Am J Transl Res 2016;8(12):5503-5511
www.ajtr.org /ISSN:1943-8141/AJTR0037500

Original Article

Micro RNA-19a suppresses thrombospondin-1 in CD35$^+$ B cells in the intestine of mice with food allergy

Li-Tao Yang[1,2,3*], Xiao-Xi Li[1*], Shu-Qi Qiu[1,2*], Lu Zeng[1], Lin-Jing Li[3,4], Bai-Sui Feng[4], Peng-Yuan Zheng[5], Zhi-Gang Liu[1], Ping-Chang Yang[1]

[1]The Research Center of Allergy & Immunology, Shenzhen University School of Medicine, Shenzhen 518060, China; [2]Shenzhen ENT Institute, Longgang ENT Hospital, Shenzhen 518116, China; [3]Brain Body Institute, McMaster University, Hamilton, ON L8N 4A6, Canada; [4]Department of Gastroenterology, The Second Hospital, Zhengzhou University, Zhengzhou, China; [5]Department of Gastroenterology, The Fifth Hospital, Zhengzhou University, Zhengzhou, China. [*]Equal contributors.

Received August 8, 2016; Accepted November 29, 2016; Epub December 15, 2016; Published December 30, 2016

Abstract: Disruption of immune tolerance is associated in the pathogenesis of allergy. Thrombospondin-1 (TSP1) plays a role in the maintenance of immune tolerance, which is compromised in allergic disorders. Micro RNA (miR) is involved in the regulation of immune responses. This study tests a hypothesis that miR-17-92 cluster is involved in the regulation of TSP1 in the intestinal CD35$^+$ B cells. In this study, a food allergy mouse model was developed. The intestinal B cells were isolated to be analyzed for the expression of a miR-17-92 cluster and TSP1. The role of miR-19a in the suppression of TSP1 in B cells was tested in a cell culture model. We observed that the levels of TSP1 were significantly decreased; the levels of miR-19a were significantly increased in intestinal CD35$^+$ B cells of mice sensitized to ovalbumin (OVA) as compared with naïve controls. Exposure to interleukin (IL)-4 suppressed the expression of TSP1 in B cells, which was abolished by inhibition of miR-19a. miR-19a mediated the effects of IL-4 on repressing TSP1 expression in B cells. We conclude that IL-4 suppresses the expression of TSP1 in the intestinal CD35$^+$ B cells via up regulating miR-19a. The miR-19a may be a target to regulate the immune tolerant status in the body.

Keywords: Micro RNA-19a, thrombospondin-1, intestine, food allergy, B cell

Introduction

Food allergy (FA) is an immune disorder that immune components in the intestine over react to the innocent food materials to induce immune inflammation in the intestine [1]. The prevalence of FA is about 2%-6% in the world [2]. The known pathogenesis of FA is the skewed Th2 polarization in the intestine. The FA response is mainly mediated by IgE antibody, which binds to the FcεRI on the surface of mast cells to make mast cells sensitized. The re-exposure to specific antigens triggers mast cell to release allergy-related mediators and initiate allergic response in the intestine [3]. Under healthy condition, the immune system has self-regulation mechanism to regulate the abnormal immune response in order to maintain the homeostasis in the body [4]. Regulatory T cells and regulatory B cells are the major immune regulatory cells. These cells may release immune regulatory molecules, such as interleukin (IL)-10 or transforming growth factor (TGF)-β to suppress the aberrant immune response [5, 6]. In allergic disorders, the frequency of regulatory T cell or regulatory B cell was decreased, or their function is compromised [7, 8].

Thrombospondin-1 (TSP1) is originally found in platelets. It is also called platelet activating factor. TSP1 is an adhesive glycoprotein and can bind to fibrinogen, fibronectin, laminin, type V collagen and integrin αVβ1 [9]. It plays roles in platelet aggregation, angiogenesis, and tumorigenesis. Published data show that TSP1 is also involved in the immune regulation; such as it is associated with the production of IL-10 and TGF-β [10, 11] in immune cells; while how is TSP1 deregulated in the immune cells has not been fully elucidated yet.

It is suggested that micro RNA (miR) is associated with the expression of TSP1 [12]. miR is a non-coding single stranded RNA with 18-22 nucleotides in length, and regulate gene expression in post transcription. The miR-17-92 cluster has multiple function in the regulation of immune response [13]. Recent reports indicate that the miR-17-92 cluster is involved in the pathogenesis of airway allergy [14]. Our previous studies showed that $CD35^+$ B cells expressed TSP1; this cell fraction contributed the immune tolerance, which was compromised in FA mice. Based on the above information, we hypothesize that miR-17-92 cluster may be involved in the pathogenesis of FA. To test this, we carried out an animal study. The results showed that the levels of miR-19a were higher, the TSP1 levels were lower, in intestinal $CD35^+$ B cells of FA mice than that in control mice.

