

L-Arabinose Attenuates Gliadin-Induced Food Allergy via Regulation of Th1/Th2 Balance and Upregulation of Regulatory T Cells in Mice

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ABSTRACT: Gliadins are the main cause of wheat allergies, and the prevalence of gliadin allergy has increased in many countries. L-Arabinose, a kind of plant-specific five-carbon aldose, possesses beneficial effects on food allergy to gliadins. This study investigated the antiallergic activities and underlying mechanisms of L-arabinose in a wheat gliadin-sensitized mouse model. BALB/c mice were sensitized to gliadin by intraperitoneal injections with gliadin followed by being given a gliadin challenge. L-arabinose-treated mice exhibited a marked reduction in the productions of total immunoglobulin E (IgE), gliadin-specific IgE, gliadin-specific IgG1, and histamine, with an increase in IgG2a level as compared with gliadin-sensitized mice. Beside that, a significant decrease in Th2-related cytokine level, IL-4, and an increase in Th1-related cytokine level, IFN- γ , in the serum and splenocytes were observed after treatment with L-arabinose. L-Arabinose treatment also improved the imbalance of Th1/Th2 immune response on the basis of the expression levels of related cytokines and key transcription factors in the small intestine and spleen of sensitized mice. In addition, gliadin-induced intestinal barrier impairment was blocked by L-arabinose treatment via regulation of TJ proteins and suppression of p38 MAPK and p65 NF- κ B inflammation signaling pathways. Notably, the results confirmed that L-arabinose treatment increased CD4⁺ Foxp3⁺ T cell populations and Treg-related factors associated with increased expression of IL-2 and activation of STAT5 in gliadin-sensitized mice. In conclusion, L-arabinose attenuated the gliadin-induced allergic symptoms via maintenance of Th1/Th2 immune balance and regulation of Treg cells in a gliadin-induced mouse model, suggesting L-arabinose could be used as a promising agent to alleviate gliadin allergy.

KEYWORDS: L-Arabinose, wheat gliadins, allergy, Th2, regulatory T cells

■ INTRODUCTION

Wheat is one of the major consumed cereals worldwide, but it can cause serious allergenic responses, and the prevalence of wheat allergy has rising over the past decade.¹ Adults and children suffer different types of clinical symptoms of wheat allergy. Atopic eczema/dermatitis syndrome (AEDS) is mainly observed in children, whereas in adults, wheat-dependent exercise-induced anaphylaxis (WDEIA) is more common and frequent.^{2–4} Gliadins and glutenins are major allergens in wheat, which vary in proportion in wheat products resulting in different allergenicity and allergy symptoms.⁵ In particular, gliadin, the major prolamin protein in wheat, is the principal allergen of wheat allergy.

Gliadin allergy is a kind of IgE-mediated immune response accompanied by allergic reactions, which is related to higher antigen-specific immunoglobulin E (IgE) from B lymphocytes.^{1,6} Since allergy is involved in impaired Th1/Th2 immune responses balance, modulating aberrant T cell immune responses has been regarded as an effective strategy for food allergies. An imbalance in Th1/Th2 immune responses has been known to lead to intestinal barrier impairment. Enhancement of tight junctions (TJs) and protection of intestinal epithelial cells are beneficial to ameliorate food allergy.⁷ As major immunosuppressive cells, regulatory T (Treg) cells responses play roles in the regulation of allergen-specific Th2 and Th1 cell responses involving

suppress allergen-specific T cell proliferation and the secretion of Th1 and Th2 cytokines.⁸ In addition, Treg cells can effectively inhibit the production of IgE and directly or indirectly suppress the function of allergic inflammation effector cells, such as eosinophils, mast cells, and basophils.⁹ In particular, transforming growth factor- β (TGF- β) and interleukin-10 (IL-10) are critical in the activation and maintenance of Treg cells.¹⁰

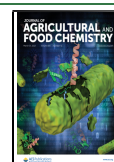
In clinical practice, strict dietary restriction to avoid allergen intake is the main cure for food allergy. However, it is difficult to avoid allergens completely, and related drugs for food allergy may have unexpected side effects such as nausea, diabetes, hypertension, and diarrhea.^{11,12} So, effective and safe strategies for the prevention and treatment of allergy to wheat gliadins have been emphasized. Natural products with immunomodulatory activities are considered to be safe immunotherapy. L-Arabinose is a kind of plant-specific aldopentose. As a component of biopolymers, it is widely found in L-arabinose-rich plant tissues such as corn husks, corn

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cobs, beet pulp, bagasse, and wheat bran.¹³ L-Arabinose has been shown to exert immunomodulatory effects to some extent as a functional food. Zhao et al. have reported that orally administered L-arabinose could reduce elevated serum C-reactive protein and TNF- α level caused by high-fat diet in C57BL/6J mice with metabolic syndrome, indicating L-arabinose has a beneficial effect on systemic inflammation.¹⁴ Our previous study confirmed that L-arabinose could protect mice from dextran sodium sulfate (DSS)-induced colitis.¹⁵ However, the beneficial effect of L-arabinose on food allergy to gliadin remains unclear and requires further investigation.

In this study, we used the gliadin sensitization model to evaluate the antiallergic properties of L-arabinose. We investigated its effects on the regulation of IgE and histamine production, Th1/Th2 cytokines, protection of intestinal barrier and differentiation of CD4⁺ T cells to Treg cells in gliadin allergy mouse model. Our data revealed that L-arabinose is a potential protector against gliadin allergy via regulation of Th1/Th2 cell response and increase in the population of Treg cells in gliadin-sensitized BALB/c mice.

MATERIALS AND METHODS

Chemicals and Reagents. L-Arabinose was purchased from Sigma-Aldrich (W325512). Aluminum hydroxide was obtained from Thermo (77161, 40 mg/mL). Gliadin from wheat S24514 was purchased from Shanghai Yuanye Biological Technology Co., LTD (Shanghai Yuanye Biological Co, Ltd., S24514). Enzyme-linked immunosorbent assay (ELISA) kits of IL-4, IFN- γ , TGF- β , histamine, IgE, gliadin-specific IgE, gliadin-specific IgG2a, and IgG1 were purchased from Nanjing jiancheng (Nanjing jiancheng, Nanjing, China). The antibody of ZO-1 and occludin were obtained from Proteintech (Rosemont, USA). Antibodies against p-p65, p65, p-p3, p38, STAT5, p-STAT5, and GAPDH were purchased from Cell Signaling Technology (MA, USA).

