



Soluble CD83 suppresses experimental food allergy via regulating aberrant T helper 2 responses

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Abstract

Aberrant T helper-2 (Th2) responses play a critical role in the pathogenesis of allergic diseases. The underlying mechanism is to be further investigated. It is reported that soluble CD83 (sCD83) has immune-regulatory effects. This study aims to investigate the role of sCD83 in the regulation of Th2 polarization. Blood samples were collected from pediatric patients with food allergy (FA). The Th2 response was analyzed by pertinent immunological approaches. An FA murine model was developed to test the role of sCD83 in the regulation of FA response. We found that the serum sCD83 levels were lower in FA patients. A negative correlation was detected between serum sCD83 levels and serum Th2 cytokine levels. The presence of sCD83 suppressed Th2 cell differentiation and antigen-specific Th2 cell activation. sCD83 upregulated the T-bet expression and suppressed the GATA3 expression in CD4⁺ T cells. Administration of sCD83 suppressed experimental FA. Pediatric FA patients have low serum sCD83 levels. Administration of sCD83 can alleviate experimental FA via suppression of aberrant Th2 polarization.

Keywords Food allergy · Th2 response · Soluble CD83 · Immune regulation · Intestine

Introduction

Food allergy (FA) is an adverse reaction to the innocuous food antigens by the immune system in the intestinal tissues [1]. FA symptoms vary from slight abdominal discomfort to the life-threatening anaphylactic shock [1]. Although the pathogenesis of FA is not fully understood, it is the consensus that aberrant T helper-2 (Th2) polarization plays a critical role in the development and maintenance of FA [2]. Currently, the prevalence of FA is about 2–4% in adults and 6–8% in children worldwide [2]. To date, the therapeutic efficacy for FA is unsatisfactory [3]. If no proper medical intervention is taken, complications may occur from FA, such as intestinal chronic inflammation [4], FA-related asthma [5], dermatitis [6], and cardiovascular disorders [1, 2]. Therefore, FA has a great

negative impact on human health and social economy. It is necessary to further investigate the pathogenesis of FA and invent novel and effective remedies for the treatment of FA.

It is recognized that FA subjects have aberrant Th2 polarization in the intestinal mucosa [1, 2]. The intestinal Th2 polarization indicates a condition that the intestinal mucosa is overpopulated by Th2 cells, which produce large amounts of Th2 cytokines [7]. The Th2 polarization condition is also reflected in the peripheral blood system. It is understood that Th2 polarization is due to Th2 overdifferentiation and the lifespan of Th2 cells expands, as Jiang et al. reported that CD4⁺ T cells are resistant to apoptosis inducers [8]. Cumulative reports indicate that the Th2 cell overdifferentiation can be induced by multiple factors, such as microbial products facilitating the development of Th2-type inflammation [9]. Recent studies indicate that overexpression of apoptosis inhibitors in CD4⁺ T cells also contributes to the Th2 polarization development [8]. Yet, therapeutic efficacy for Th2 polarization-related diseases is not satisfactory currently. Therefore, it is necessary to develop new remedies for regulating Th2 polarization.

In general, immune responses are tightly regulated by the immune-regulatory system in the body. Regulatory T and B cells are important cellular components in the immune-regulatory system. Inflammation due to Th2 or Th1

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polarization can be an indicator of immune-regulatory system dysfunction. Thus, to expand the immune-regulatory cell function is one of the approaches to suppress immune inflammation [10]. Besides, other factors also play roles in immune regulation. Published data indicate that soluble CD83 (sCD83) has immune-suppressive functions [11]. CD83 is mainly expressed in dendritic cells (DC) as a maturation marker. The extracellular part of the CD83 molecule has an immunoglobulin-like domain and may foliate out to be sCD83 or a splice variant of CD83 secreted by DCs, T cells, B cells, macrophages, and neutrophils [12]. Cumulative studies indicate that sCD83 is associated with immune-regulatory activities [11, 12]. Whether sCD83 is involved in FA development is to be investigated.

Based on the above information, we hypothesize that sCD83 is associated with the pathogenesis of FA. In this study, the role of sCD83 in the inhibition of Th2 polarization was analyzed by a variety of immunological approaches.

Materials and methods

Reagents

Antibodies of T-bet, CD83, GATA3, and IRF1 were purchased from abcam (Cambridge, MA). T-bet and IRF1 CRISPR reagents were provided by GenScript (Nanjing, China). Recombinant sCD83 (with a His-tag) was provided by the Shanghai Sangon Biotech company. ELISA kits of CD83, IL-4, IL-5, IL-13, mMCP1, and OVA-specific IgE were purchased from BioMart (Beijing, China). Fluorescence-conjugated antibodies for flow cytometry, including IL-4, CD4, IL-4, CD154, and CD62L, were purchased from BD Biosciences (Franklin Lakes, NJ). Reagents and materials for immunoprecipitation (IP), real-time quantitative RT-PCR (RT-qPCR), chromatin IP, and Western blotting were purchased from Invitrogen (Carlsbad, CA).

FA patients

Pediatric FA patients (sensitized to egg) were recruited into this study at our outpatient clinic. The diagnosis of FA was based on the disease history, ovalbumin (OVA) skin tests, and OVA-specific IgE measurements. The diagnosis and management of FA were carried out by our physicians. Patients with any of the following conditions were excluded from this study: cancers, autoimmune diseases, severe organ diseases, and under treatment of corticosteroids or other immune-suppressive agents for any reasons. Gender- and age-matched healthy control (HC) subjects were also recruited. The demographic data are presented in Table 1. The experimental procedures were approved by the Human Ethics Committee at Jiaozuo Women and Children Hospital. An

informed written consent was obtained from the guardian of each human subject.

Collection and processing of blood samples

Blood samples were obtained from FA patients and HC subjects through ulnar vein puncture. The blood was collected into tubes with or without heparin. The serum was isolated from the samples without heparin by centrifugation at 10,000g for 10 min at 4 °C. The serum was stored at − 80 °C until use. Mononuclear cells were isolated from the samples with heparin by Percoll gradient density centrifugation.

Cell culture

Immune cells were cultured in RPMI1640 medium. The medium was supplemented with antibiotics (penicillin and streptomycin), fetal calf serum, and glutamine. The medium was changed in 2–3 days. Cell viability was more than 99% before handling over further experiments by Trypan blue exclusion assay.