Materials and methods

Mice and ethic statement

Male BALB/c mice (6-8 week old) were purchased from the Guangdong Experimental Animal Center. Mice were maintained in a pathogen-free facility with accessing food and water freely. The experimental procedures were approved by the Animal Ethic Committee at Shenzhen University. The study was carried out in accordance with the approved guidelines.

Development of food allergy in mice

Following our established procedures [15], mice were gavage-fed with ovalbumin (OVA, 1 mg/mouse; Sigma Aldrich) and cholera toxin (20 μg/mouse; Sigma Aldrich) in 0.3 ml saline once a week for 5 consecutive weeks. The mice were sacrificed one day after the last time of antigen challenge. Mouse diarrhea and core temperature change in response to the antigen challenge was recorded 30 min after the challenge.

Assessment of serum levels of cytokines with enzyme-linked immunosorbent assay (ELISA)

The blood was collected from each mouse. The sera were isolated from the blood by centrifugation and stored at -80°C until use. The levels of IL-4, IL-5 and IL-13 in the sera and intestinal extracts were determined by ELISA with commercial reagent kits (R&D Systems) following the manufacturer's instructions.

Isolation of $CD35^+$ B cells from the mouse intestine

Following our established procedures [11], the lamina propria mononuclear cells (LPMC) were isolated from the mouse intestine. $CD35^+$ $CD19^+$ B cells were purified from the LPMCs by magnetic cell sorting (MACS) with cell isolating kits (Miltenyi Biotech) following the manufacturer's instructions. The purity of the isolated cells was greater than 98% as checked by flow cytometry.

Protein extraction from the intestinal tissue

Small intestinal segments were collected at the sacrifice. The tissue was homogenized at 4°C in a protein extraction buffer. The samples were maintained at 4°C for 30 min, and then centrifuged for 10 min (×13,000 rpm) at 4°C. The supernatant was collected as protein extracts. The protein in the extracts was quantified by the BCA method.

Cell culture

The isolated B cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 2 mM L-glutamine. The medium was changed in 2-3 days. The cell viability was greater than 99% before use in the further experiments as checked by Trypan blue exclusion assay.

Assessment of allergen specific $CD4^+$ T cells in the intestine

$CD4^+$ T cells and dendritic cells (DC) were isolated from LPMCs by MACS with the reagent kits purchased from Mitenyi Biotech following the manufacturer's instructions. The cell purity was greater than 98%. The $CD4^+$ T cells were labeled with CFSE (carboxyfluorescein diacetatesuccinimidyl ester) and cultured with DCs (T cell:DC = 10^5:2 × 10^4/well) in the presence of specific allergen OVA (5 μg/ml) or an irrelevant allergen bovine serum albumin (5 μg/ml) for 3 days. The cells were analyzed by flow cytometry (CFSE-dilution assay). The proliferating cell population was regarded as allergen specific $CD4^+$ T cells.

