



Formononetin attenuated allergic diseases through inhibition of epithelial-derived cytokines by regulating E-cadherin

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ABSTRACT

Radix Astragali, has long been used to alleviate allergic diseases (ADs). Formononetin is one of the major active components in Radix Astragali, but its mechanism on ADs is not definitively known. The fluorescein isothiocyanate isomer-induced atopic contact dermatitis mouse model and poly I:C or lipopolysaccharide-treated HaCaT cells were used to examine thymic stromal lymphopoietin (TSLP)/interleukin (IL)-33 production and expression of E-cadherin. After administration of formononetin, TSLP/IL-33 levels decreased both *in vitro* and *in vivo*, while E-cadherin was increased *in vivo* and restored *in vitro*. Furthermore, small interference RNA silencing of E-cadherin resulted in elevated levels of TSLP, whereas the inhibitory effect of formononetin on TSLP was no longer observed. In addition, TSLP resulted in no detectable changes in delocalization or protein expression of E-cadherin in HaCaT cells. These results indicated that formononetin showed a protective effect in ADs, which was correlated with decreasing TSLP/IL-33 production *via* regulation of E-cadherin.

1. Introduction

Allergic disease (AD) is a common condition, which impacts the quality of life, depending on severity and the degree of control. The World Health Organization estimates that approximately 400 million people worldwide suffer from allergic rhinitis, and > 180,000 people die because of asthma each year. Furthermore, the number of AD patients is relatively high and is rising, making it important to explore the mechanism of action of this disease [1]. The major drugs used in the treatment of AD currently include IgE antagonists, immune modulators, and glucocorticoids. Although these drugs rapidly reduce symptoms when AD occurs, they cannot always prevent recurrence, so a curative therapy for allergy is still lacking.

Formononetin, an isoflavone phytoestrogen, is a major component in Radix Astragali (known as Huangqi in Chinese), which has long been used to attenuate airway inflammation and allergic disease [2, 3]. In our previous study, we showed that formononetin exerted potent anti-allergic effects on the initial stage of the Th2-mediated atopic contact dermatitis (ACD) mouse model [4]. However, the mechanism responsible for the effect of formononetin on the recurrence of ADs is not known.

The epithelium not only serves as a barrier against allergens, but

also plays an important role in immune responses by its exposure to the external environment. Among various products secreted by epithelial cells, thymic stromal lymphopoietin (TSLP) and interleukin (IL)-33 have been identified as key initiation factors in the pathophysiology of ADs [5]. Early studies showed that overexpression of TSLP was detected in airway epithelial cells of asthmatic patients and in a mouse model of asthma [6]. Similarly, elevated levels of TSLP were expressed in both asthmatic airway and nasal epithelial cells of allergic rhinitis patients [7, 8]. Moreover, TSLP was correlated with the expression of Th2-attracting chemokines such as TARC and CCL17 [9], and by creating a microenvironment, TSLP promoted Th2 differentiation [7]. Thus, TSLP may act as a master switch that triggers the initiation and maintenance of allergic responses. IL-33, another critical epithelium-derived cytokine, activates group 2 innate lymphoid cells (ILC2s) to drive type 2 inflammation [9]. Clinical findings showed that IL-33 levels were elevated in allergic rhinitis patients, suggesting its essential role in ADs [10, 11].

Epithelial barrier impairment is a common feature of allergic responses, which has been postulated to contribute to antigen sensitization. As a main component of the cell adhesion connection system, E-cadherin is an important molecule in the maintenance of epithelial integrity. Expression of E-cadherin is lower not only in skin epithelial

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cell (ECs) of patients with allergic dermatitis [12], but also in bronchial ECs of patients with asthma [13], when compared with healthy subjects. E-cadherin is regarded as a “gatekeeper” in both airway mucosa and allergic sensitization, because it contributes to the structural and immunological functions of the airway epithelium [14]. E-cadherin controls the response to allergens through its interaction with regulatory T cells and dendritic cells (DCs) [14, 15]. The deficiency and redistribution of E-cadherin result in upregulated TSLP and contribute to allergic pathogenesis [16, 17].