Cytokine level determination

Levels of Th2 cytokine, human CD83, serum specific IgE, and mMCP1 levels were determined by enzyme-linked immunosorbent assay (ELISA) with purchased specific ELISA kits according to the manufacturer's instructions. To determine mouse serum CD83 levels, microplates were coated with the mouse serum (diluted 1:10) or bovine serum albumin (BSA, used as a negative control) or recombinant CD83 (no His tag attached; used to create a standard curve), incubated at 37 °C for 1 h. The plates were washed with TPBS (PBS/0.05% Tween-20 wash buffer) 3 times, followed by blocking with 1% skim milk (in TPBS), incubated for 1 h at room temperature, and washed with TPBS 3 times. TPBS-diluted anti-CD83 antibody (1:5000; rat anti-mouse) was added at 100 µl per well, incubated at room temperature for 1 h, and washed with TPBS 3 times. HRP-labeled anti-rat second antibody (diluted 1:1000) was incubated at room temperature for 1 h and washed with TPBS 3 times. TMP solution was added to the plates for 10–30 min; the reaction was terminated by adding 1 M H₂SO₄. The plates were read with a microplate reader (BioTek Synergy LX) at 450 nm. The readouts from samples were subtracted the readout from negative controls. The results were calculated with the readouts against the standard curve.

Immune cell isolation

Peripheral blood mononuclear cells (PBMC) were incubated with fluorescence-labeled antibodies of interest. Targeted immune cells were isolated from PBMCs by flow cytometry cell

Table 1 Demographic data

	FA	Healthy control subjects
Number	20	20
Age (years)	12.5 ± 2.2	12.7 ± 2.1
Gender	Male, 10; female, 10	Male, 10; female, 10
Body weight	44.5 ± 10.2	42.6 ± 9.8
Egg-allergy history	20	0
Egg Ag STP positive	20	0
Serum total IgE (IU/ml)	Median, 35.4 (16.6 to 122.5)	Median, 0.14 (0.11 to 0.28)*

* $p < 0.01$ (Mann–Whitney U test)

sorting (FCCS). The purity of isolated cells was assessed by flow cytometry. If the purity did not reach 95%, FCCS was repeated with the cells.

Th2 polarization

CD4⁺ CD62L⁺ T cells were prepared and cultured in a Th2 polarization condition. Cells were cultured with plate-bound anti-CD3 (5 µg/ml) plus soluble anti-CD28 (2 µg/ml), 20 ng/ml IL-2, 10 µg/ml IL-4, and 10 µg/ml anti-IFN-γ. The medium together with the reagents was changed in 2 days.

Flow cytometry

Cells were collected from relevant experiments. In surface staining, cells (10⁶ cells/ml) were stained with antibodies (labeled with fluorochromes; diluted 1:100) of interest or isotype IgG on ice for 30 min. In intracellular staining, the cells were fixed with 1% paraformaldehyde (containing 0.1% Triton X-100) for 1 h, and then stained with antibodies (labeled with fluorochromes) of interest or isotype IgG on ice for 30 min. After being washed with phosphate-buffered saline (PBS) thoroughly, the cells were analyzed with a flow cytometer (FACSCanto II, BD Biosciences). Data were analyzed with FlowJo. Data obtained from isotype IgG staining were used as gating references.

OVA-specific Th2 cell detection and isolation

To detect antigen (OVA)-specific Th2 cells, PBMCs were stained with anti-CD154-FITC first (the increase in CD154 expression can be an indicator of specific antigen-induced CD4⁺ T cell activation [13]). The CD154⁺ cells were selected out by FCCS. The cells were then cultured with OVA (5 µg/ml) for 3 days and analyzed by flow cytometry. CD154⁺ cells were gated first. CD4⁺ IL-4⁺ cells in the gated CD154⁺ cells were assessed and regarded as antigen-specific Th2 cells. The CD154⁺ CD4⁺ cells were sorted by FCCS, in

which IL-4⁺ cells were more than 95% (assessed by flow cytometry) and used as antigen-specific Th2 cells.

Real-time quantitative RT-PCR (RT-qPCR)

The total RNA was extracted from cells with TRIzol reagents and converted to cDNA with a reverse transcription kit following the manufacturer's instructions. The samples were amplified in a qPCR device (Applied Biosystems QuantStudio 3 and 5 Real-Time PCR Systems; Thermo Fisher Scientific) with the SYBR Green Master Mix and the presence of relevant primers (Table 2). The results were calculated by the $2^{-\Delta\Delta C_t}$ method and are presented as relative quantification.

Protein extraction

Intestinal tissues were cut into small pieces and homogenized with a homogenizer (containing cell lysis buffer). Cells were lysed with a lysis buffer (10 mM HEPES; 1.5 mM MgCl₂; 10 mM KCl; 0.5 mM DTT; 1 mM EDTA; 0.05% NP40). Lysates were centrifuged (10,000g; 10 min). Supernatants were collected as the cytosolic proteins and stored at −80 °C until use. The pellets were resuspended in a nuclear lysis buffer (5 mM HEPES; 1.5 mM MgCl₂SO₄; 4.6 M NaCl; 0.2 mM EDTA; 0.5 mM DTT; 26% glycerol) for 30 min and centrifuged for 10 min at 10,000g. Supernatants were collected as

Table 2 Primers used in the present study

	Forward	Reverse
T-bet (<i>Homo</i>)	accagatgattgtgctcca	ggactcaagttctcccga
T-bet (<i>Mus</i>)	tccgggagaactttgagtc	tggaaggctcgggtagaag
GATA3 (<i>Homo</i>)	cccttccaagacgtccat	aggcactctttctgctctg
GATA3 (<i>Mus</i>)	cggagaggtggacgtact	atggacgtctggagaagg
IRF1 (<i>Homo</i>)	atgctccacctcacc	tgtagctgtgtggtcatca
IRF1 (<i>Mus</i>)	actccgtgtgcatgaac	ggtaggtagagctgctgag
<i>Il4</i> promoter (<i>Homo</i>)	gggtggatcatttgaggcc	ttgatctctgggctcaagg

nuclear proteins and stored at -80°C until use. The procedures were carried out at 4°C .

Western blot

Proteins were fractioned ($50\text{ }\mu\text{g/well}$) by SDS-PAGE and electrically transferred onto a PVDF membrane. After blocking with a skim milk solution (5%) for 30 min, the membrane was incubated with the primary antibodies (diluted in 1:300) of interest for 16 h at 4°C , washed with Tris-buffered saline with 0.1% Tween 20 (TBST) 3 times, incubated with the second antibodies (conjugated with horseradish peroxidase; diluted in 1:2000) for 2 h at room temperature, and washed with TBST 3 times. Immunoblots on the membrane were developed by enhanced chemiluminescence and photographed in an imaging device.