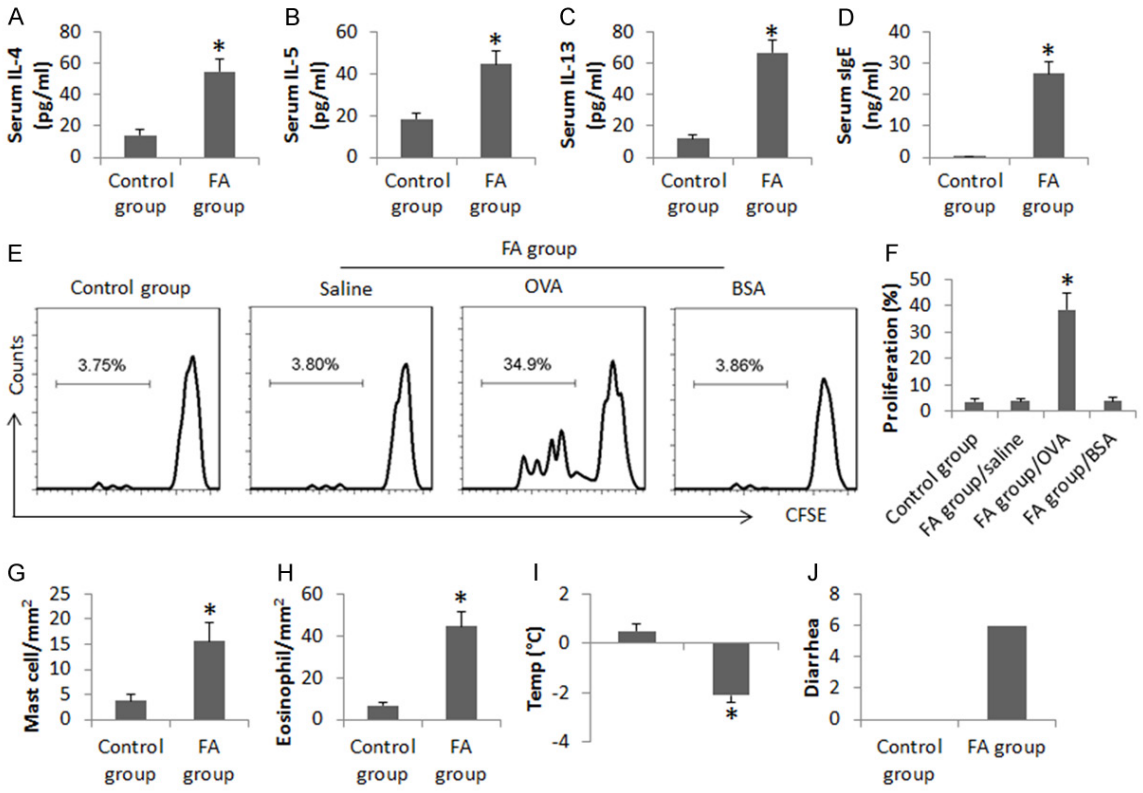

Figure 1. Assessment of intestinal allergic inflammation. (A-D) The bars indicate the serum levels of Th2 cytokines and specific IgE in control mice or FA mice. (E) The histograms indicate the $CD4^+$ T cell proliferation in the culture after exposure to specific allergen OVA or BSA. (F) The bars indicate the summarized data of (E). (G, H) The bars indicate the mast cell (G) and eosinophil (H) infiltration in the intestinal mucosa. (I) The bars indicate the core temperature change after allergen challenge. (J) The bars indicate the number of mice had diarrhea after allergen challenge. Data of bars are summarized from 6 experiments and presented as mean ± SD. *, $P<0.01$, compared with the saline group. Each group consists of 6 mice. Samples from individual mice were processed separately. The data of (E) are from one experiment out of 6 independent experiments.

Counts of mast cells and eosinophils in the intestinal tissue

A segment of the small intestine was fixed with 4% paraformaldehyde overnight and processed for paraffin sections. The sections were stained with 0.5% toluidine blue to stain mast cells or with eosin & hematoxylin to stain eosinophils. Mast cells and eosinophils were counted on the sections under a light microscope. Twenty image windows were counted for each sample. The results were converted to cell number per mm^2. The sections were coded. The observers were not aware of the code to avoid the observer bias.

Assessment of levels of TSP1 mRNA and miR-17-92 cluster in B cells by real time RT-PCR

Intestinal $CD35^+$ B cells were isolated as described above. Total RNA was extracted from the B cells with TRIzol reagents (Invitrogen). The cDNA was synthesized with the RNA and a reverse transcription kit (Invitrogen) following the manufacturer's instructions. Real time PCR was carried out with the SYBR Green Master Mix (Invitrogen) in a real time PCR device (Bio-Rad). The primers of miR-17-92, IL-4 receptor and TSP1 were provided by Enke Biotech (Shenzhen, China). The results were calculated with the $2^{-\Delta\Delta Ct}$ method and presented as fold change against RNA U6B (Invitrogen) (for miR-17-92 cluster) or control group (for TSP1).

Assessment of TSP1 protein in B cells by western blotting

Total proteins were extracted from the isolated $CD35^+$ B cells. The proteins were fractioned by SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk for 30 min at room temperature, incubated with an anti-TSP1 antibody (1:300; Santa Cruz Biotech) overnight at 4°C, washed

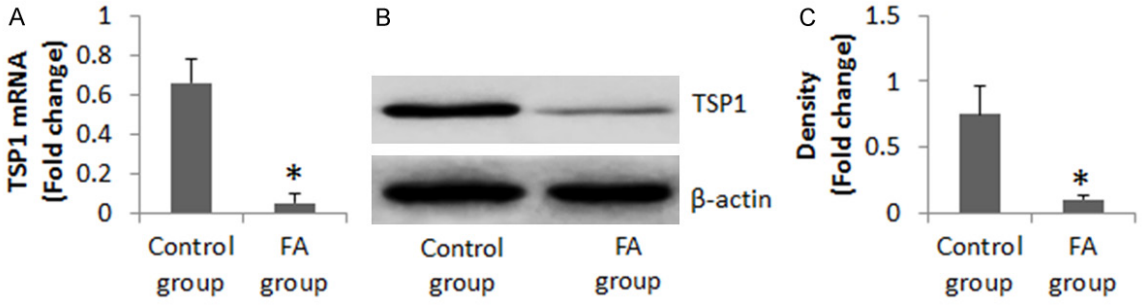

Figure 2. Assessment of TSP1 expression in CD35$^+$ B cells. (A) The bars indicate the mRNA levels of TSP1 in intestinal CD35$^+$ B cells of control mice and FA mice. (B) The immune blots indicate the protein levels of TSP1 in intestinal CD35$^+$ B cells of control mice and FA mice. (C) The bars indicate the integrated density of the immune blots of (B). Data of bars are presented as mean ± SD. *, P<0.01, compared with the control group. Each group consists of 6 mice. The data represent 6 independent experiments.

with TBST (Tris-buffered saline Tween 20) for 3 times, incubated with a peroxidase-labeled second antibody for 1 h at room temperature, washed with TBST for 3 times, developed with enhanced chemiluminescence. The results were photographed with an image station (UVI, Cambridge, UK). The integrated density of the immune blots was analyzed with software Photoshop CC 2015 and presented as fold change against the internal control β-actin.