Animal. Female BALB/c mice (3–4 weeks old, 18–20 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and housed in specific pathogen-free conditions with a 12 h dark-light cycle. The temperature and humidity were maintained at 24 ± 2 °C and $50\% \pm 10\%$, respectively. All mice received food and water ad libitum and were acclimated for 2 week before immunizations. All animal experiments were approved by the Laboratory Animal Ethics Committee of Jiangnan University (Wuxi, China). Ethical approval number: JN.No20190615b0200810 [173].

Sensitization and Allergic Challenge of Mice. Mice were randomly divided into three groups ($n = 10$ for each group): a control group, gliadin-sensitized group (Gliadin), and L-arabinose-treatment group (Gliadin+L-Ara). The protocol was performed as previously described for sensitization and elicitation with slight modification to induce allergic reactions.^{16,17} Briefly, mice were immunized with 50 μ g of gliadin absorbed on 1 mg of aluminum hydroxide by intraperitoneal injection on days 0 and 14. Mice in the control group were sensitized with aluminum hydroxide diluted in PBS. From day 28 to 38, the mice were orally challenged with 20 mg of gliadins every other day. From day 27, sensitized mice in the L-arabinose group were intragastrically administered with L-arabinose (400 mg/kg body weight) 1 h before the challenge and L-arabinose was given daily, while other two groups received distilled water of equal volume.¹⁵ Body weight was monitored before and after the allergic challenge. One hour after the last intragastric challenge with gliadins, anaphylactic symptoms were scored as described previously.¹⁸ Then, all mice were sacrificed and the spleen index was calculated (mg/g body weight). Small intestines and plasma were collected for further analysis.

Allergenicity Analysis. One hour after the last challenge, serum samples were collected to detect the following biomarkers. Mouse total IgE and histamine levels in the serum were assayed respectively by ELISA kit according to the manufacturer's instructions. ELISA

analysis of IgE specific to gliadin in the serum was conducted as previously described.¹⁹ The concentrations of IL-4, IFN- γ , and TGF- β in the serum were measured using Mouse IL-4, IFN- γ , and TGF- β ELISA kits.

Measurement of Cytokine Production from Cultured Splenocytes. After mice were sacrificed, single cell suspensions of spleen were prepared and treatment was as previously described.¹⁶ After the lysis of red blood cells, splenocytes were incubated in RPMI-1640 medium containing 10% fetal bovine serum, 100 mg/mL streptomycin, and 100 IU/mL penicillin (Gibco, Invitrogen Inc.) at 37 °C and 5% CO₂. Cells were exposed to gliadin (20 μ g/mL in PBS) or PBS for 72 h in 6-well culture plates. Cell supernatants were collected, and IL-4, IFN- γ , and TGF- β levels were assayed by Mouse IL-4, IFN- γ , and TGF- β ELISA kits following the manufacturer's instructions.

Quantitative Real-Time PCR analysis (qRT-PCR). Total RNA from the spleens and small intestines was extracted using Trizol reagent (Thermo Fisher Scientific, CA, USA). cDNA was synthesized using the Prime Script RT system (Takara, Japan). After cDNA synthesis, the mRNA expression levels of TSLP, GM-CSF, Th1/Th2/Th17/Treg related cytokine and critical transcription factors (IL-2, IFN- γ , IL-4, IL-5, TGF- β , T-bet, GATA-3, Foxp3, and CTLA-4), and tight junction proteins (ZO-1, occludin, claudin-1, and JAM-1) were assayed by qRT-PCR using an ABI 7900 RT-PCR system (Applied Biosystem, USA). The mRNA expression level of 18S was used as an internal control. The sequences of qRT-PCR primers are shown in Table S1.

Western bolt analysis. Western blotting was performed as previously described.²⁰ In brief, proteins were extracted from spleen and small intestine tissues using RIPA lysis buffer (Beyotime, Shanghai, China). The concentration of the protein was measured using the BCA protein assay reagent (Beyotime, Shanghai, China). Samples with equal amounts of protein were loaded and separated on 10% SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were incubated with specific primary antibodies and HRP-conjugated secondary antibodies. ImageJ software was used for the quantification of the band intensity, and the results were normalized to the β -actin.

Flow cytometry analysis. Single cells were isolated from spleen, mesenteric lymph nodes (MLNs), and Peyer's patches (PPs). To detect Tregs, staining protocols were performed according to the manufacturer's instructions (eBioscience, San Diego, CA). Cells were first labeled with anti-CD4-FITC (eBioscience). After being surface-stained, cells were fixed and permeabilized using Foxp3/Transcription Factor Fixation/Permeabilization agents (eBioscience); then, cells were stained with anti-Foxp3-PE (eBioscience). Single cell suspensions were also stained with anti-CD11b-PE-Cy7 and anti-Siglec-F-PE (BD Biosciences, San Jose, CA, USA) to detect eosinophils. Data was acquired using BD FACSAria III system (BD Biosciences) and analyzed by FlowJo software (Tree Star, Inc., San Carlos, CA, USA).

Histological analysis. Hematoxylin and Eosin (H&E) and toluidine blue staining of jejunum tissue were carried out as previously described.²¹ Briefly, small intestine tissues were fixed in 10% neutral formalin, embedded in paraffin, and sectioned at 5 μ m thickness. Tissue sections were stained with toluidine blue to investigate mast cells. The section images were observed by an inverted light microscope (Axio Vert.A1, Carl Zeiss Microscopy GmbH, Germany).

Statistical Analysis. Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software). Data were presented as the mean $\pm$ SEM with at least three independent experiments. Statistical significance between the two groups was determined by Student's *t* test. Differences were taken as statistically significant at $p < 0.05$.

RESULTS

L-Arabinose ameliorates wheat gliadin-induced allergic reactions in vivo. First, we evaluated whether L-arabinose