Co-immunoprecipitation (co-IP)

Proteins were precleared by incubation with protein A/G ($2\text{ }\mu\text{g/ml}$) for 1 h. After centrifugation, the supernatant was collected and incubated with antibodies (final concentration, $1\text{ }\mu\text{g/ml}$) of interest or isotype IgG for 16 h to form immune complexes, which were precipitated by incubation with protein A/G ($2\text{ }\mu\text{g/ml}$) for 1 h. The immune complexes were eluted and analyzed by Western blot. The procedures were carried out at 4°C .

Chromatin IP (ChIP)

Cells were fixed with 1% formalin for 15 min, lysed with a lysis buffer (10 mM HEPES; 1.5 mM MgCl_2 ; 10 mM KCl; 0.5 mM DTT; 1 mM EDTA; 0.05% NP40), and sonicated to shear the DNA into small pieces. The samples were then processed with IP procedures. DNA was recovered from the samples with a DNA extracting kit following the manufacturer's instructions and analyzed by qPCR in the presence of *I14* promoter primers (Table 2).

Mice

Male BALB/c mice were purchased from the Beijing Experimental Animal Center (Beijing, China). The mice were maintained in a specific pathogen-free facility; they were allowed to access food and water freely. The experimental procedures were approved by the Animal Ethics Committee of Jiaozuo Women and Children Hospital.

FA mouse model development

The mice were subcutaneously sensitized by injecting OVA solution (OVA 0.1 mg/mouse in 0.1 ml alum) on day 0 and boosted one time on day 7. The mice were then gavaged

with OVA (0.1 mg/mouse) and cholera toxin (CT, $20\text{ }\mu\text{g/mouse}$) in 0.1 ml saline once a week for 4 consecutive weeks.

Treating FA mice with sCD83

One week after the last OVA/CT gavage, the FA mice were treated with sCD83 (0.1 mg/mouse ; saline was used as a control) by intraperitoneal injection daily for one week.

Assessment of FA response in mice

FA response in mice was assessed following published procedures [8], including FA symptoms, serum levels of OVA-specific IgE and mouse mast cell protease-1 (mMCP1), Th2 cytokines in intestinal tissue protein extracts, and intestinal epithelial barrier permeability.

Isolation of lamina propria mononuclear cells (LPMC)

Jejunal segments were excised from mice upon the sacrifice. The tissues were cut into small pieces and incubated with collagenase IV (1 mg/ml) at 37°C for 30 min with mild agitation. Single cells were filtered through a cell strainer and collected by centrifugation at $1000\times g$ for 5 min.

Assessment of antigen-specific CD4^+ T cells

CD4^+ T cells and DCs were isolated from the intestine. The CD4^+ T cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) and cultured in the presence of OVA ($5\text{ }\mu\text{g/ml}$) for 3 days and analyzed by flow cytometry and the CFSE-dilution assay. The proliferating T cell frequency was recorded and regarded as an indicator of antigen-specific CD4^+ T cells.

Statistics

Each sample was analyzed in triplicate. Data are presented as mean $\pm$ SEM. The difference between two experimental groups was determined by Student *t* test. Analysis of variance (ANOVA) followed by Dunnett's test or Bonferroni test was performed for multiple comparisons. $P < 0.05$ was considered statistical significance.

Results

Serum sCD83 levels are negatively correlated with Th2 cytokine levels of FA patients

We collected blood samples from FA patients and healthy control (HC) subjects. The serum was isolated from the samples and analyzed by ELISA. We found that the serum sCD83

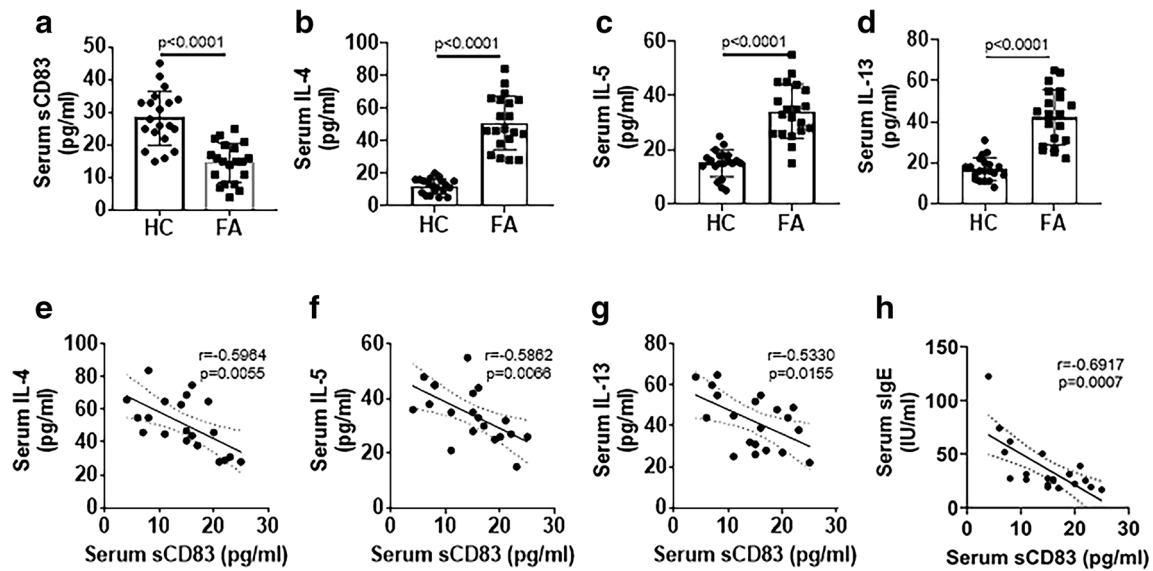


Fig. 1 FA serum sCD83 levels are negatively correlated with serum Th2 cytokines of FA patients. Peripheral blood samples were collected from 20 FA patients and 20 healthy control (HC) subjects. The serum was prepared from the samples and analyzed by ELISA. **a–d** Serum levels of sCD83, IL-4, IL-5, and IL-13. **e–h** Negative correlation between serum

sCD83 and serum Th2 cytokines and serum OVA-specific IgE. Data of bars are presented as mean $\pm$ SEM. Statistics of **a–d**, t test; **e–h**, Pearson correlation assay. (sIgE data are presented in Table 1). Each sample was analyzed in triplicate

levels were lower in FA patients than in HC subjects (Fig. 1a). The serum levels of Th2 cytokines in FA patients were higher than those in HC subjects (Fig. 1b–d). Serum sCD83 levels were negatively correlated with serum Th2 cytokines as well as serum-specific IgE levels (Fig. 1e–h). The results suggest that the serum sCD83 levels may be associated with the pathogenesis of FA.