Knockdown of miR-19a in B cells by RNA interference (RNAi)

B cells were isolated from the naïve mouse spleen and treated with shRNA of miR-19a or control shRNA (provided by the Enke Biotech, Shenzhen, China) following the manufacturer's instructions. The effects of the RNAi on miR-19a expression in B cells were assessed by real time RT-PCR.

Statistics

Data are presented as mean ± SD. The difference between two groups was determined by Student t test or ANOVA followed by Bonferroni correction if more than two groups. P< 0.05 was set as a significant criterion.

Results

TSP1 levels in intestinal CD35$^+$ B cells are less in FA mice

To evaluate the condition of CD35$^+$ B cell in the allergic environment, we developed a mouse FA model. The sensitized mice showed FA-like response, including elevation of serum specific IgE, serum Th2 cytokines, infiltration of mast cells and eosinophils in the intestinal mucosa, allergen-specific CD4$^+$ T cells, drop of the core temperature and diarrhea (**Figure 1**). The CD35$^+$ CD19$^+$ B cells were isolated from the intestinal mucosa. The expression of TSP1 in the B cells of FA mice was significantly lower than that in control mice (**Figure 2**). The results suggest that allergic condition alters the property of CD35$^+$ B cells in the intestine.

Expression of miR-19a is associated with the low levels of TSP1 in intestinal B cells of FA mice

To observe the role of miR-17-19a in the regulation of the nature of CD35$^+$ B cells, we next assessed the levels of miR-17-92 cluster in intestinal CD35$^+$ B cells since this miR family is associated with allergic disorders [14]. The results showed that the levels of miR-19a were higher in intestinal CD35$^+$ B cells in FA mice than that in control mice, while the levels of the rest 5 members of the miR-17-92 cluster, including miR-17, miR-18a, miR-19b, miR-20a and miR-92, were not different from those in control mice (**Figure 3**). A correlation assay was performed with the data of TSP1 mRNA and miR-19a in intestinal CD35$^+$ B cells; the results showed a negative correlation ($r = 0.6845$, $P<0.01$). The results indicate that the allergic environment increases the levels of miR-19a in B cells, which may affect the expression of TSP1 in the B cells.

IL-4 up regulates the expression of miR-19a in B cells

To elucidate possible causative factors of the elevation of miR-19a in intestinal B cells, we