Epithelial cells therefore play a pivotal role in the initiation of ADs. However, the effects of anti-allergic drugs on TSLP/IL-33 or E-cadherin remain unclear. Several studies have reported a protective effect of isoflavone phytoestrogens in intestinal barrier function [18, 19], suggesting that formononetin may also repair the barrier function. Additionally, in our previous study, we initially found that formononetin was an active component that interacted with ECs, and formononetin given in the initial stage of the ACD mouse model significantly decreased ear thickness and infiltration of inflammatory cells. These results suggested that formononetin interfered with the initial stage of the allergic process. In the present study, we hypothesized that formononetin restored epithelial barrier function and subsequently reduced the expression of TSLP/IL-33, as well as inhibiting the occurrence and development of ADs. The objectives of the present study were therefore to: 1) determine whether formononetin affected levels of TSLP/IL-33; 2) determine whether an impaired epithelial barrier function could be restored by formononetin treatment; and 3) determine the relationship between an impaired epithelial barrier function and the expression of TSLP/IL-33. To accomplish these objectives, we explored the possible mechanism of action of formononetin on AD, and assessed the possibility of TSLP/IL-33 and epithelial barrier proteins as therapeutic targets of ADs.

2. Materials and methods

2.1. Materials

Formononetin (purity: 99%) was purchased from Tianjin Wanxiang Hengyuan Technology (Tianjin, China). Fluorescein isothiocyanate isomer (FITC) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

BALB/c mice were purchased from Shanghai SLAC Laboratory Animal Company (Shanghai, China). All animals were housed at Nanjing University of Chinese Medicine, at 22 °C and 50%–60% humidity with a 12 h light-dark cycle, and with food and water freely available. All procedures involving animals were approved by the Animal Care and Use Committee of Nanjing University of Chinese Medicine and were strictly performed according to the Guide for the Care and Use of Laboratory Animals.

2.3. The initial stage of the Th2-mediated ACD model

Both the left and right ears of BALB/C mice were sensitized with 20 µL of 0.6% FITC (Sigma-Aldrich) dissolved in acetone and dibutylphthalate (1:1 as vehicle) on days 1 and 2. The mice were treated with formononetin (0.4, 2, or 10 mg/kg, intragastrically) once daily, or with normal saline, repeatedly from day 1 to day 3. On day 3, the mice were euthanized for further analysis. The study design is summarized in Fig. 1A.

2.4. Enzyme-linked immunosorbent assay (ELISA) of cytokines in ear homogenates

Both ears of each mouse were removed and ground to homogenates in ice-cold phosphate-buffered saline (PBS), and the homogenates were

centrifuged at 4000 × g for 15 min at 4 °C. The supernatants were stored at −80 °C before analysis. The levels of TSLP and IL-33 in the ear tissues were measured with a human TSLP ELISA kit (eBioscience, San Diego, CA, USA) and human IL-33 ELISA kit (Becton Dickinson, USA) according to the manufacturer's instructions. All measurements were made in triplicate.

2.5. Quantitative real-time polymerase chain reaction (qPCR) assay for the mRNA expression of TSLP and IL-33

Ear tissues of each group were homogenized in 1 mL of TRIzol (Life Technologies, USA) using a glass homogenizer. The total RNA was isolated according to the manufacturer's protocol. The SYBR green PCR Master Mix (Thermo Scientific, USA) was used for real-time PCR analysis. All reactions were run on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The oligonucleotide sequences of the PCR primers (Sangon Biotech, Shanghai, China) were 5'-TACTATACTCTCAATCCTATCCCTG-3' (sense, S) and 5'-ACTTCTTG TGCCATTTCCTG-3' (antisense, AS) for TSLP (If-TSLP); 5'-TCCAATC CAAGATTTCCTG-3' (S) and 5'-CATGCAGTAGACATGGCAGAA-3' (AS) for IL-33; 5'-GGTTGTCTCCTGCGACTTCA-3' (S) and 5'-TGGTCCA GGGTTTCTTACTCC-3' (AS) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The cycle time of the gene was normalized with GAPDH in the same sample, and the fold induction of gene expression was calculated using the $\Delta\Delta C_t$ method. The results obtained from each PCR were pooled and statistically analyzed.

2.6. Electron microscopy

The ear tissue specimen was first fixed with 2.5% glutaraldehyde in PBS for > 4 h, washed three times in PBS, then postfixed with 1% OsO₄ for 1 h and washed four times in PBS. The specimen was dehydrated by a graded series of ethanol (30%, 50%, 70%, 80%, 90%, and 100%) for approximately 15 min at each step and transferred to absolute acetone for 20 min. The specimen was then placed in a 1:1 mixture of absolute acetone and resin for 1 h at room temperature, then transferred to a 1:3 mixture of absolute acetone and resin for 3 h, then to a final resin solution overnight. The specimen was then placed in capsules containing embedding medium and heated at 70 °C for 48 h. The 70 nm specimen sections were stained by acetate and alkaline lead citrate for 15 min and observed using a transmission electron microscope (JEOL, Akishima, Japan).