sCD83 restricts Th2 cell differentiation

Data of Fig. 1 imply that sCD83 may be able to restrict Th2 cell differentiation. To test this, blood samples were collected from HC subjects. Naive $CD4^+$ T cells were isolated from the samples by flow cytometry cell sorting (FCCS) with $CD4^+ CD62L^{\text{high}}$ as the cell markers. The cells were cultured in the presence or absence of sCD83 under the Th2-polarization condition

for 6 days. As analyzed by flow cytometry, $IL-4^+$ T cell differentiation was suppressed by the presence of sCD83 in a dose-dependent manner (Fig. 2a, b). The results indicate that sCD83 can restrict Th2 cell differentiation.

sCD83 prevents $CD4^+$ T cell activation

We next tested the effects of sCD83 on suppressing $CD4^+$ T cell activation. Blood samples were collected from FA patients (sensitized to eggs). $CD4^+$ T cells and dendritic cells (DC) were isolated from the samples by FCCS. $CD4^+$ T cells (labeled with CFSE) and DCs were cocultured at a ratio of 5:1 in the presence of OVA, the specific antigen, with or without the presence of sCD83 for 3 days. As shown by flow cytometry analysis, the presence of OVA markedly induced $CD4^+$ T cell

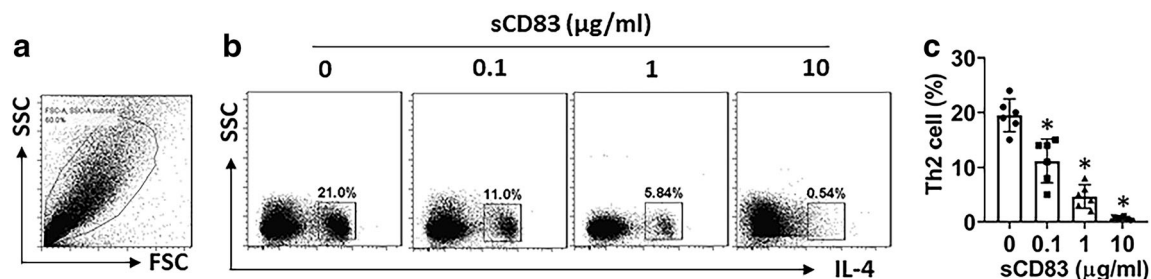


Fig. 2 sCD83 suppresses Th2 cell differentiation. Naive $CD4^+$ T cells were isolated from blood samples collected from 6 healthy subjects by flow cytometry and cultured under Th2 polarization condition in the presence of sCD83 at indicated concentrations above each subpanel. **a** SSC and FSC plots were gated first. **b** The gated plots were further

analyzed to determine the $IL-4^+$ T cell frequency. **c** Summarized Th2 cells in panel **b**. Data of bars are presented as mean $\pm$ SEM. Statistics: ANOVA followed by Dunnett's test. * $p < 0.01$, compared with group "0." Each sample was analyzed in triplicate

Fig. 3 sCD83 suppresses antigen-specific CD4⁺ T cell activation. Blood samples were collected from 6 egg-allergy patients; CD4⁺ T cells and DCs were isolated from blood samples. CD4⁺ T cells were labeled with CFSE and cultured with DCs (T cell:DC = 5:1) in the presence of OVA (5 µg/ml) or OVA and sCD83 (10 µg/ml) for 3 days. The cells were analyzed by flow cytometry. **a** Gated histograms show frequency of proliferating CD4⁺ T cells. **b** Summarized data of panel **a**. **c–e** Bars show Th2 cytokines (IL-4, IL-5, and IL-13) in culture supernatant (by ELISA). Data of bars are presented as mean ± SEM. Statistics: ANOVA followed by the Bonferroni test). Each sample was analyzed in triplicate

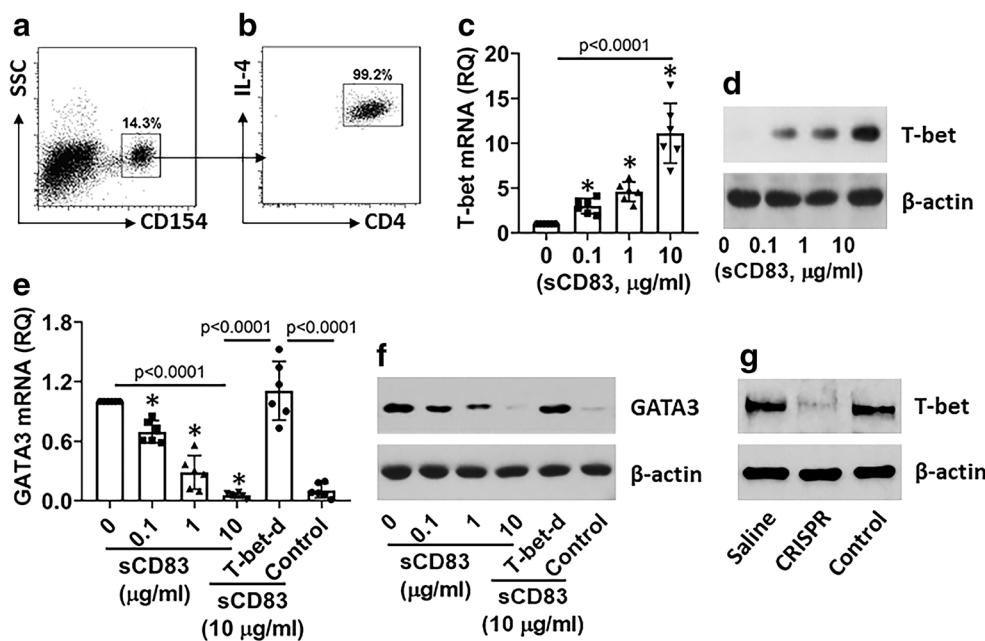
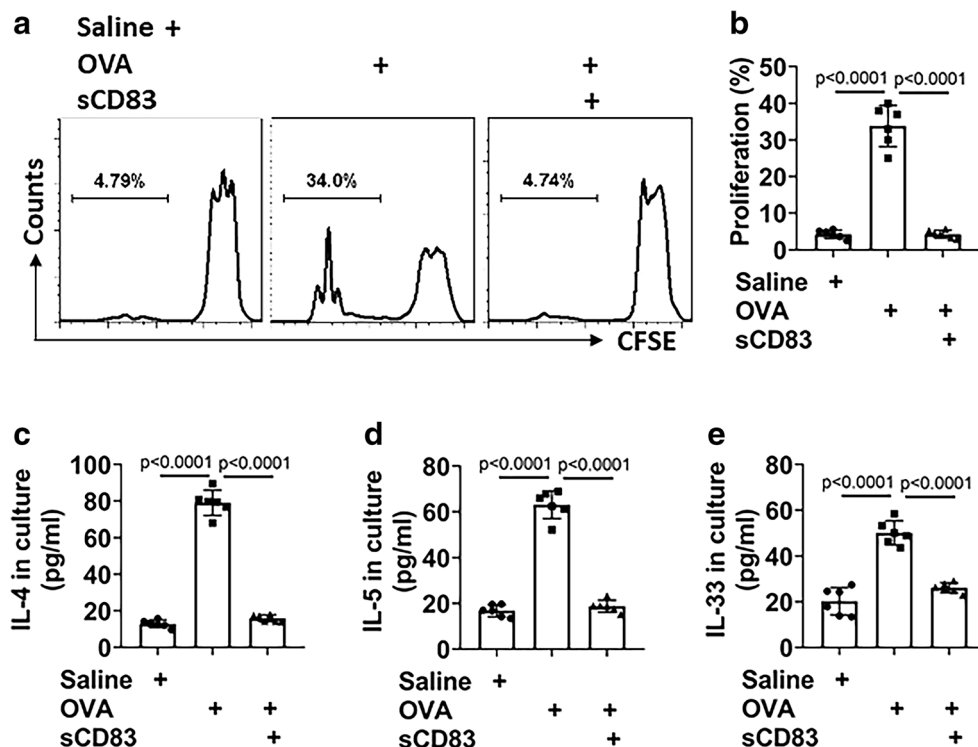