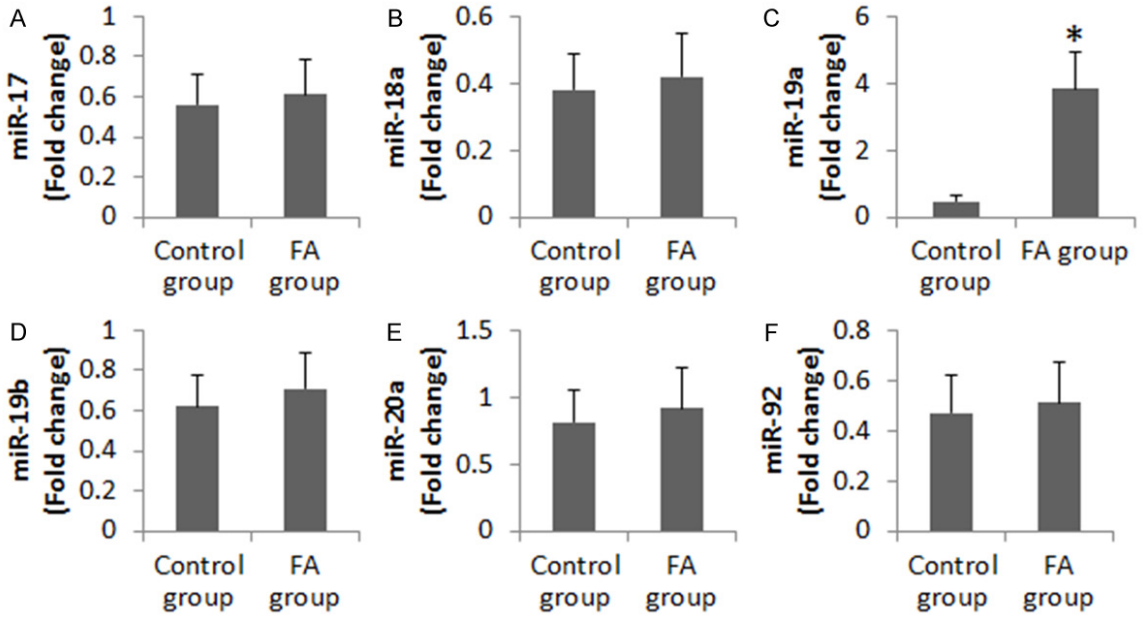

Figure 3. Assessment of miR-17-92 cluster in intestinal CD35$^+$ B cells. The bars indicate the levels of the 6 members of the miR-17-92 cluster in intestinal CD35$^+$ B cells of control mice and FA mice. Each group consists of 6 mice. The data are summarized from 6 independent experiments. Data are presented as mean ± SD. *, P<0.01, compared with the control group.

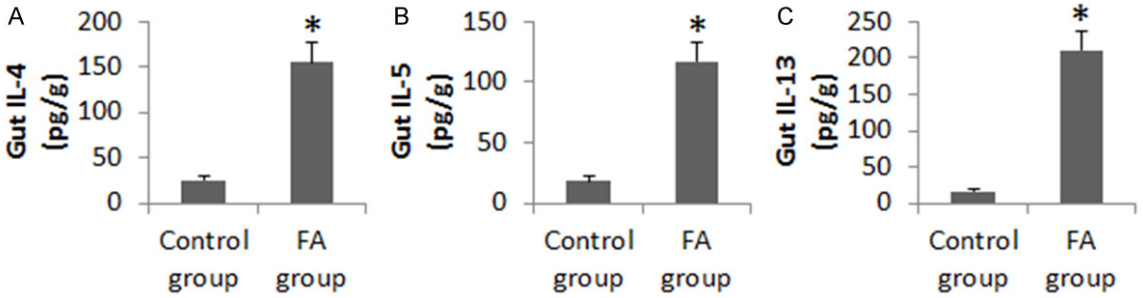

Figure 4. Assessment of Th2 cytokine in intestinal tissue. The bars indicate the Th2 cytokine levels in intestinal extracts. Each group consists of 6 mice. The data are summarized from 6 independent experiments. Data are presented as mean ± SD. *, P<0.01, compared with the control group.

prepared intestinal tissue extracts and analyzed by ELISA. The results showed that the levels of IL-4, IL-5 and IL-13 in the extracts were significantly higher in FA group than in control group (**Figure 4**). The results implicate that the Th2 cytokines may affect the expression of miR-19a in B cells. To test this, we treated B cells (from the naive mouse spleen) with IL-4, or IL-5, or IL-13 in the culture, respectively. The results showed that exposure to IL-4, but not IL-5 or IL-13, significantly up regulated the expression of miR-19a in B cells (**Figure 5A-C**). In addition, we also observed that intestinal B cells expressed IL-4 receptors, which was increased in FA mice (**Figure 5D**, **5E**). This results suggest that IL-4 modulates the expression of miR-19a in CD35$^+$ B cells.

miR-19a mediates the effects of IL-4 on suppression of TSP1 in B cells

We next investigated the role of IL-4 in suppression of TSP1 in B cells. In the first step, we treated naive B cells with LPS in the culture (B cells express TLR4 [16]). The exposure to LPS increased the expression of TSP1 in B cells in a LPS dose-dependent manner (**Figure 6A**). The