2.7. Cell culture and treatments

HaCaT cells (a human keratinocyte cell line) were obtained from the Model Animal Research Center of Nanjing University (Nanjing, China). The cells were cultured in Minimal Essential Medium (Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Wisent) at 37 °C under 5% CO₂. The cells were pretreated with formononetin (0.1 µM, 1 µM, or 10 µM) or medium with 0.05% dimethyl sulfoxide as vehicle (control) for 6 h. To induce TSLP and IL-33 expression in keratinocytes, the cells were incubated with 10 µg/mL poly I:C (Sigma-Aldrich) or 10 µg/mL lipopolysaccharide (LPS) (Sigma-Aldrich) for 24 h.

2.8. Immunofluorescence

HaCaT cells were seeded into 12-well plates with coverslips at a density of 1×10^5 cells/mL and incubated at 37 °C under 5% CO₂. The cells on the coverslips were fixed in methanol for 20 min at −20 °C and washed with PBS. For cytokine detection, the cells were permeabilized with Triton X-100 (Genview Scientific, USA) for 10 min, while this procedure was not needed for E-cadherin. The endogenous peroxidase was blocked by incubating in 3% H₂O₂ for 20 min, and nonspecific binding sites were blocked with 10% bovine serum albumin for 1 h at 37 °C. The cells were probed with rabbit monoclonal antibodies against