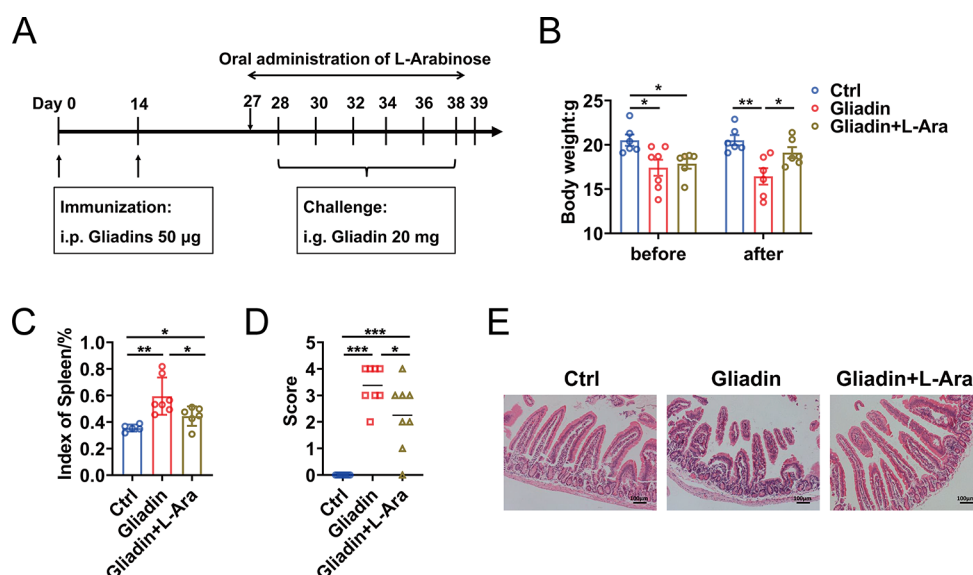


Figure 1. L-Arabinose suppresses gliadin-induced food allergic responses. Protocol for the sensitization and allergic challenge of mice (A). Female mice were sensitized twice by intraperitoneal injection of 50 µg gliadins on days 0 and 14, and followed by orally challenged with 20 mg gliadins for six times from day 28 to 38. L-Arabinose was orally administered 1 h prior to gliadins challenge from day 27 to 38. Body weight was monitored before and after L-arabinose treatment (B). Spleen index (C). Anaphylactic response scores were evaluated 1 h after the last challenge (D). Representative histologic images of hematoxylin and eosin (H&E) staining of jejunum sections (E). Data were shown as the mean $\pm$ SEM of three independent experiments for all panels. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's t test). Ctrl: control; Gliadin: gliadin-sensitized mice; Gliadin + L-Ara: gliadin-sensitized mice treated with L-arabinose.

could suppress allergic reactions in gliadin-sensitized mice. BALB/c mice were sensitized with gliadins, followed by orally administered L-arabinose as shown in Figure 1A. The L-arabinose-treated group showed a significantly lower body weight loss and spleen index compared with that in gliadin-sensitized mice (Figure 1B,C). Additionally, severe anaphylactic symptoms were observed in gliadin-sensitized mice, whereas L-arabinose supplement could alleviate anaphylactic responses induced by gliadins (Figure 1D). The histological assessment of jejunum was shown in Figure 1E. More severe intestinal alterations were observed in jejunum of gliadin-sensitized mice, whereas L-arabinose treatment was helpful to recovery of the histopathological change. Taken together, these results suggested that L-arabinose could alleviate allergy caused by gliadin antigen.

L-Arabinose modulates different immune markers production. Allergen-specific IgE mediates allergic reactions, and we determined gliadin-specific IgE and total IgE levels in the serum using the Elisa assay. Total IgE and gliadin-specific IgE levels in the serum were significantly increased in the gliadin-sensitized mice compared to the control group. However, after treatment with L-arabinose, significantly lower total IgE and gliadin-specific IgE levels were detected (Figure 2A,B). In addition, as a critical mediator of mast-cell degranulation and allergic symptoms, histamine concentration was measured to evaluate its potential antiallergic activities. As expected, gliadin antigens induced the release of histamine in the gliadin-sensitized mice, and L-arabinose treatment significantly decreased the serum histamine concentration in the gliadin-sensitized mice (Figure 2C). As shown in Figure 2D,E, L-arabinose-treated mice exhibited a marked reduction in IgG1 level, with an increase in IgG2a level as compared with gliadin-sensitized mice. These results indicated that administration of L-arabinose could ameliorate IgE and mast cell-mediated

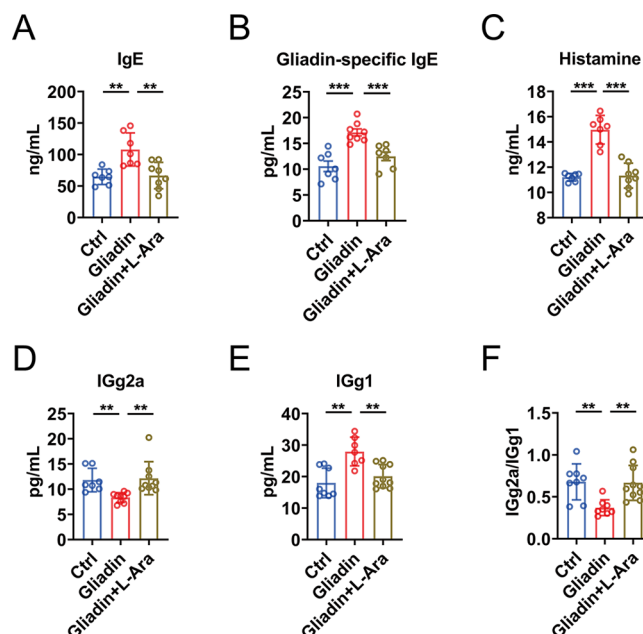


Figure 2. L-Arabinose inhibits the production of gliadin-specific antibodies and allergic mediators. Mice were sensitized and challenged with gliadins followed by L-arabinose treatment as described in Figure 1A. The levels of total IgE (A), gliadin-specific IgE (B), histamine (C), gliadin-specific IgG2a (D), gliadin-specific IgG1 (E), and gliadin-specific IgG2a/IgG1 ratio (F) in the serum were measured. Data were presented as the mean $\pm$ SEM of three independent experiments for all panels. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's t test). Ctrl: control; Gliadin: gliadin-sensitized mice; Gliadin + L-Ara: gliadin-sensitized mice treated with L-arabinose.

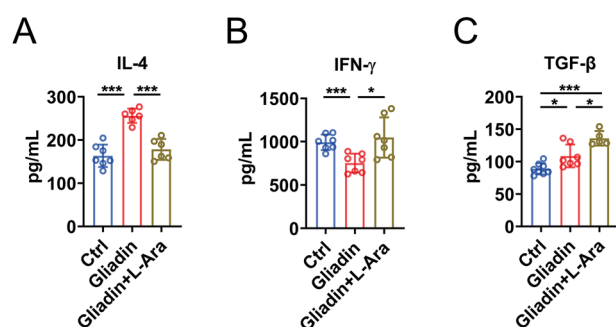
allergic disorders and play roles in improving the Th1/Th2 imbalance.