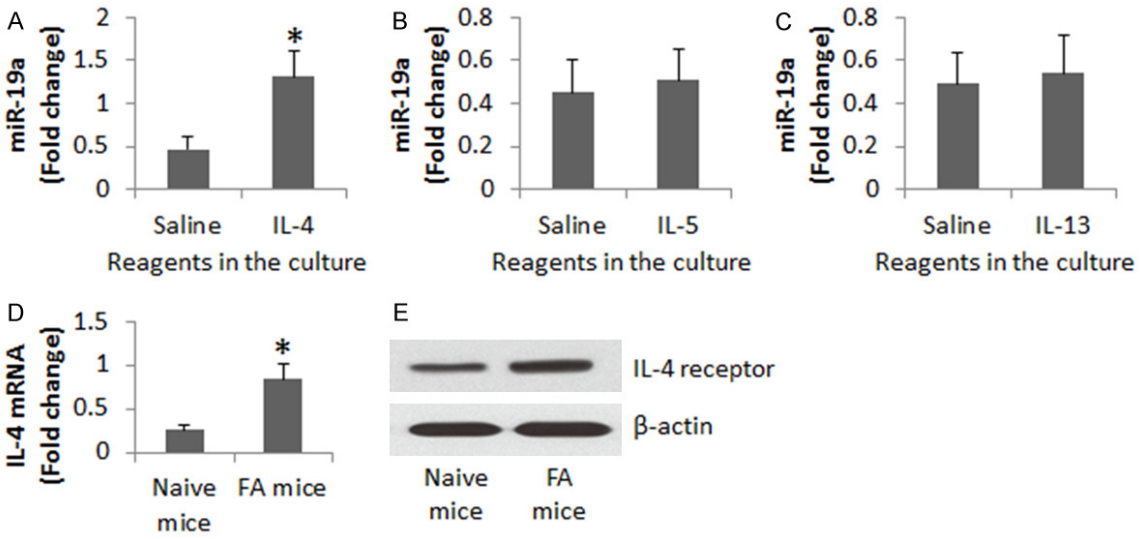

Figure 5. Assessment of the effects of Th2 cytokines on expression of miR-19a in B cells. A-C. The bars indicate the levels of miR-19a in B cells (isolated from the naïve mouse spleen) after exposure to the cytokines (denoted on the X axis) for 48 h. The concentrations of IL-4, or IL-5, or IL-13 were 100 pg/ml of each (the recombinant cytokines were purchased from R&D Systems). D, E. The results of IL-4 receptor assessment in intestinal B cells. Data are presented as mean ± SD. *, $p<0.01$, compared with the saline group. The data were summarized from 3 independent experiments.

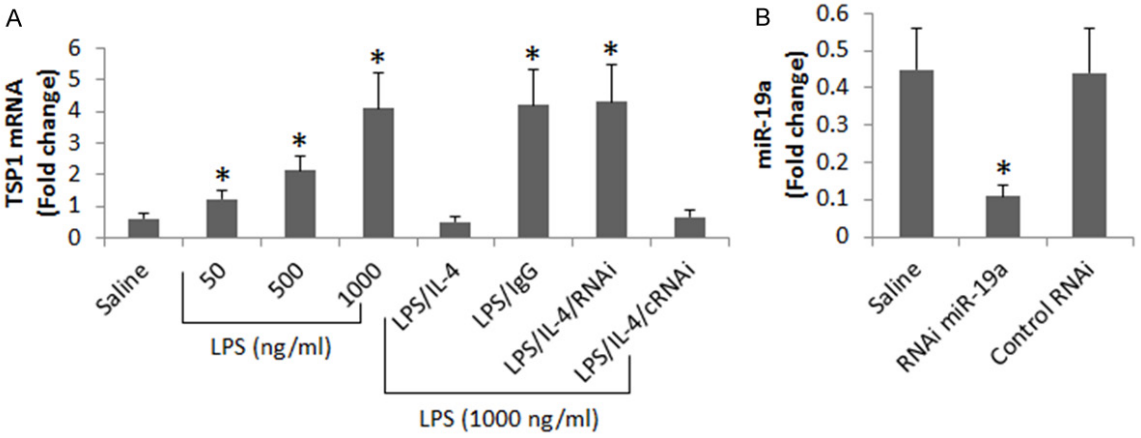

Figure 6. Assessment of the role of miR-19a in the suppression of TSP1 in B cells. (A) The bars indicate the levels of TSP1 mRNA in B cells; the B cells were isolated from the naive mouse spleen and were treated for 48 h in the culture as denoted on the X axis of (A). (B) The bars indicate the levels of miR-19a in B cells treated with or without RNAi of miR-19a. The IgG is an isotype IgG used as a negative control. Data of bars are presented as mean ± SD. *, $P<0.01$, compared with the saline group. The data were summarized from 3 independent experiments.

LPS-increased TSP1 expression in B cells could be abolished by the presence of IL-4 in the culture. The effects of IL-4 on suppression of TSP1 in B cells could be abolished by knocking down the gene of miR-19a (**Figure 6A**, **6B**). The results demonstrate that miR-19a plays a crucial role in the regulation of TSP1 expression in B cells.

Discussion

Immune tolerance is compromised in the intestine of FA subjects [17]. The mechanism is unclear. Our previous studies indicate that TSP1-producing $CD35^+$ B cells contribute to the immune tolerance in the intestine [11]. The present data have expanded our previous

studies by showing that lower levels of TSP1 and higher levels of miR-19a were found in intestinal CD35$^+$ B cells of FA mice. The analysis showed a negative correlation between TSP1 and miR-19a in CD35$^+$ B cells, implicating the increase in miR-19a may be associated with the suppression of TSP1 in the B cells. The inference is supported by further experiments, which showed that the increase in miR-19a (induced by IL-4) indeed inhibited the expression of TSP1 in B cells.