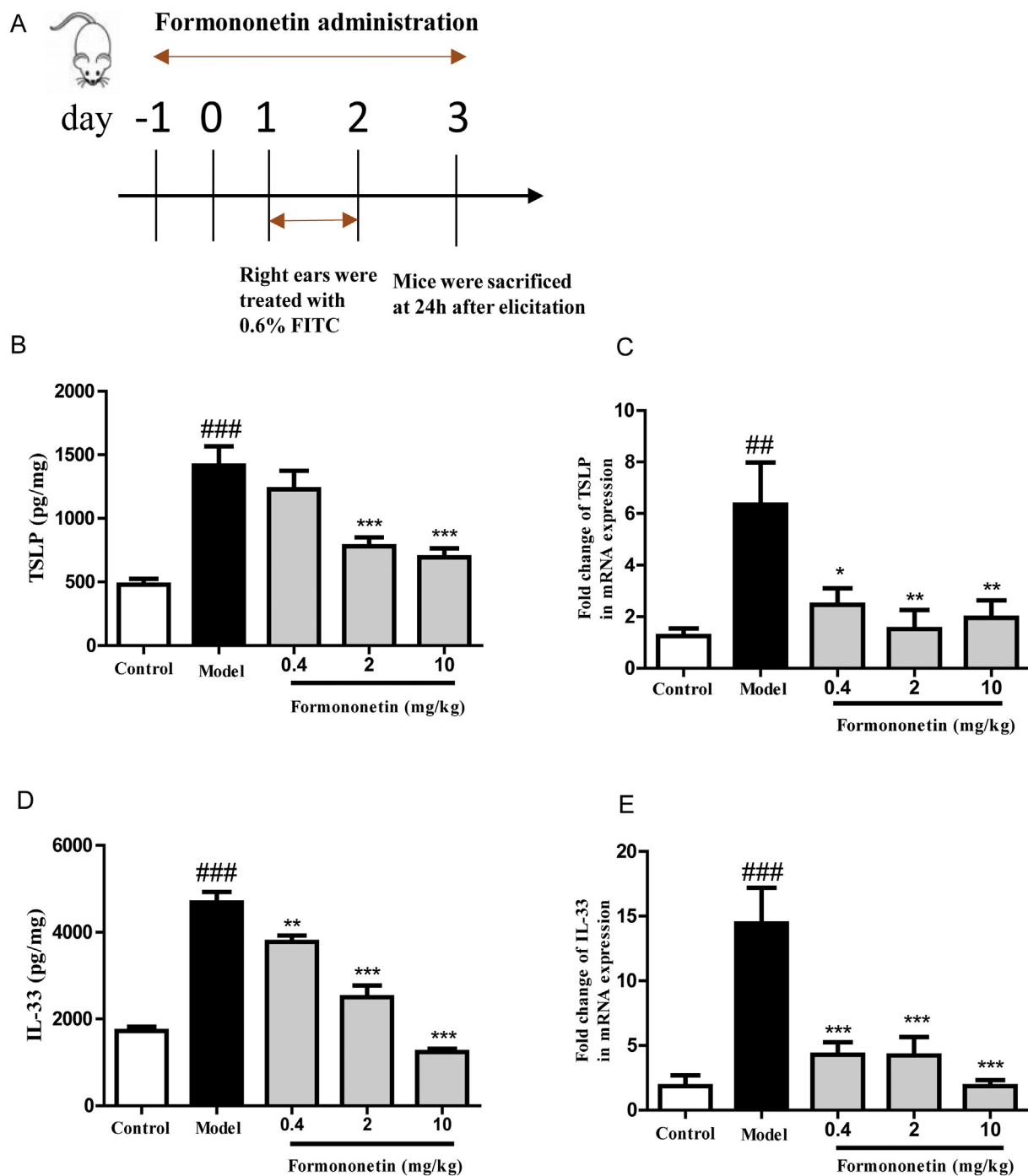


Fig. 1. Formononetin inhibited thymic stromal lymphopoietin (TSLP) and interleukin (IL)-33 expression during the initial stage of sensitization *in vivo*. (A) Flow charts of the fluorescein isothiocyanate isomer (FITC)-induced sensitization phase of the atopic contact dermatitis model. The model mice ears were sensitized with 20 μ L 0.6% FITC dissolved in acetone and dibutylphthalate (1:1: vehicle). The control mice were treated with the same volume of vehicle only. Formononetin (0.4, 2, or 10 mg/kg) was given intragastrically during the model process. (B) The protein level of TSLP in ear tissue homogenates. (C) The mRNA expression of TSLP in ear tissue homogenates. (D) Protein levels of IL-33 in ear tissue homogenates. (E) The mRNA expression of IL-33 in ear tissue homogenates (mean $\pm$ SEM, $n = 7$). ^{###} $P < 0.001$ vs. control; ^{*} $P < 0.05$; ^{**} $P < 0.01$; ^{***} $P < 0.001$ vs. model.

TSLP and IL-33 (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or E-cadherin (1:50 dilution; BD Biosciences, San Jose, CA, USA) at 4°C overnight. After repeated washes with PBS, the cells were probed with goat anti-rabbit IgG conjugated to FITC (1:200 dilution; Santa Cruz Biotechnology) and 4',6-diamidino-2-phenylindole (Biotword, China) at a concentration of 0.1 μ g/mL for 10 min. The labeled sections were observed with fluorescence optical microscopy (Zeiss, Axio Vert.A1, USA).

2.9. Transfection with E-cadherin small interfering RNA (siRNA)

Downregulation of E-cadherin expression in HaCaT cells was conducted using 50 nM siRNA (TranSheepBio, China). HaCaT cells were seeded into 24-well plates with coverslips at a density of 1×10^4 cells/mL and incubated at 37°C under 5% CO₂ for 12 h, then transfected with the indicated siRNAs for human E-cadherin or with control siRNA using Lipofectamine 2000 (Invitrogen Life Technologies, USA). E-cadherin was knocked down with the following siRNA duplexes: GGCCUGAAG