L-Arabinose regulates Th1 and Th2 cell responses in gliadin-sensitized mice. Food allergies are associated with excessive Th2 immune response, and the imbalance of Th1 and Th2 immune response is an effective indicator of food allergies.^{18,22} Therefore, improving Th1 and Th2 cell imbalance can effectively alleviate allergic reactions. To further determine whether L-arabinose could affect the Th1 and Th2 cell responses in gliadin-sensitized mice, we determined the serum IFN- γ and IL-4 levels of gliadin-sensitized mice after L-arabinose administration. As expected, Th1/Th2 cell imbalance induced by gliadin was observed in sensitized mice. Gliadin-sensitized mice displayed a significantly increase in IL-4 level compared with the control mice. By contrast, we found that the IL-4 level in the serum was decreased under L-arabinose administration (Figure 3A). The decrease of IFN- γ , a

the serum and splenic cells (Figure 3C,F). These results indicated that L-arabinose could enhance Th1 and inhibit Th2 cell immune responses to alleviate the allergic reactions.

Allergic Inflammatory Response Is Altered by L-Arabinose in Jejunum and Spleen. To further identify whether L-arabinose could regulate Th1 and Th2 cell responses, we measured the gene expressions of Th1 and Th2 cytokines and their key transcription factors T-bet and GATA-3 in the jejunum, which play crucial roles in the development of allergic responses. Decreases in the mRNA expression of Th1 cytokine IFN- γ was observed in the jejunum of gliadin-sensitized mice compared with the control group, but L-arabinose intervention upregulated the expression of IFN- γ (Figure 4A). In addition, L-arabinose treatment could

Serum



Spleen

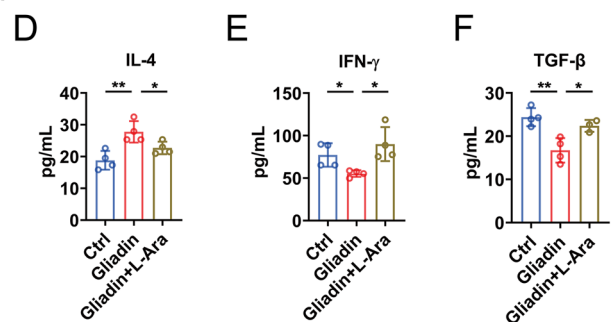
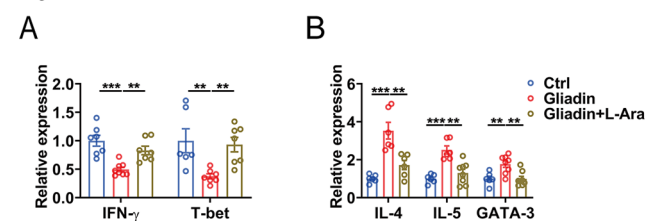


Figure 3. Effects of L-arabinose on the production of IL-4, IFN- γ , and TGF- β in gliadin-sensitized mice. Mice were sensitized and challenged with gliadins and treatment with L-arabinose as described in Figure 1A. (A–C) The amounts of IL-4 (A), IFN- γ (B), and TGF- β (C) in the serum were determined. (D–F) Cells isolated from spleen were stimulated with gliadins (20 μ g/mL in PBS) or PBS up to 72 h, and the levels of IL-4 (D), IFN- γ (E), and TGF- β (F) in the cell supernatant were assessed using ELISA assay. Data were shown as the mean $\pm$ SEM of three independent experiments for all panels. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's t test). Ctrl: control; Gliadin: gliadin-sensitized mice; Gliadin + L-Ara: gliadin-sensitized mice treated with L-arabinose.

typical Th1 cytokine, caused by food allergy was suppressed by L-arabinose administration (Figure 3B). In addition, splenocytes isolated from gliadin-sensitized mice were incubated with gliadins to further elucidate potential mechanisms via which L-arabinose exerted antiallergic effect. Consistent with the results in vivo, an increased IFN- γ level and a decreased IL-4 concentration of culture supernatant were observed in the L-arabinose group (Figure 3D,E). Furthermore, the L-arabinose-treated group exhibited a marked increase in TGF- β level in

Jejunum



Spleen

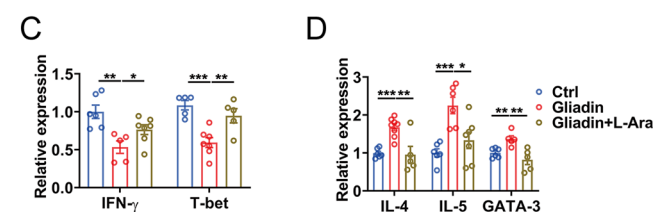


Figure 4. L-Arabinose regulates Th1 and Th2 cell responses in gliadin-sensitized mice. Mice were sensitized and challenged with gliadins and treated with L-arabinose as described in Figure 1A. The mRNA expression levels of key inflammatory markers in jejunum sections were determined by RT-qPCR analysis. Th1-related cytokine and transcription factor, IFN- γ and T-bet (A). Th2-related cytokine and transcription factor, IL-4, IL-5, and GATA-3 (B). qRT-PCR analysis of mRNA levels indicated in spleen of sensitized mice. Th1-related cytokine and transcription factor, IFN- γ and T-bet (C). Th2-related cytokine and transcription factor, IL-4, IL-5, and GATA-3 (D). The mRNA expression levels were normalized to 18S and were shown relative to mice in control group. Data were shown as the mean $\pm$ SEM of three independent experiments for all panels. $n = 6$ –8 mice per group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's t test). Ctrl: control; Gliadin: gliadin-sensitized mice; Gliadin + L-Ara: gliadin-sensitized mice treated with L-arabinose.

reverse abnormal elevated expression of Th2 cytokines IL-4 and IL-5 in the jejunum of gliadin-sensitized mice (Figure 4B). Consistently, the mRNA expression of T-bet, key transcription factor of Th1 cells, was significantly increased, whereas GATA-3 mRNA expression level was significantly decreased after L-arabinose intervention (Figure 4A,B). Beside that, L-arabinose intervention could restore Th1 and Th2 cell responses in a similar manner in spleen. The mRNA level of IFN- γ and T-bet was increased, while IL-4, IL-5, and GATA-3 mRNA expression levels were inhibited by L-arabinose administration in gliadin-sensitized mice spleens (Figure 4C,D). These results suggested that the beneficial effects of L-arabinose on gliadin allergy were exerted by modulating the Th1/Th2 imbalance.