Fig. 4 sCD83 promotes T-bet expression to suppress GATA3 in antigen-specific Th2 cells. PBMCs were isolated from blood samples collected from FA patients (sensitized to eggs) and cultured in the presence of OVA (5 µg/ml) overnight. **A**, CD154⁺ cells (specific antigen-activated cells) were gated first. **B**, the CD154⁺ cells in panel **A** are also CD4⁺ IL-4⁺. **C–F**, The CD154⁺ cells were isolated by FCCS and analyzed by RT-qPCR and Western blotting. **C**, **D**, T-bet expression in antigen-specific Th2

cells. **E**, **F**, GATA3 expression in antigen-specific Th2 cells. **G**, results of the T-bet CRISPR. T-bet-d: T-bet-deficient T cells (prepared by the T-bet CRISPR). Data of **C** and **E** are presented as mean ± SEM. Statistics: C, ANOVA; E, ANOVA followed by the Bonferroni test. **p* < 0.01, compared with dose “0” group. Each sample was analyzed in triplicate. RQ, relative quantification

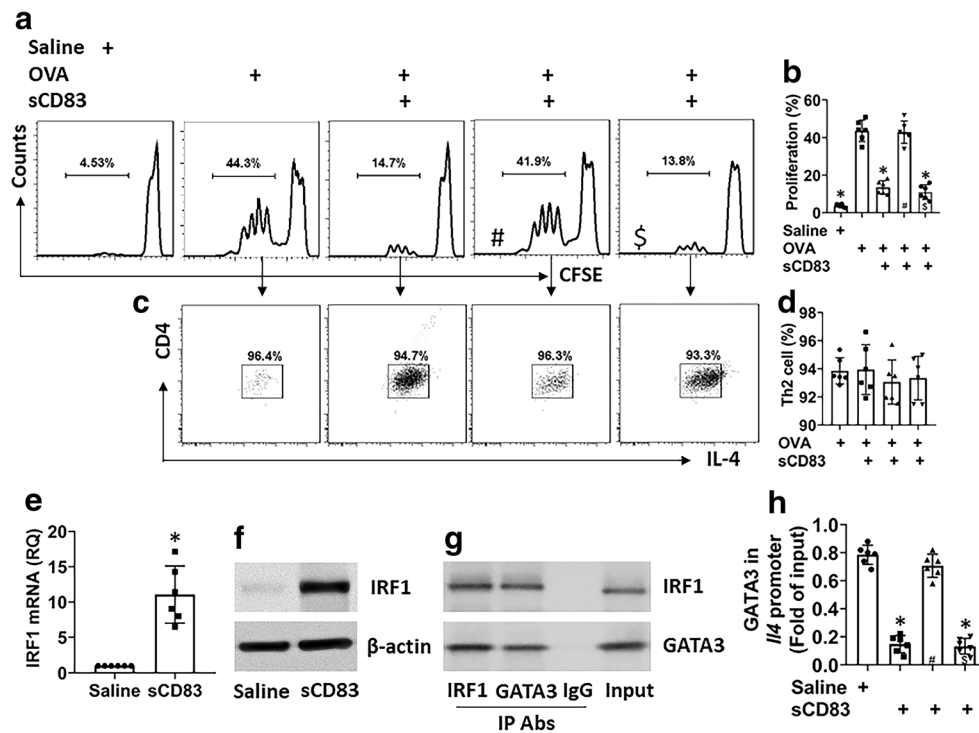


Fig. 5 IRF1 mediates the effects of sCD83 on modulating Th2 response. A–D, $CD4^+$ T cells and DCs were isolated from blood samples collected from 6 egg-allergy patients and labeled with CFSE and cultured with DCs in the presence of OVA (the specific antigen, 5 μ g/ml) or the presence of sCD83 (10 μ g/ml) for 3 days. A, gated histograms show proliferating $CD4^+$ T cells. B, summarized data of panel A. C, gated dot plots show $CD4^+$ IL-4 $^+$ T cells in the proliferating cells of panel A, indicating that these cells are antigen-specific Th2 cells. D, summarized data of panel C. E–H, OVA-specific Th2 cells were prepared and cultured in the presence

of sCD83 (10 μ g/ml) for 48 h. E, F, IRF1 expression in antigen-specific Th2 cells (by RT-qPCR and Western blotting). G, a complex of IRF1/GATA3 (by co-immunoprecipitation). H, GATA3 levels in the *IL4* promoter (by ChIP). Data of bars are presented as mean $\pm$ SEM. Statistics: *t* test (E) and ANOVA followed by the Bonferroni test (B, H). *compared with OVA-alone group (B) or saline group (E, H). Each sample was analyzed in triplicate. #, $CD4^+$ T cells were treated with IRF1 CRISPR reagent to deplete the IRF1 expression; \$, $CD4^+$ T cells were treated with control CRISPR reagent used as controls

proliferation (Fig. 3a, b); the presence of sCD83 efficiently suppressed $CD4^+$ T cell proliferation. On the other hand, exposure to OVA in the culture markedly induced Th2 cytokine release into culture supernatant, which was abrogated by the presence of sCD83 in the culture (Fig. 3c–e). The results demonstrate that sCD83 is capable of suppressing antigen-specific $CD4^+$ T cell activation.

sCD83 activates T-bet in $CD4^+$ T cells

Since T-bet counteracts Th2 cell functions [14], the above results imply that sCD83 may strengthen T-bet activities to restrict Th2 cell responses. To test this, OVA-specific Th2 T cells were prepared (Fig. 4 A, B) from blood samples collected from egg-allergic patients and cultured in the presence of sCD83 for 3 days. As analyzed by RT-qPCR and Western blotting, exposure to sCD83 increased the expression of T-bet at both mRNA and protein levels in a dose-dependent manner (Fig. 4 C, D). The results imply that T-bet may mediate the effects of sCD83 on suppressing Th2 cell activation. Indeed, the expression of GATA3 was suppressed in OVA-specific Th2 cells after exposure to sCD83 in the culture; such

an effect was abolished by depleting T-bet expression in $CD4^+$ T cells by CRISPR (Fig. 4 E–G).