Our previous work showed that a fraction of B cells, the CD35$^+$ B cells, expressed TSP1, which contributed to the immune tolerance in the intestine [11]. Other investigators also found that CD35$^+$ T cells produced IL-10, which played a critical role in the allergen specific immunotherapy [18]. In patients with systemic sclerosis, the frequency of peripheral CD35$^+$ B cells is lower than healthy subjects [19]. The present data show further evidence that the CD35$^+$ B cells in the FA mouse intestine have lower TSP1 expression, suggesting that these B cells may be less competent to induce immune regulatory cells [11].

TSP1 is originally found in platelets [20]. It is latterly found in other cells. Astrocyte-derived TSP1 functions as a modulator of synaptogenesis and neurogenesis [21]. Human microvascular endothelial cells produce TSP1 in response to tumor necrosis factor-α to increase endothelial permeability, apoptosis, and reduced proliferation [22]. Intestinal epithelial cell-produced TSP1 converts naive monocytes to tolerogenic monocytes [23]. Intestinal CD35$^+$ B cell-derived TSP1 plays a role in the induction of regulatory T cells [11]. The present data confirmed that intestinal CD35$^+$ B cells can produce TSP1, which was down regulated in an allergic environment.

The data also showed that miR-19a was detected in intestinal B cells, which was negatively correlated with the expression of TSP1, implicating that miR-19a may be one of the causative factors in the suppression of TSP1 in B cells. miR-19a has multiple functions in affecting immune responses. This miR is associated with the pathogenesis of cancer [24]. Akhtar et al indicate that inhibition of the endothelial HIF-1α/miR-19a pathway can be a therapeutic option against atherosclerosis [16]. Haj-Salem et al suggest that miR-19a is a critical factor in the pathogenesis of the severe phenotype of asthma and downregulating miR-19a expression may be explored as a potential new therapy to modulate epithelial repair in asthma [25]. Our data reveal another aspect of miR-19a that plays a role in the suppression of TSP1 in B cells, implicating that miR-19a may be associated with the disruption of immune tolerance in the intestine in the allergic environment; this needs to be further investigated.

In summary, the present study reveals that miR-19a expression is higher in intestinal CD35$^+$ B cells, which is negatively correlated with the expression of TSP1 in the B cells. Exposure to IL-4 in the culture can up regulate the expression of miR-19a in B cells, which suppresses the expression of TSP1 in the B cells. Since TSP1-producing B cells play a role in the immune tolerance in the intestine, miR-19a may be a target in the regulation of intestinal immune tolerance disruption.

Acknowledgements

This study was supported by grants from the innovation of science and Technology Commission of Shenzhen Municipality (JCYJ20150402090413008, JCYJ20160422101725667, JCYJ20140418095735611, JCYJ20160429-9-1935720 and ZDSYS201506050935272), the Natural Science Foundation of China (81373176, 31570932, 31400856, 81571790 and 81501573).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Ping-Chang Yang and Zhi-Gang Liu, Room 722, Medical School Building, Shenzhen University, 3688 Nanhai Blvd, Shenzhen 518060, China. Tel: 86 755 8668 1907; Fax: 86 755 8668 1906; E-mail: pcy2356@szu.edu.cn (PCY); lzg@szu.edu.cn (ZGL)

References

[1] Brandtzaeg P. Food allergy: separating the science from the mythology. Nat Rev Gastroenterol Hepatol 2010; 7: 380-400.

[2] Allen KJ and Koplin JJ. Prospects for prevention of food allergy. J Allergy Clin Immunol Pract 2016; 4: 215-220.