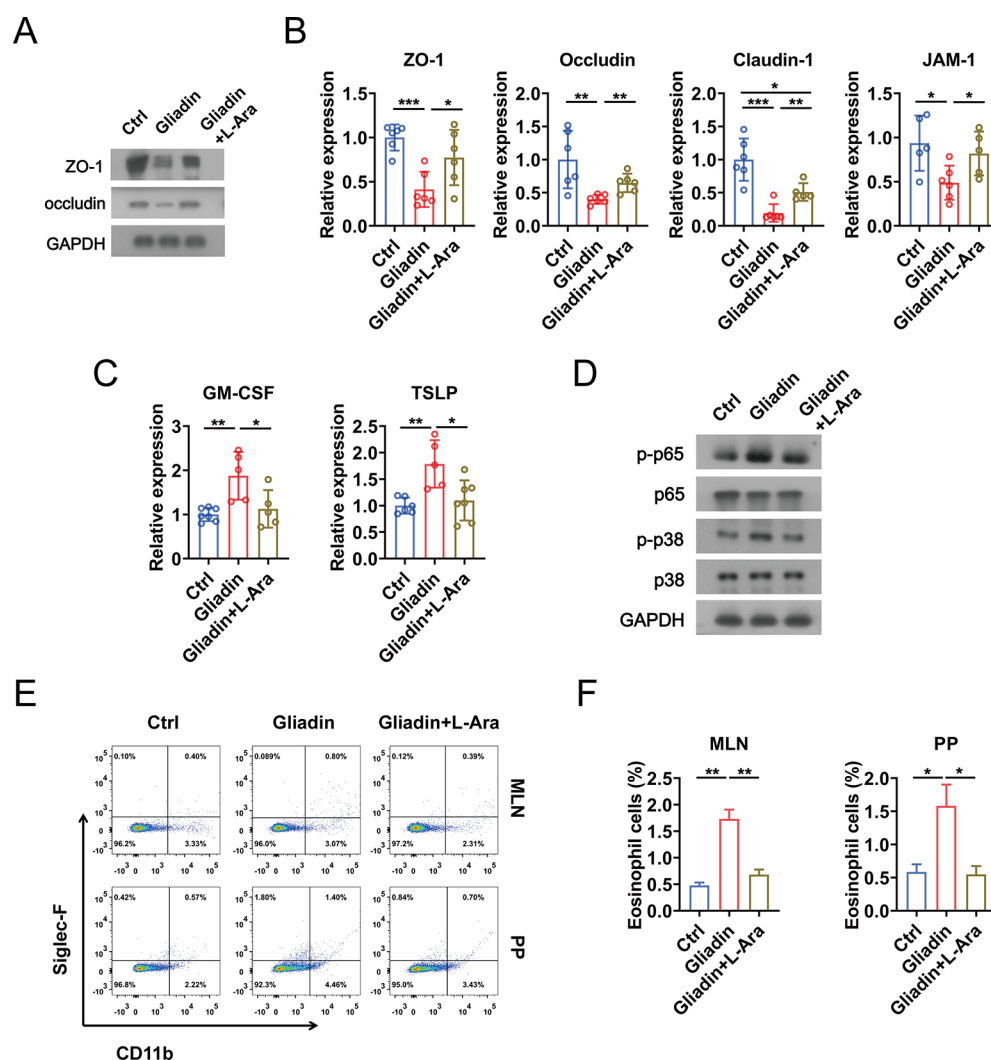


Figure 5. L-Arabinose inhibits gliadin-induced damage of intestinal barrier. The protein levels of tight junction protein genes ZO-1 and occludin (A). qRT-PCR analysis of ZO-1, occludin, claudin-1, and JAM-1 mRNA levels of jejunum sections in each group (B). The mRNA expression levels of GM-CSF and TSLP in jejunum sections (C). The mRNA expression levels were normalized to 18S and were shown relative to mice in control group. The protein levels of p-p38 and p-p65 in jejunum sections (D). GAPDH was used as an internal reference. Representative images of flow cytometry analysis (E) and frequency of eosinophils in MLNs and PPs (F). Data were shown as the mean $\pm$ SEM of three independent experiments for all panels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's t test). Ctrl: control; Gliadin: gliadin-sensitized mice; Gliadin + L-Ara: gliadin-sensitized mice treated with L-arabinose.

L-Arabinose Protects Intestinal Barrier from Gliadin-Induced Damage. The allergic response to food is induced by allergen permeation into the intestinal epithelium. Tight junction (TJ) proteins are known to play roles in maintenance of intestinal barrier integrity, and the protection of intestinal barrier function contributes to amelioration of food allergy.²³ The mRNA expression of TJ proteins, as well as the protein expression of ZO-1 and occludin increased in the intestinal epithelium of L-arabinose group (Figure 5A,B). Additionally, the expression of cytokines produced by intestinal epithelial cells, such as granulocyte macrophage colony-stimulating factor (GM-CSF) and TSLP, were suppressed in L-arabinose-treated mice (Figure 5C). Beside that, the protein levels of p-p38 and p-p65 were downregulated in the jejunum of gliadin-sensitized mice treated with L-arabinose (Figure 5D). Moreover, based on the decreased expression of IL-5, we detected the frequency of eosinophils in the MLNs and PPs. As shown in Figure 5E,F, L-arabinose intervention could reduce the accumulation of eosinophils in the small intestine of allergic

mice, which contributed to its positive effects on epithelial barrier function. Together, these data indicated that L-arabinose could relieve allergic reactions through protecting intestinal barrier impairment.

L-Arabinose Enhances the Induction of Treg Cells. It has been reported that Treg cells regulate the balance of allergen specific Th1 and Th2 cells. This could occur by favoring Th1 skewing and inhibiting the development of Th2-type allergic responses.²⁴ According to the results of regulation Th1 and Th2 cell responses by L-arabinose, the proportion of Treg cells was measured to explore whether L-arabinose could modulate the differentiation of CD4⁺ T cells to Treg cells in spleen, MLNs, and PPs of gliadin-induced mice. The results showed that the population of Treg cells decreased in gliadin-induced mice compared to the control group. However, sensitized mice treated with L-arabinose exhibited a significant increase in Treg cells numbers in the spleen, MLNs, and PPs (Figure 6A,B). We next verified the induction of Treg cells by L-arabinose. The phosphorylation of STAT5 and the

