shows, the adjusted clearance index regarding to the control group was higher than the model group due to the immune-suppression by CTX. SBOSs treatment significantly increased the adjusted clearance index compared to this model group (*p* < 0.01). The carbon particle clearance of the SBOSs treated groups remained close to the control group. There was a reasonable match between the LMS positive control group and the control group. These results suggested that SBOSs can improve monocyte/macrophage function of immunosuppressed mice, but did not restore the levels to normal.

3.2.5. Impact of SBOSs on the activity of NK cell form immunosuppressed mice

As an important part of the nonspecific immune response, the activity of NK cells was evaluated to determine the impact of SBOSs on immunologic function. As presented in Table 6, the killing activity of the NK cells of the model group on target YAC-1 cells was far below the control group (*P* < 0.01). Middle- and high-doses of SBOSs both increased the killing activity of NK cells (*P* < 0.05), however, the increased NK cell killing activity of these groups still did not reach a normal level, which suggested that SBOSs do not increase the innate immune defense or antitumor and anti-infection abilities.

Table 4Effect of SCP on the serum hemolysin level of immunosuppressed mice (mean \pm SD, n = 10).

Group	Dosage (mg/kg)	Serum Hemolysin (OD ₅₄₀)
Normal control	0	0.416 \pm 0.058
Model control	0	0.131 \pm 0.065 ^{##}
Positive control	80	0.320 \pm 0.062**
High dosage	500	0.233 \pm 0.072*
Middle dosage	200	0.219 \pm 0.036**
Low dosage	50	0.228 \pm 0.073*

Compared With normal control [#]*P* < 0.05, ^{##}*P* < 0.01; Compared With model control, **p* < 0.05, ***p* < 0.01.**Table 5**Effect of SBOS on the adjusted clearance index of immunosuppressed mice (mean \pm SD, n = 10).

Group	Dosage (mg/kg)	Adjusted clearance index(α)
Normal control	0	4.53 \pm 0.32
Model control	0	3.86 \pm 0.69 [#]
Positive control	80	4.50 \pm 0.27* ^{##}
High dosage	500	4.23 \pm 0.44**
Middle dosage	200	4.07 \pm 0.43*
Low dosage	50	3.91 \pm 0.23*

Compared With normal control [#]*P* < 0.05, ^{##}*P* < 0.01; Compared With model control, **p* < 0.05, ***p* < 0.01.**Table 6**Effect of SBOS on NK cell activity of immunosuppressed mice (mean \pm SD, n = 10).

Group	Dosage (mg/kg)	NK cell activity (%)
Normal control	0	30.743 \pm 4.786
Model control	0	22.409 \pm 4.516 ^{##}
Positive control	80	28.918 \pm 4.405*
High dosage	500	24.287 \pm 4.643*
Middle dosage	200	23.788 \pm 4.432*
Low dosage	50	22.884 \pm 2.756*

Compared With normal control [#]*P* < 0.05, ^{##}*P* < 0.01; Compared With model control, **p* < 0.05, ***p* < 0.01.

3.2.6. Impact of SBOSs on cytokine secretion in immunosuppressed mice

TNF- α produced by activated monocyte/macrophage, has capacity to inhibit and kill tumor cells with its strong antitumor activity. TNF- α can promote neutrophil phagocytosis, cell proliferation and differentiation, as well as the increasing anti-infection activity. In addition, TNF- α participates in the pathological damage of some autoimmune diseases. IFN is a family of cytokines comprised of IFN- α , IFN- β , IFN- γ , and some subtypes of IFN. IFN- α and IFN- β belong to the type I interferon family that inhibit virus replication, inhibit the proliferation of a variety of cells and participate in antitumor immune regulation. IFN- γ produced by activated monocyte/macrophages, belongs to the type II interferon family of protein that inhibit virus replication suppress activate macrophages, induce MHC molecule expression and promote Th1 cell differentiation. Interleukin (IL) produced by leukocytes and other cells, mediates the interactions between cells. Thirty-three of IL forms have been found to participate in immunoregulation, hematopoiesis and inflammatory reactions. IL-4, secreted by Th2 cells, regulates some hematopoietic cells. IL-4 can promote the proliferation of activated B cells to mediate the humoral immune response.

In comparison to the normal group, the levels of serum TNF- α , IFN- γ , and IL-4 regarding to the model group were markedly reduced (*P* < 0.01), which suggested that the activity of monocyte/macrophages, T and B cells, and NK cells were killed and restricted by the cytotoxicity of CTX, proceeding to the decrease of cytokine

Table 7
Effect of SBOS on cytokine content in serum of immunosuppressed mice (mean \pm SD, n = 10).