[3] Chinthrajah RS, Hernandez JD, Boyd SD, Galli SJ and Nadeau KC. Molecular and cellular mechanisms of food allergy and food tolerance. J Allergy Clin Immunol 2016; 137: 984-997.
[4] Rochman Y, Spolski R and Leonard WJ. New insights into the regulation of T cells by gamma(c) family cytokines. Nat Rev Immunol 2009; 9: 480-490.
[5] Hawrylowicz CM and O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. Nat Rev Immunol 2005; 5: 271-283.
[6] Flavell RA, Sanjabi S, Wrzesinski SH and Licona-Limon P. The polarization of immune cells in the tumour environment by TGFbeta. Nat Rev Immunol 2010; 10: 554-567.
[7] Gong P, Li Y, Tan YP and Li H. Pretreatment with inactivated bacillus calmette-guerin increases CD4+CD25+ regulatory T cell function and decreases functional and structural effects of asthma induction in a rat asthma model. Artif Organs 2016; 40: 360-367.
[8] Noval Rivas M, Burton OT, Wise P, Charbonnier LM, Georgiev P, Oettgen HC, Rachid R and Chatila TA. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. Immunity 2015; 42: 512-523.
[9] Isenberg JS, Martin-Manso G, Maxhimer JB and Roberts DD. Regulation of nitric oxide signalling by thrombospondin 1: implications for anti-angiogenic therapies. Nat Rev Cancer 2009; 9: 182-194.
[10] Kawakami T, Tokunaga T, Hatanaka H, Tsuchida T, Tomii Y, Osada H, Onoda N, Morino F, Nagata J, Kijima H, Yamazaki H, Abe Y, Osamura Y, Ueyama Y and Nakamura M. Interleukin 10 expression is correlated with thrombospondin expression and decreased vascular involvement in colon cancer. Int J Oncol 2001; 18: 487-491.
[11] Zhang HP, Wu Y, Liu J, Jiang J, Geng XR, Yang G, Mo L, Liu ZQ, Liu ZG and Yang PC. TSP1-producing B cells show immune regulatory property and suppress allergy-related mucosal inflammation. Sci Rep 2013; 3: 3345.
[12] Maier KG, Ruhle B, Stein JJ, Gentile KL, Middleton FA and Gahtan V. Thrombospondin-1 differentially regulates microRNAs in vascular smooth muscle cells. Mol Cell Biochem 2016; 412: 111-117.
[13] Mogilyansky E and Rigoutsos I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. Cell Death Differ 2013; 20: 1603-1614.
[14] Simpson LJ, Patel S, Bhakta NR, Choy DF, Brightbill HD, Ren X, Wang Y, Pua HH, Baumjohann D, Montoya MM, Panduro M, Remedios KA, Huang X, Fahy JV, Arron JR, Woodruff PG, Ansel KM. A microRNA upregulated in asthma airway T cells promotes TH2 cytokine production. Nat Immunol 2014; 15: 1162-1170.
[15] Yang B, Li LJ, Xu LZ, Liu JQ, Zhang HP, Geng XR, Liu ZG and Yang PC. Histone acetyltransferease p300 modulates TIM4 expression in dendritic cells. Sci Rep 2016; 6: 21336.
[16] Akhtar S, Hartmann P, Karshovska E, Rinderknecht FA, Subramanian P, Gremse F, Grommes J, Jacobs M, Kiessling F, Weber C, Steffens S and Schober A. Endothelial hypoxia-inducible factor-1alpha promotes atherosclerosis and monocyte recruitment by upregulating MicroRNA-19a. Hypertension 2015; 66: 1220-1226.
[17] Feng BS, Chen X, He SH, Zheng PY, Foster J, Xing Z, Bienenstock J and Yang PC. Disruption of T-cell immunoglobulin and mucin domain molecule (TIM)-1/TIM4 interaction as a therapeutic strategy in a dendritic cell-induced peanut allergy model. J Allergy Clin Immunol 2008; 122: 55-61, 61, e51-57.
[18] Torok K, Dezso B, Bencsik A, Uzonyi B and Erdei A. Complement receptor type 1 (CR1/CD35) expressed on activated human CD4+ T cells contributes to generation of regulatory T cells. Immunol Lett 2015; 164: 117-124.
[19] Soto L, Ferrier A, Aravena O, Fonseca E, Berendsen J, Biere A, Bueno D, Ramos V, Aguillón JC and Catalán D. Systemic sclerosis patients present alterations in the expression of molecules involved in B-Cell regulation. Front Immunol 2015; 6: 496.
[20] Starlinger P, Haegele S, Offensperger F, Oehlberger L, Pereyra D, Kral JB, Schrottmaier WC, Badrnya S, Reiberger T, Ferlitsch A, Stift J, Luf F, Brostjan C, Gruenberger T and Assinger A. The profile of platelet alpha-granule released molecules affects postoperative liver regeneration. Hepatology 2016; 63: 1675-1688.
[21] Son SM, Nam DW, Cha MY, Kim KH, Byun J, Ryu H and Mook-Jung I. Thrombospondin-1 prevents amyloid beta-mediated synaptic pathology in Alzheimer's disease. Neurobiol Aging 2015; 36: 3214-3227.
[22] Fairaq A, Goc A, Artham S, Sabbineni H and Somanath PR. TNFalpha induces inflammatory stress response in microvascular endothelial cells via Akt- and P38 MAP kinase-mediated thrombospondin-1 expression. Mol Cell Biochem 2015; 406: 227-236.
[23] Fang LL, Yu HQ, Wu RJ, He C, Li M, Yan H, Li JJ, Wang S, Liu ZG, Liu ZJ and Yang PC. Throm-

bospondin 1 modulates monocyte properties to suppress intestinal mucosal inflammation. J Innate Immun 2015; 7: 601-611.

[24] Stuopelyte K, Daniunaite K, Jankevicius F and Jarmalaite S. Detection of miRNAs in urine of prostate cancer patients. Medicina (Kaunas) 2016; 52: 116-124.

[25] Haj-Salem I, Fakhfakh R, Berube JC, Jacques E, Plante S, Simard MJ, Bosse Y and Chakir J. MicroRNA-19a enhances proliferation of bronchial epithelial cells by targeting TGFbetaR2 gene in severe asthma. Allergy 2015; 70: 212-219.