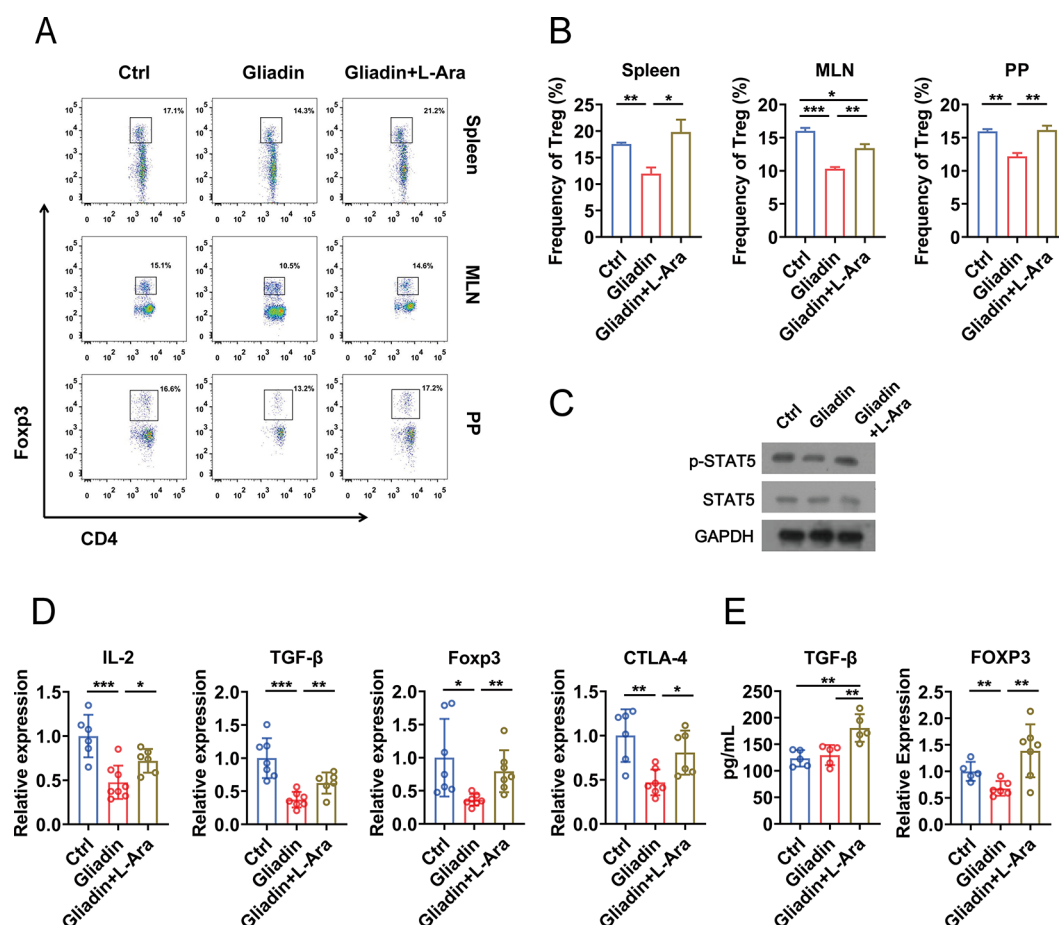


Figure 6. Effects of L-arabinose on Treg cells in the spleen, MLNs, and PPs of gliadin-induced allergic mice. Gliadin-induced allergic mice were administered with L-arabinose as described above. Spleen, MLNs, PPs were collected to measure Treg cell population by flow cytometry analysis. Representative images (A) and the frequency of Treg cells in CD4 positive population of spleen, MLNs, PPs (B) were shown. The protein levels of p-STAT5 and STAT5 in jejunum sections (C). GAPDH was used as an internal reference. qRT-PCR analysis of Treg-related cytokine and transcription factor, IL-2, TGF- β , Foxp3, and CTLA-4 mRNA levels in jejunum sections (D). The mRNA expression levels of TGF- β and Foxp3 in spleens (E). The mRNA expression levels were normalized to 18S and were shown relative to mice in control group. Data were shown as the mean $\pm$ SEM of three independent experiments for all panels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's t test). Ctrl: control; Gliadin: gliadin-sensitized mice; Gliadin + L-Ara: gliadin-sensitized mice treated with L-arabinose.

expressions of IL-2 were elevated in response to the L-arabinose treatment (Figure 6C,D), suggesting L-arabinose treatment activated STAT5 pathway and promoted the transcription of Foxp3. Consistently, the expressions of TGF- β , Foxp3 and CTLA-4 increased in gliadin-sensitized mice treated with L-arabinose and these molecules play an important role in regulating Treg cell function and homeostasis (Figure 6D,E). These results demonstrated that L-arabinose attenuated gliadin-induced food allergy by inducing CD4⁺ Foxp3⁺ T cells to modulate the Th1/Th2 imbalance.

DISCUSSION

L-Arabinose, an aldopentose extracted from plants, is known to have beneficial effects on antihyperglycemia, improving insulin resistance, reducing triglyceride and fat accumulation.^{25,26} In addition, L-arabinose has been reported to exert anti-inflammatory effects in DSS-induced colitis.¹⁵ However, the effects of L-arabinose on food allergy to wheat gliadins have not been studied, and we evaluated the inhibitory effects of L-arabinose on gliadin-induced allergy. Gliadins are major principal allergens of wheat, and gliadin allergy are IgE-mediated allergic responses accompanied by IgE-dependent

degranulation of mast cells and recruitment of eosinophils in inflammation sites.²⁷ Therapeutic approaches through controlling immune function for wheat allergy have been attempted.^{17,28} According to Pérot et al., polyphenol extracted from cranberry interacted with gliadins and mask epitopes, which reduced the immunogenicity and allergenicity of wheat gliadins, and consequently, the reduction mouse anti-gliadin IgE recognition inhibited IgE mediated allergic reactions.²⁹ In the current study, gliadin-sensitized mice exhibited a typical Th2 response with high levels of IgE, histamine, and intestinal alterations, which were the same as the existing research results.^{19,29} L-Arabinose had a certain relieving effect on adverse symptoms caused by an allergy with the regulation of IgE production and histamine release, and maintenance of intestinal barrier integrity, which corroborated the antiallergic effect of L-arabinose.

Since the imbalance between Th1 and Th2 cell subsets caused by food allergy is considered to be responsible for allergic reactions, the regulation of Th1/Th2 cell balance by natural products to attenuate food allergy symptoms in mice has been reported. Shin et al. reported that skullcap treatment attenuated OVA-induced food allergy symptoms by inhibiting the Th2 cell response.³⁰ Hsu et al. demonstrated that an ip

injection of dioscorins proteins extracted from yam tubers suppressed the allergic reactions through modulation of the Th1/Th2 imbalance by the promotion of Th1 cell response in OVA-sensitized BALB/c mice.³¹ As an important Th2 cytokine, IL-4 stimulates B cells differentiation and IgE production, which induces the degranulation of MCs and aggravates allergic reactions. On the contrary, IFN- γ secreted by Th1 cells inhibits the secretion of IgE and the production of eosinophils, therefore, alleviating allergic reactions.³² In the current study, the serum IFN- γ level increased and the IL-4 level decreased by treatment with L-arabinose in sensitized mice, which was consistent with the detection of IgG2a and IgG1 levels. Additionally, in correlation with the decrease of IgE and histamine levels in serum, this result was confirmed by cytokine production by gliadin-reactivated splenocytes, suggesting that L-arabinose suppressed the allergic reaction by improving Th1/Th2 imbalance. Moreover, L-arabinose could modulate the expressions of Th1 and Th2 cytokines and the transcription factors, T-bet and GATA-3, to balance the Th1/Th2 immune response in small intestine and spleen of sensitized mice. Taken together, even if sensitized mice developed significant Th1/Th2 imbalance responses, L-arabinose treatment could restore Th1/Th2 imbalance to some extent.