IRF1 interacts with GATA3 to prevent antigen-specific Th2 response

The above data indicate that sCD83 promotes T-bet activities in $CD4^+$ T cells. Published data show that interferon regulatory factor 1 (IRF1) mediates interferon (IFN)- γ 's function [15]. We wondered if IRF1 was involved in the suppression of Th2 responses by sCD83. To this end, $CD4^+$ T cells were isolated from blood samples collected from egg-allergic patients. We observed that exposure to OVA significantly induced IL-4 $^+$ $CD4^+$ T cell proliferation, which was abolished by depleting the IRF1 expression in $CD4^+$ T cells (Fig. 5 A–D, Fig. S1 in supplemental materials). We also found that exposure to sCD83 increased IRF1 in $CD4^+$ T cells (Fig. 5 E, F); the IRF1 formed a complex with GATA3 (Fig. 5 G), which prevented GATA3 from binding to the IL-4 promoter (Fig. 5 H). The results demonstrate that IRF1 mediates the effects of sCD83 on suppressing antigen-induced Th2 cell activation.

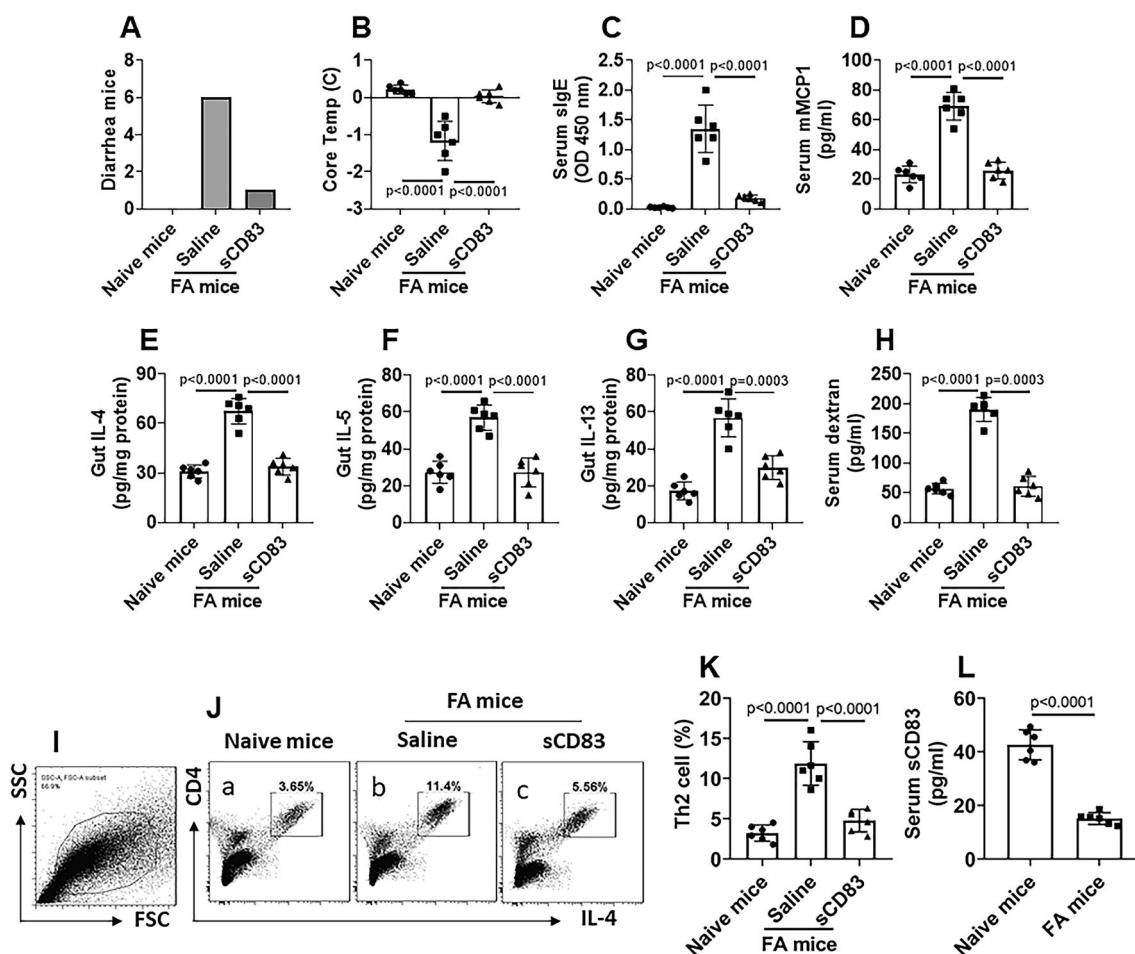


Fig. 6 sCD83 suppresses experimental FA. FA mice were developed. Mice (6 mice per group) were treated with sCD83 (0.1 mg/mouse, i.p.) daily for 7 days. **a, b** FA symptoms (diarrhea and core body temperature drop). **c, d** Serum levels of specific IgE (sIgE) and mouse mast cell protease-1 (mMCP1). **e–g** Th2 cytokines in intestinal protein extracts. **h** Serum FITC-dextran levels used as an indicator of mouse intestinal

epithelial barrier permeability. **i** Intestinal mononuclear cells were isolated and analyzed by flow cytometry; the FSC and SSC plots were gated first. **j** Gated plot shows Th2 cell frequency (also CD3⁺, not shown). **k** Summarized Th2 cells of panel **j**. **l** Mouse serum sCD83 levels. Data of bars are presented as mean $\pm$ SEM. Statistics: ANOVA followed by the Bonferroni test. Each sample was analyzed in triplicate