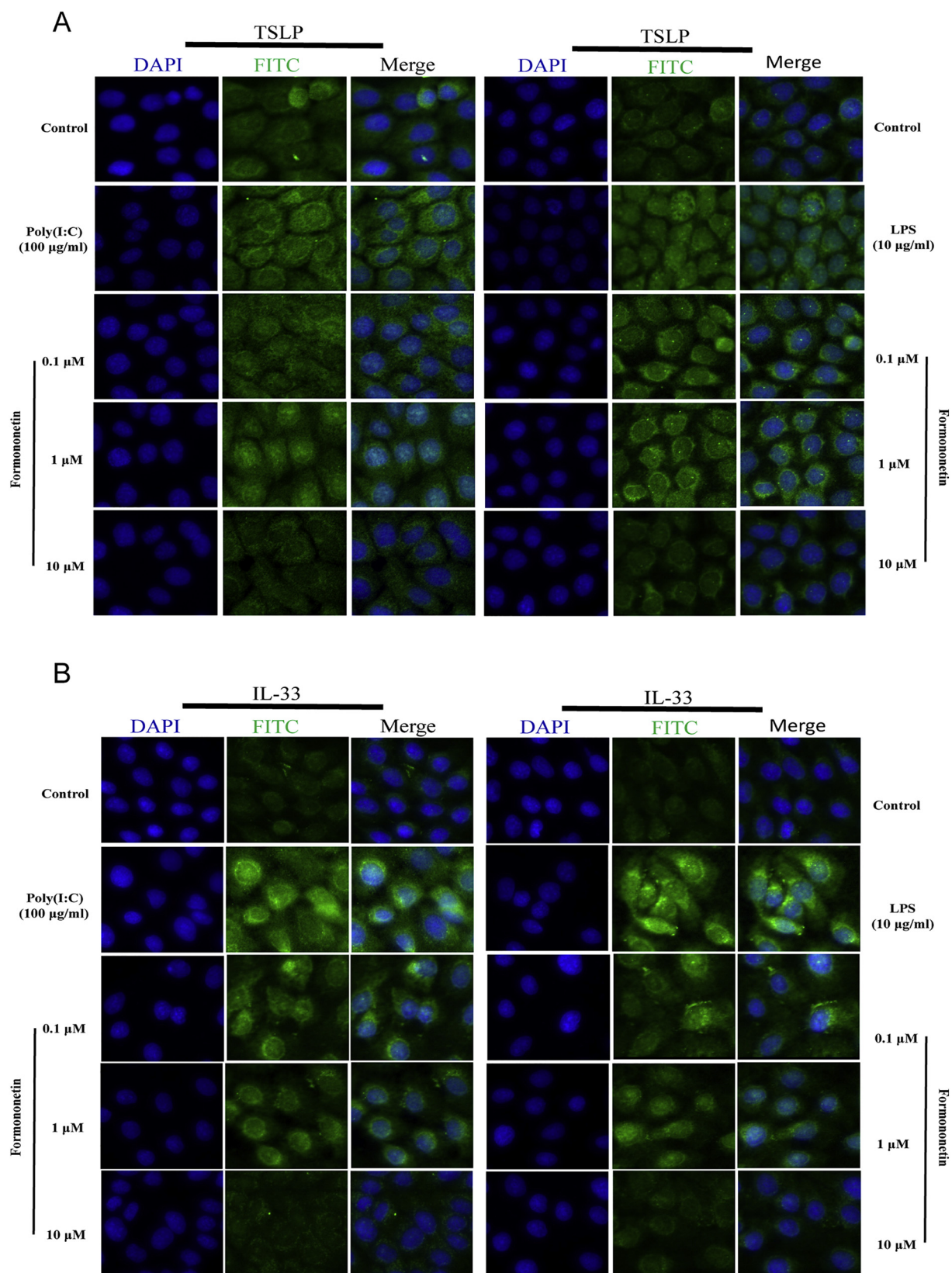


Fig. 2. Formononetin dramatically reduced poly I:C- or lipopolysaccharide (LPS)-induced thymic stromal lymphopoietin (TSLP) and interleukin (IL)-33 expression in HaCaT cells. Cells were treated with different concentrations of formononetin and stimulated with 100 µg/mL poly I:C or 10 µg/mL LPS for 24 h. (A) Immunofluorescence assay for TSLP expression in HaCaT cells ($n = 3$; magnification, 400 $\times$). (B) Immunofluorescence assay of IL-33 expression ($n = 3$; magnification: 400 $\times$).