Dendritic cells (DCs) sense and process wheat proteins and then activate specific Th cells. CD4⁺ T lymphocytes can be activated to differentiate into Th1, Th2, Th17, and Treg cell subsets according to their distinct profile of cytokine secretion and functional properties. It has been reported that modifications of gliadins by deamidation and Alcalase hydrolysis altered their processing by DCs and might affect Th cell stimulation in mesenteric lymph nodes (MLNs) and Peyer's patch cells (PPs).³³ Therefore, we detected whether the immunosuppressive effects of L-arabinose were due to the differentiation of Treg cells and found that L-arabinose was able to promote the differentiation of CD4⁺ T cells into Treg cells, which enhanced intestinal mucosal immunity. In addition, we detected that L-Arabinose effectively maintained intestinal barrier homeostasis by increasing TJ protein expression and suppressing inflammatory cytokine expression in intestinal epithelial cells. Beside that, our results revealed that beneficial effects of L-arabinose on intestinal barrier have correlations with the inactivation of p65 NF- κ B and p38 MAPK inflammation signaling pathways. Moreover, IL-5 is known to mediate eosinophil proliferation and survival, which play a crucial role in allergic processes and inflammation. The expression of IL-5 exacerbates the inflammatory response related to the eosinophil in food allergy.^{34,35} In this study, L-arabinose could inhibit the infiltration of eosinophils in inflammatory sites. Taken together, L-arabinose offers protection against the gliadin-induced damage of intestinal barrier which contributes to the attenuation of food allergy.

Dietary L-arabinose appears to affect the composition, diversity, and abundance of intestinal microbiota, and microbial degradation of L-arabinose increases the production of its metabolites, such as acetate, lactate, propionate, succinate, and molecular hydrogen according to previous reports.^{36,37} Furthermore, the gut microbiota and short chain fatty acids (SCFAs) are critical for gut homeostasis and Treg cell development.³⁸ It has been reported that dietary-fiber-derived SCFAs might promote differentiation of regulatory T (Treg) cells.³⁹ Consistently, our results revealed that L-arabinose could enhance the differentiation of Treg cells in

gliadin sensitized mice, which may be one of the possible mechanisms of the benefits of L-arabinose on gliadin allergy. Immunosuppressive Treg cells regulate Th2 and Th1 cell responses and maintain immune homeostasis; therefore, Treg cell-mediated immune suppression is regarded as a potential therapeutic approach for food allergy.⁴⁰ Katayama et al. demonstrated that oral administration of rutinoylated ferulic acid attenuated OVA-induced allergic response and DSS-induced colon inflammation by upregulating regulatory T cells in mouse models.⁴¹ Beside that, IL-2R signaling regulates Treg cell growth, function, and homeostasis. Increased phosphorylation of STAT5 promotes the transcription of Foxp3 in Treg cells, through which IL-2 affects the differentiation of Treg cells.⁴² In this study, increased IL-2 expression and activation of STAT5 were associated with the promotion of Treg differentiation, which revealed parts of the mechanism of L-arabinose effect on the induction of CD4⁺ Foxp3⁺ T cells. Our results indicated that enhanced Treg activity favored Th1 skewing and inhibited the development of Th2-type allergic responses, which was helpful to regulate the Th1/Th2 immune response and suppress allergic reactions.

In summary, the present study showed that dietary intervention with L-arabinose could alleviate gliadin-induced allergic reactions with the suppression of gliadin-specific IgE, histamine, intestinal barrier impairment, and reduced recruitment of eosinophils. Moreover, L-arabinose effectively recovered imbalanced Th1/Th2 immune responses and the population of Treg cells of sensitized mice, which may play an important role in the amelioration of gliadin allergy symptoms. Our findings indicate that L-arabinose can be a promising candidate against gliadin allergy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.0c07167>.

Primer Sequences; qRT-PCR analysis of IL-2, TGF- β , IL-17, and ROR γ t mRNA levels in spleen; mRNA expression levels of IL-17 and ROR γ t in jejunum sections; gate strategies of eosinophils in MLNs, and PPs; data were shown as the mean $\pm$ SEM of three independent experiments for all panels; * p < 0.05, ** p < 0.01, *** p < 0.001 (Student's t test); Ctrl: control; Gliadin: gliadin-sensitized mice; Gliadin + L-Ara: gliadin-sensitized mice treated with L-arabinose; gate strategies of Treg cells in Spleen, MLNs, and PPs; Ctrl: control; Gliadin: gliadin-sensitized mice; Gliadin + L-Ara: gliadin-sensitized mice treated with L-arabinose (PDF)

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Notes

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Table S1. Primer Sequences

gene	forward primer	reverse primer
ZO-1	ATAGGAGTGCAAGCAGGGAGA	CCATCTCTTGCTGCCAAACTATC
occludin	TGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
Claudin-1	GACTGTGGATGTCCTGCGTTTC	CAATTACCATCAAGGCTCGGG
JAM-1	AGTGGAAGTTCGTCCAAGGC	ACTCGGTCCGCATAGGGAG
TSLP	CAGCTTGTCTCCTGAAAATCG	AAATGTTTTGTCGGGGAGTG
GM-CSF	TCGTCTCTAACGAGTTCTCCTT	CGTAGACCCTGCTCGAATATCT
IL-2	GTGCTCCTTGTCAACAGCG	GGGGAGTTTCAGGTTCTGTGA
IFN- γ	TCAAGTGGCATAGATGTGGAAGA A	TGGCTCTGCAGGATTTTCATG
T-bet	CCTCTTCTATCCAACCAGTATC	CTCCGCTTCATAACTGTGT
IL-4	CTCATGGAGCTGCAGAGACTCTT	CATTCATGGTGCAGCTTATCGA
IL-5	TGACCGCCAAAAAGAGAAGTG	GAACTCTTGCAGGTAATCCAGGA A
GATA-3	CAGAACCGGCCCTTATCA	ACAGTTCGCGCAGGATGTC
TGF- β	CACCGGAGAGCCCTGGATA	TGTACAGCTGCCGCACACA
Foxp3	CCCATCCCCAGGAGTCTT	ACCATGACTAGGGGCACTGTA
CTLA-4	TTTTGTAGCCCTGCTCACTCT	CTGAAGGTTGGGTCACCTGTA
IL-17	AAAGCTCAGCGTGTCCAAAC	TTCTGGAGCTCACTTTTGCG
ROR γ t	ACAGCCACTGCATTCCCAGTTT	TCTCGGAAGGACTTGCAGACAT
18s	ACCGCAGCTAGGAATAATGGA	CAAATGCTTTCGCTCTGGTC