Administration of sCD83 inhibits experimental FA

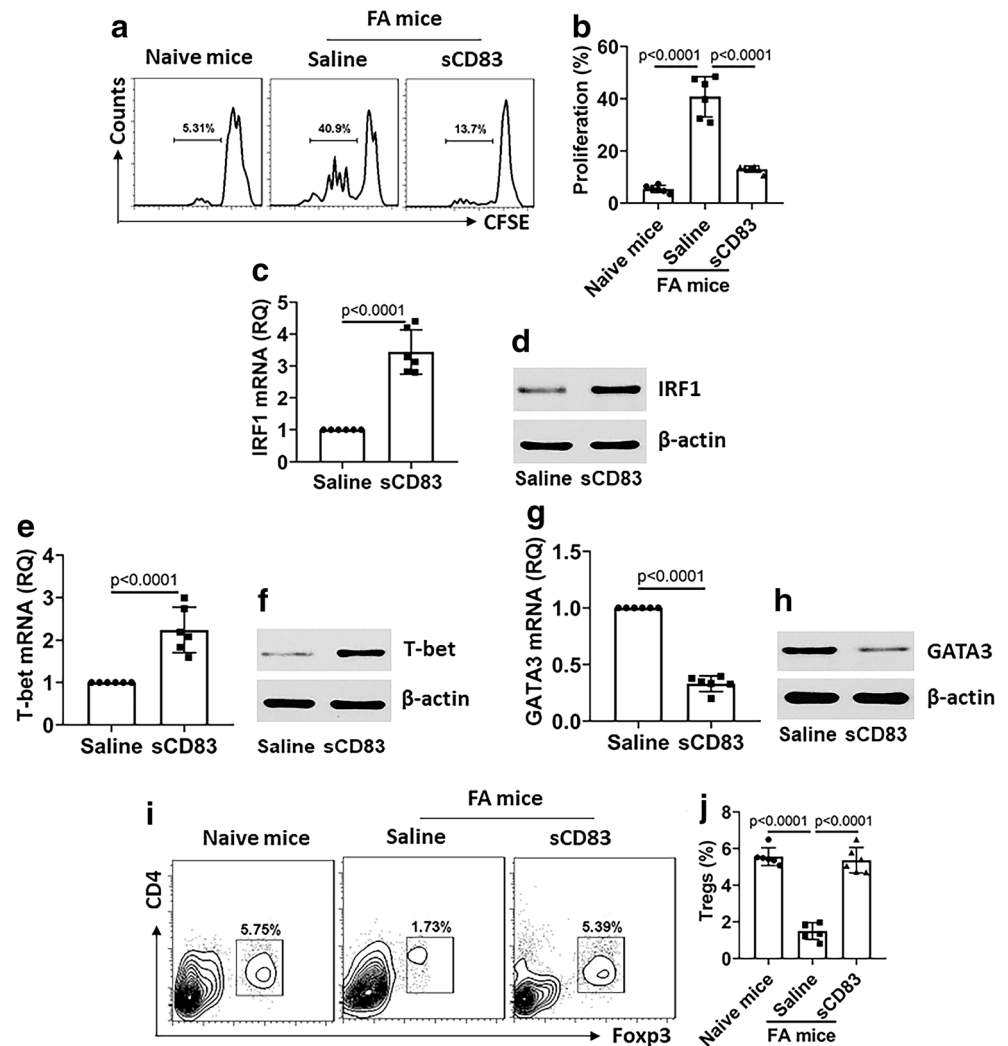
Finally, we developed a murine FA model with OVA as the specific antigen and cholera toxin (CT) as an adjuvant. After the last garage feeding with OVA/CT, the FA mice were treated with sCD83 (or saline, used as a control) via intraperitoneal injection daily for 7 days. One day after the last injection of sCD83, the FA mice were challenged with OVA. The FA response was assessed and recorded. We found that, upon challenging with OVA, the FA mice showed FA symptoms (drop of the core body temperature and diarrhea) (Fig. 6a, b); increases in serum levels of OVA-specific IgE and mMCP1 (Fig. 6c, d); elevation of IL-4, IL-5, and IL-13 contents in the intestinal tissue extracts (Fig. 6e–g); and increased intestinal epithelial barrier permeability (Fig. 6h), which were significantly downregulated by the administration of sCD83. The inhibitory effects of sCD83 administration on IL-4⁺ CD4⁺ T cell differentiation were also detected in FA mice (Fig. 6i–k).

We also found that serum sCD83 levels were markedly decreased in FA mice (Fig. 6l). Furthermore, after the treatment of sCD83, the frequency of OVA-specific CD4⁺ T cells was reduced (Fig. 7a, b), the levels of IRF1 and T-bet in CD4⁺ T cells were increased (Fig. 7c–f), the levels of GATA3 (Fig. 7g, h) were decreased in CD4⁺ T cells, and Treg counts were increased (Fig. 7i, j) in the intestinal tissues; CD83⁺ DC counts were lower in FA mice than in naive control mice (Fig. S2 in supplemental materials). The results demonstrate that sCD83 is able to inhibit experimental FA.

Discussion

The present data show that the serum sCD83 levels in egg-allergy patients are significantly lower than those in HC subjects; this phenomenon was verified in an FA animal study. The serum sCD83 levels are negatively correlated with the

Fig. 7 sCD83 modulates CD4⁺ T cell activities in the intestine. FA mice were prepared. Mice (6 mice per group) were treated with sCD83 (0.1 mg/mouse, i.p.) or saline (control) daily for 7 days. **a**, **b** CD4⁺ T cells (labeled with CFSE) and DCs were isolated from the intestine and cultured in the presence of OVA (5 µg/ml) for 3 days and analyzed by flow cytometry. **a** Gated histograms indicate proliferating CD4⁺ T cells. **b** Summarized proliferating CD4⁺ T cells. **c–h** Isolated CD4⁺ T cells were analyzed by RT-qPCR and Western blotting. **c**, **d** IRF1 expression. **e**, **f** T-bet expression. **g**, **h** GATA3 expression. **i**, **j** Treg frequency in lamina propria mononuclear cells (the Tregs were also CD25⁺; not shown). Data of bars are presented as mean ± SEM. Statistics: ANOVA followed by the Bonferroni test (**b**, **j**) and *t* test (**c**, **e**, **g**). Each sample was analyzed in triplicate. RQ, relative quantification



serum Th2 cytokine levels. sCD83 suppresses Th2 cell differentiation and inhibits antigen-specific Th2 cell activation through upregulating the expression of IRF1 and T-bet, and downregulating GATA3. Administration of sCD83 inhibits experimental FA. A recent publication also found that sCD83 could suppress Th2 response by facilitating Th2 cell apoptosis and could suppress experimental allergic rhinitis in mice [16]. Our data are in line with this study.