Group	Dosage (mg/kg)	IFN- γ concentration (ng/mL)	TNF- α concentration (ng/mL)	IL-4 concentration (pg/mL)
Normal control	0	93.00 \pm 7.24	50.68 \pm 3.01	16.63 \pm 1.78
Model control	0	77.95 \pm 5.86##	45.78 \pm 3.13##	13.40 \pm 1.07##
Positive control	80	92.38 \pm 7.27**	50.04 \pm 2.31**	16.50 \pm 1.86**
High dosage	500	87.46 \pm 7.95**	47.63 \pm 2.83**	15.83 \pm 1.42**
Middle dosage	200	84.88 \pm 6.93**	46.78 \pm 3.11*	14.24 \pm 1.56*
Low dosage	50	85.90 \pm 7.41*	45.99 \pm 2.65#	13.59 \pm 1.37*

Compared With normal control # $P < 0.05$, ## $P < 0.01$; Compared With model control, * $p < 0.05$, ** $p < 0.01$.

secretion. SBOSs treatment which was decreased the serum cytokine level of the model group and was increased the serum INF- γ content in the low-, middle- and high-dose groups ($P < 0.05$), however, the INF- γ content of these groups were lower compared to the normal group. The level of serum IL-4 and TNF- α was all markedly raised in the middle-dose group and high-dose group ($P < 0.05$) but lower than that of control group, a finding that is consistent with the levels of monocyte/macrophages, T and B cells, and NK cells. In general, SBOSs are antagonists of cytotoxicity caused by CTX such as a decrease of the immune cell number and activity. SBOSs can promote the proliferation and activation of immune cells to secrete more cytokines, but levels were not returned to normal (see Table 7).

4. Discussion

The Impacts of SBOSs on immune function of immunosuppressive mice were studied, including the changes of immune organs, immune cells and immune molecules. SBOSs showed a varying degree of regulation on the non-specific and specific immunity of immunosuppressive mice, but the immune indexes did not return to normal levels (Gill et al., 2001; Takeda et al., 2006; Feng et al., 2010). The results showed that SBOSs could regulate monocyte/macrophage phagocytosis, promote the formation of antibodies and antibody forming cells, improve the secretion of cytokines and proliferation of splenic lymphocytes, and increase the killing rate of NK cells in immunosuppressive mice. SBOSs act on the intestine following oral delivery, promote the regulation of the intestinal microenvironment, improve the cytokine reaction induced by microorganisms, stimulate the proliferation and activation of immune cells, and release a variety of immune molecules (e.g., antibodies and cytokines) (Arseneau et al., 2007).

The immune system consists of three levels that can be divided into the immune organs, immune cells and immune molecules. The specific implementation of the immune function is executed by various types of immune cells, including lymphocytes, granulocytes as and so on. T and B lymphocytes and NK cells are lymphocytes, while monocyte/macrophages are antigen presenting cells. Different immune cells play different roles in the immune response. T and B lymphocytes are the key cells that participate in the specific immune response, exhibiting cellular immunity and humoral immunity effects, respectively. The main function of T lymphocytes is to adjust the immune response caused by protein antigens and eliminate bactericidal effects. B lymphocytes can produce antibodies. NK cells can dissolve various cells infected by viruses without antigen stimulation. Monocyte/macrophages could intake, process antigens, and present the processed antigen to antigen-specific T cells.

The Impacts of SBOSs on the four immunocyte were studied in this research. The lymphocyte proliferation test is commonly used to determine the T cell function. T cells can proliferate and transform to lymphoblasts under the action of mitogens, and their conversion rate could reflect the body's cellular immune function. B

cells divide, proliferate, differentiate and mature to antibody generating cells, which then secrete the corresponding antibody stimulated by the antigen or mitogen.

The analysis of the serum antibody forming cells and serum hemolysin reflects the proliferation and differentiation and the secretion of hemolysin to body fluids. SBOSs treatment increased the conversion function belonging to T and B lymphocytes. The determination of the serum hemolysin and antibody forming cell levels in immunosuppressed mice suggested that SBOSs could improve the proliferation, differentiation of B lymphocytes and antibody secretion. The carbon clearance ability of any dose group did not reach the normal level. Thus, analysis of monocyte/macrophage phagocytosis suggested that the a killing efficiency on in tumor and virus-infected cells was not significant.

NK cells are crucial effector cells in the immunologic defense system of human body that remove foreign matter and kill between pathogens and tumor cells. We found that SBOSs could not recover the killing activity of NK cells of immunosuppressed mice to the normal level, which may result from the following two reasons: first, SBOSs could not facilitate the activation and proliferation of lymphocytes that may finally differentiate to NK cells. Second, the secretion of cytokines including IL-2, IFN- γ and TNF- α remained at a low level. SBOSs may improve the intestinal microenvironment in immunotherapy.