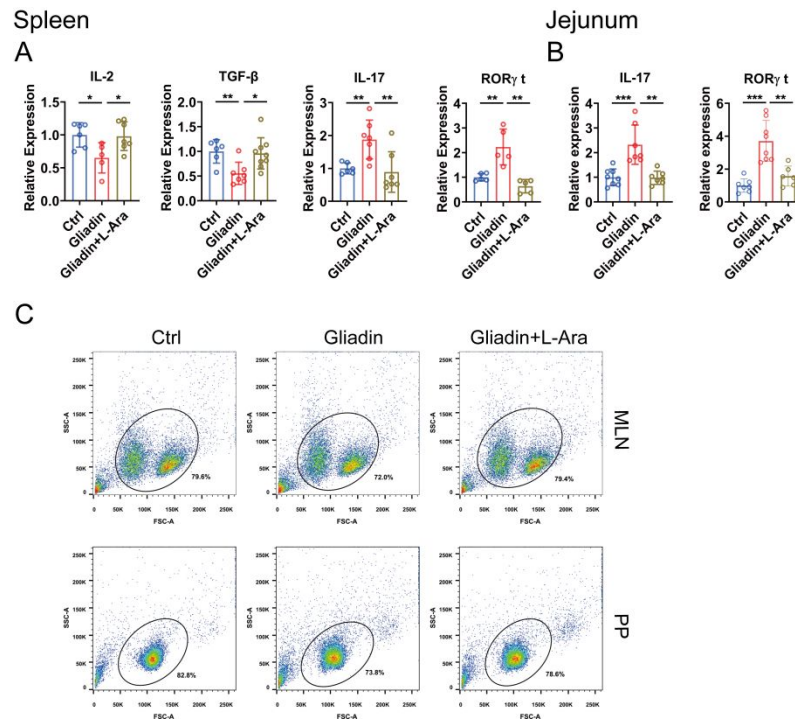


Figure S1. qRT-PCR analysis of IL-2, TGF- β , IL-17 and ROR γ t mRNA levels in spleen (A). The mRNA expression levels of IL-17 and ROR γ t in jejunum sections (B). Gate strategies of eosinophils in MLNs, and PPs (C). Data were shown as the Mean $\pm$ SEM of three independent experiments for all panels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's t-test). Ctrl: control; Gliadin: gliadins-sensitized mice; Gliadin + L-Ara: gliadins-sensitized mice treated with L-arabinose.

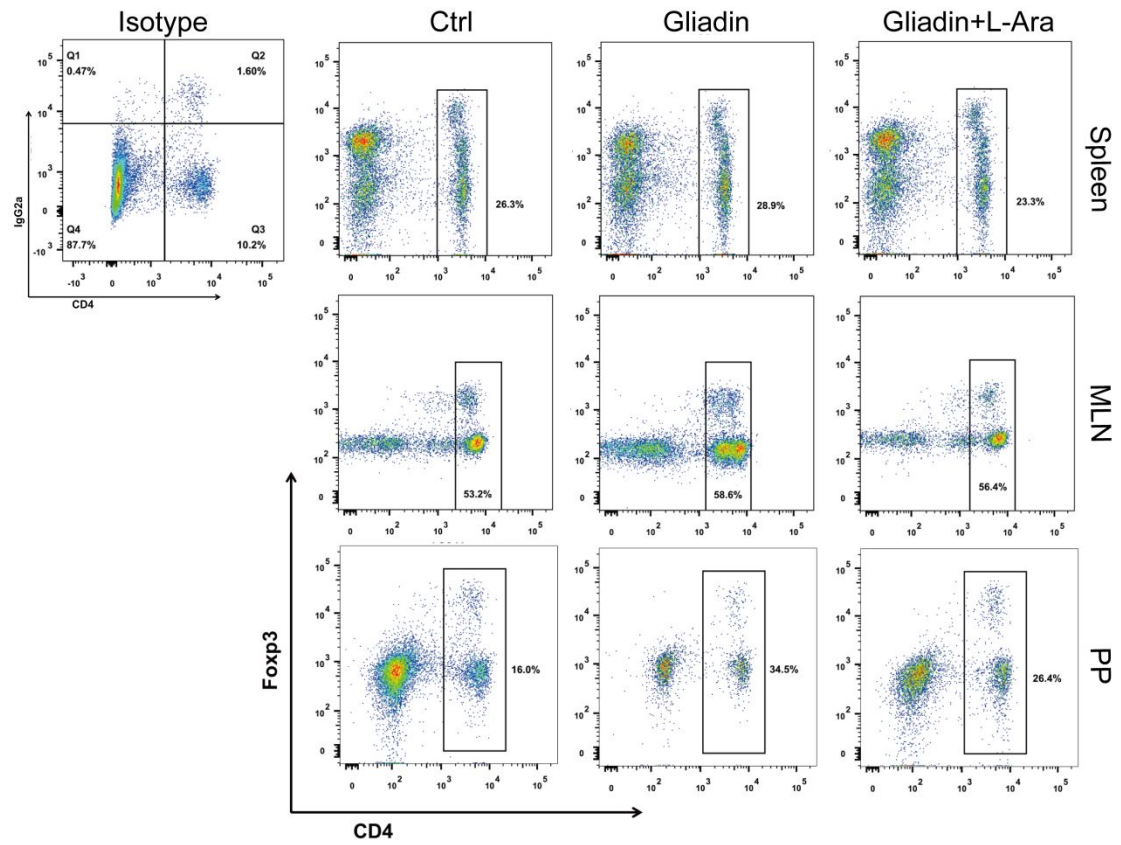


Figure S2. Gate strategies of Treg cells in Spleen, MLNs, and PPs. Ctrl: control; Gliadin: gliadins-sensitized mice; Gliadin + L-Ara: gliadins-sensitized mice treated with L-arabinose.