The results showed that FA patients had lower serum sCD83 levels. Although CD83 is usually expressed on the surface of DCs and B cells, sCD83 can be detected in the serum [12]. Previous studies showed that serum sCD83 levels were altered in patients with multiple sclerosis [17]; rheumatoid arthritis [18]; some malignant tumors, such as Hodgkin lymphoma [19]; and allergic rhinitis [16]; these diseases are immune disorders, indicating that sCD83 may be associated with the pathogenesis of immune diseases. Our data add novel information to this area that lower serum sCD83 levels are one of the immune pathological features of the FA. This is

in line with Wu et al.'s recent report, in which the authors observed lower serum sCD83 levels in patients with allergic rhinitis [16].

The data show that serum sCD83 levels are negatively correlated with the serum Th2 cytokine levels. The high serum Th2 cytokine levels are a canonical phenomenon of allergic diseases, including the FA [20]. Negative correlation between the serum sCD83 and the serum Th2 cytokine levels suggests that there may be a link between these two parameters. Th2 polarization plays a critical role in the pathogenesis of allergic diseases [20] and some autoimmune diseases [21]. Thus, to downregulate Th2 cytokine levels has therapeutic significance. This has been demonstrated by published data. Lundell et al. indicate that sCD83 suppresses allergen-induced human neonatal Th2 differentiation [22]. Previous reports indicated that sCD83 suppressed inflammatory bowel disease activities by increasing indolamine 2,3-dioxygenase and expanded regulatory T cell activities [23]. Our data are in line with this report by showing that administration of sCD83 increased the Treg frequency in the mouse intestine.

The present data show that the presence of sCD83 also has the capacity to downregulate Th2 cytokines both in vitro and in vivo, suggesting that sCD83 may be a new remedy for regulating the skewed Th2 response.

The results also show the underlying mechanism by which sCD83 suppresses Th2 polarization. Since Th2 cytokines are produced by Th2 cells, the suppression of Th2 cytokines must be obtained by inhibiting Th2 cells from producing Th2 cytokines. There are reports showing inhibitory approaches on aberrant Th2 responses, such as corticosteroid agents [24], antigen-specific immunotherapy [25] [26], and some alternative medicines, such as Chinese traditional medicine [27]. The present data show that sCD83 can restrict the development of Th2 cells and inhibit antigen-specific Th2 cell activation through increasing IRF1 and T-bet expression and suppressing GATA3 activities. In other words, we have found a novel effective method to be used in the regulation of skewed Th2 response.

The therapeutic efficacy of FA is not satisfactory currently. By specific antigen immunotherapy (SIT), the protective antigen-specific IgG may be induced. However, the treatment period of SIT is about 3 years. Poor compliance of SIT was reported in patients with allergic diseases. Administration of corticosteroids usually can get good control effect on the treatment of FA and other allergic diseases, but the complications induced by corticosteroids need to be paid much attention. The present data demonstrate that administration of sCD83 can significantly suppress FA symptoms and the pathological changes in the intestinal tissues. Others also found that sCD83 had immune-regulatory functions. For example, Horvatinovich et al. indicate that sCD83 can inhibit T cell activation [28]. Lin et al. show that sCD83 alleviates experimental autoimmune uveitis [29]. Zinser et al. provided evidence that sCD83 can prevent and treat experimental autoimmune encephalomyelitis [30]. These studies demonstrate that sCD83 may also be a candidate for the treatment of FA and other immune disorders.

Published data indicate that administration of sCD83 can inhibit inflammatory bowel disease-like response in the intestine, in which the immune response has the Th1 pattern [23]. Our data show that sCD83 can suppress FA response with the Th2 pattern of inflammation. The underlying mechanism needs to be further investigated. sCD83 can promote regulatory T cell function [31]. Our data also show that sCD83 expands regulatory T cells in the FA mouse intestine. Regulatory T cells can suppress both Th1 and Th2 patterns of inflammation. Thus, the inhibitory effects of sCD83 on experimental FA can be the effects of both regulation of Th1/Th2 response and expansion of regulatory T cells in the intestine.

In summary, the present data indicate that sCD83 can suppress FA symptoms and pathological changes in the intestine through modulating the skewed Th2 response. The data suggest that sCD83 has the translational potential to be used in the treatment of FA.

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Author contributions WS, HL, and BJ performed the experiments, analyzed the research data, and reviewed the manuscript. ZW designed the project, supervised the experiments, and wrote the manuscript. All of the authors reviewed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Supplemental materials

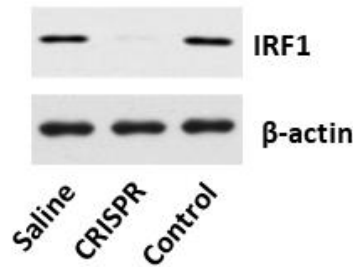


Figure S1. Depletion of IRF1 in CD4⁺ T cells. CD4⁺ T cells were prepared and treated with IRF1 CRISPR reagents (or control reagents) following the manufacturer's instruction. The effects of CRISPR were assessed by Western blotting 48 h after the treatment. The immunoblots show IRF1 levels in CD4⁺ T cells.

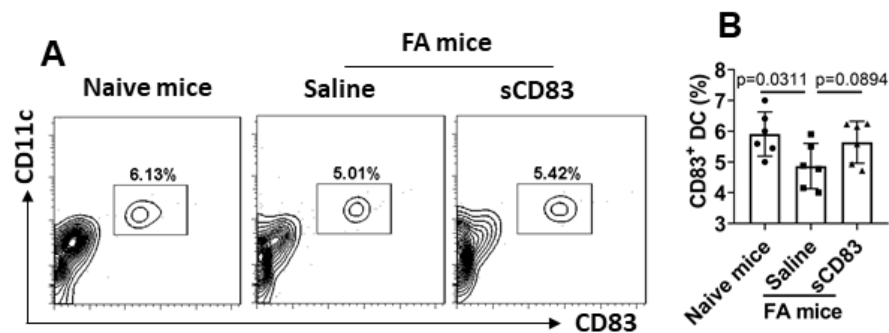


Figure S2. Effects of sCD83 on intestinal DCs. FA mice were treated with sCD83 or saline as described in figure 6 legend. After the sacrifice, jejunal segments were excised; lamina propria mononuclear cells (LPMC) were isolated from the intestinal tissues and analyzed by flow cytometry. A, gated flow cytometry plots show CD83⁺ DC counts in LPMCs. B, bars show summarized CD83⁺ DC counts (mean ± SEM). Each sample was analyzed in triplicate. Statistics: ANOVA + Bonferroni test.