T cells are a complex group of cells that can be differentiated to different sub-groups with different immunocompetences, including "helper" and "cytotoxic" T-cells. With the aid of T helper cells (Th), B cells have the ability to produce cells and enlarge the cellular immunologic response following differentiating into antibody. Activated T helper cells release cytokines, regulating T cells activity, B cells activity, monocytes/macrophages activity and other immune cells activity. According to cytokine differences, T helper cells can be divided into 3 types: Th1, Th2 and Th0. Th1 can secrete IFN- γ , IL-2 and TNF- β increase the cellular immune response and promote the synthesis of IgM and IgG2 by B cells. Activated Th2 cells can produce IL-10, IL-6, IL-5 and IL-4, facilitate the secrete of IgG1 and IgE, and increase the amount of eosinophils in the partial circulation.

Th0 cells have the biological activity of Th2 and Th1 cells. The dominant cell factors secreted by Th1 and Th2 cells are IL-4 and IFN- γ . Normally, Th2 and Th1 cells self-regulate are regulated by cytokines secreted by other immune cells to maintain normal immune function. The balance of Th1/Th2 is the core pillar of immune balance of the human body and regulates the internal environment, especially the stability of the immune system. IFN- γ produced by Th1 cells mediates the cellular immune response, has antiviral activity and regulates the immune system. IL-4 secreted by Th2 cells can facilitate the proliferation of activated B cells as well as mediating the humoral immune response. The amount of IFN- γ derived from IL-4 and Th1 cells produced by Th2 cells is crucial for regulating the proportion of Th1/Th2.

Serum IL-4 and IFN- γ regarding to the three-dose groups of immunosuppressed mice were lower than the control groups,

which suggested that increasing the Th1 and Th2 cytokine activity was adverse for maintaining the balance of Th1/Th2 and immune function recovery.

The secretion level of Th1 cells was low, which may influence the activation and secretion of TNF- α . The results of the carbon clear test suggested that SBOSs could not promote the proliferation and activation of monocytes/macrophages compared with the control groups. Though IL-4 secreted by Th2 cells is a superactive anti-inflammatory cytokine and can decrease the expression of inflammatory cell factors TNF- α , IL-1 and IL-8, thus, the serum TNF- α in the immunosuppressed mice was not recovered to a normal level.

In conclusion, to detect SBOS-induced improvements on the proliferation of intestinal flora and immune function of immunosuppressed mice, we simulated human clinical methods and orally administered the prebiotic for a 29 day period. We found that SBOSs selectively improved intestinal flora proliferation and changed the flora composition. Colonization of the intestinal flora promoted the formation of the intestinal mucosa and then changed the immune cytokine levels and immune function, suggesting that the change in the number of intestinal flora was not related to improving immune function.

In recent years, with the application of immunosuppressive therapy, some encouraging treatment results have been achieved in immunity hematology. However, immunosuppressive therapy can also damage the normal immune response and can be cytotoxic. Attention must be given to the toxic side effects caused by immunosuppressive therapy. A gastrointestinal reaction is the most common side effect. The multiple side effects and complications, including diarrhea, nausea, vomiting, and anorexia, may influence therapy continuation.

5. Conclusion

Some related studies have also proved that soybean oligosaccharide has rich sources, no harmful residues, and obvious probiotics. When the digestive physiological function of infants is not yet mature, intestinal immune stress is often caused due to weaning, food changes and dysfunction, which results in dyspepsia and growth retardation. The physiological characteristics of piglets during weaning are very close to those of infants and young children. Via using weaning piglets around the river fragrance as experimental animals, the microecology and immune function of soybean oligosaccharide to intestinal tract were analyzed in vivo and in vitro. The regulating effect has obtained a new idea for improving the nutrition of infants and young children, and is the theoretical basis for the comprehensive development and utilization of soybean oligosaccharide.

When further studying the characteristic composition of intestinal microorganisms, it was found that the changes of intestinal microecology in special people who needed immunosuppressive agents for life were often different from those before operation, which would affect the changes of intestinal microecology and change the structure of flora after operation. The results showed that soybean oligosaccharide could significantly increase the diversity of intestinal microecology, increase the number of beneficial bacteria in intestinal tract, inhibit the proliferation of harmful bacteria, and thus reduce the catabolism of protein in large intestine. Furthermore, SBOS can improve intestinal morphology and structure, regulate of immune function, enhance of intestinal mucosal barrier function, and promote nutrient metabolism. When the intestinal mucous membrane barrier and intestinal permeability are normal, there will be no serious clinical infection even if there are some conditional pathogenic bacteria. This conclusion will help

us to understand the role of microorganisms in human health and disease more deeply and widely.

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