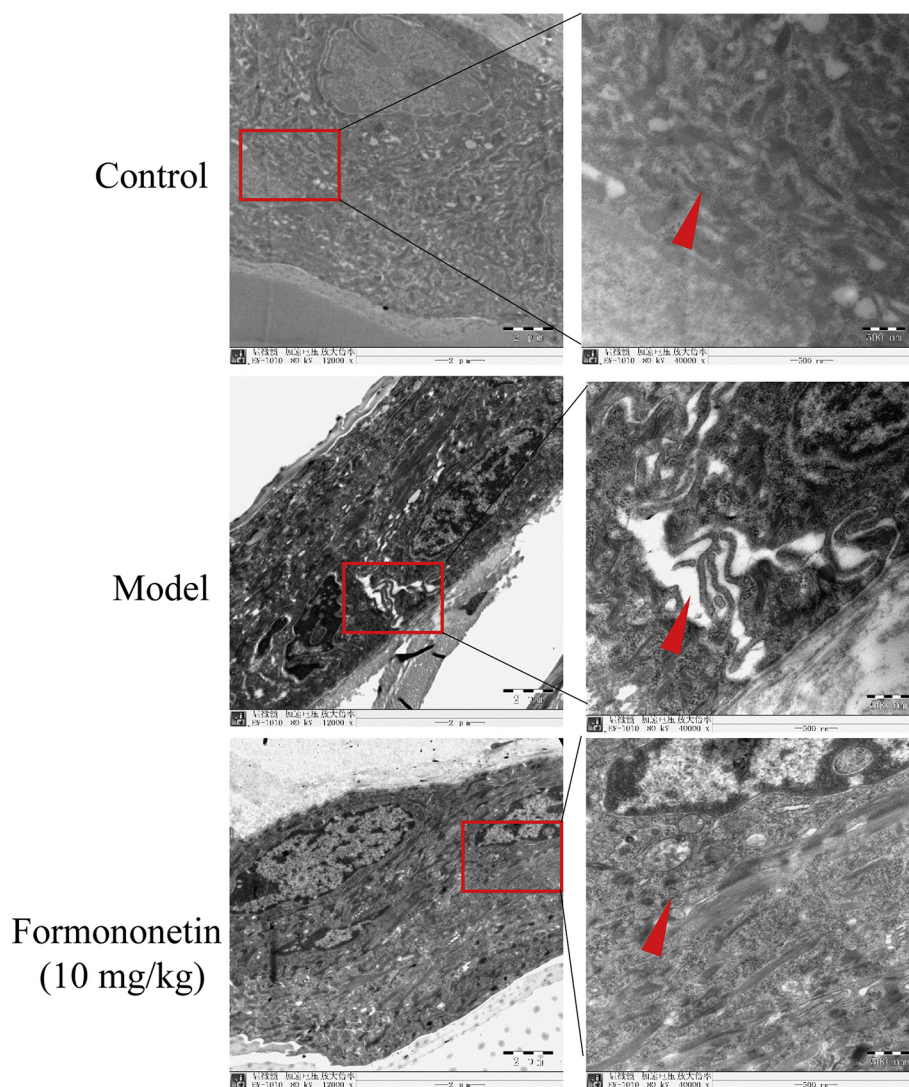


Fig. 3. Effect of formononetin on the integrity of ear skin epithelial cells during the initial stage of the atopic contact dermatitis model. The model mice ears were sensitized with 20 μ L 0.6% fluorescein isothiocyanate isomer dissolved in acetone and dibutylphthalate (1:1; vehicle). The control mice were treated with vehicle only with the same volume. Formononetin was given intragastrically during the model process. Arrows denote junctional structure of epithelial cells ($n = 3$, magnification: 12,000 $\times$ and 40,000 $\times$).

UGACUCGUAATT and UUACGAGUCACUUCAGGCCTT. Six hours after transfection, cells were cultured for another 48 h before stimulation, then measured using a human TSLP ELISA kit (see “ELISAs”).

2.10. Statistical analysis

The data are expressed as the means $\pm$ SD. Multiple group comparisons were analyzed using one-way analysis of variance, and Dunnett's test was used for comparisons between two groups using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The statistical significance was set at $P < 0.05$.

3. Results

3.1. Formononetin inhibited TSLP and IL-33 expression in vivo and in vitro

3.1.1. Formononetin inhibited TSLP and IL-33 production in the initial stage of sensitization in ACD mice

Mice were treated with formononetin in the initial stage of the Th2-mediated ACD model for 5 days. Ear tissues were collected 24 h after the final FITC sensitization (Fig. 1A). We examined mRNA and protein

levels of TSLP and IL-33 in ear tissue homogenates. Compared with the control, increased TSLP and IL-33 expression levels in FITC-sensitized mice (model) were observed, whereas formononetin reduced both protein (Fig. 1B, D) or mRNA expression levels (Fig. 1C, E) of TSLP and IL-33 induced by FITC.

3.1.2. Formononetin reduced poly (I:C) or LPS-induced TSLP and IL-33 expression in HaCaT cells

Cells were treated with different concentrations of formononetin and stimulated with 100 μ g/mL poly I:C or 10 μ g/mL LPS for 24 h. TSLP and IL-33 in the supernatants increased after stimulations with poly I:C or LPS. Formononetin significantly reduced the TSLP (Fig. 2A) and IL-33 (Fig. 2B) expression levels either induced by poly I:C or LPS in HaCaT cells, which was consistent with the *in vivo* results. Taken together, these results indicated that formononetin inhibited epithelial-derived TSLP and IL-33 expression.

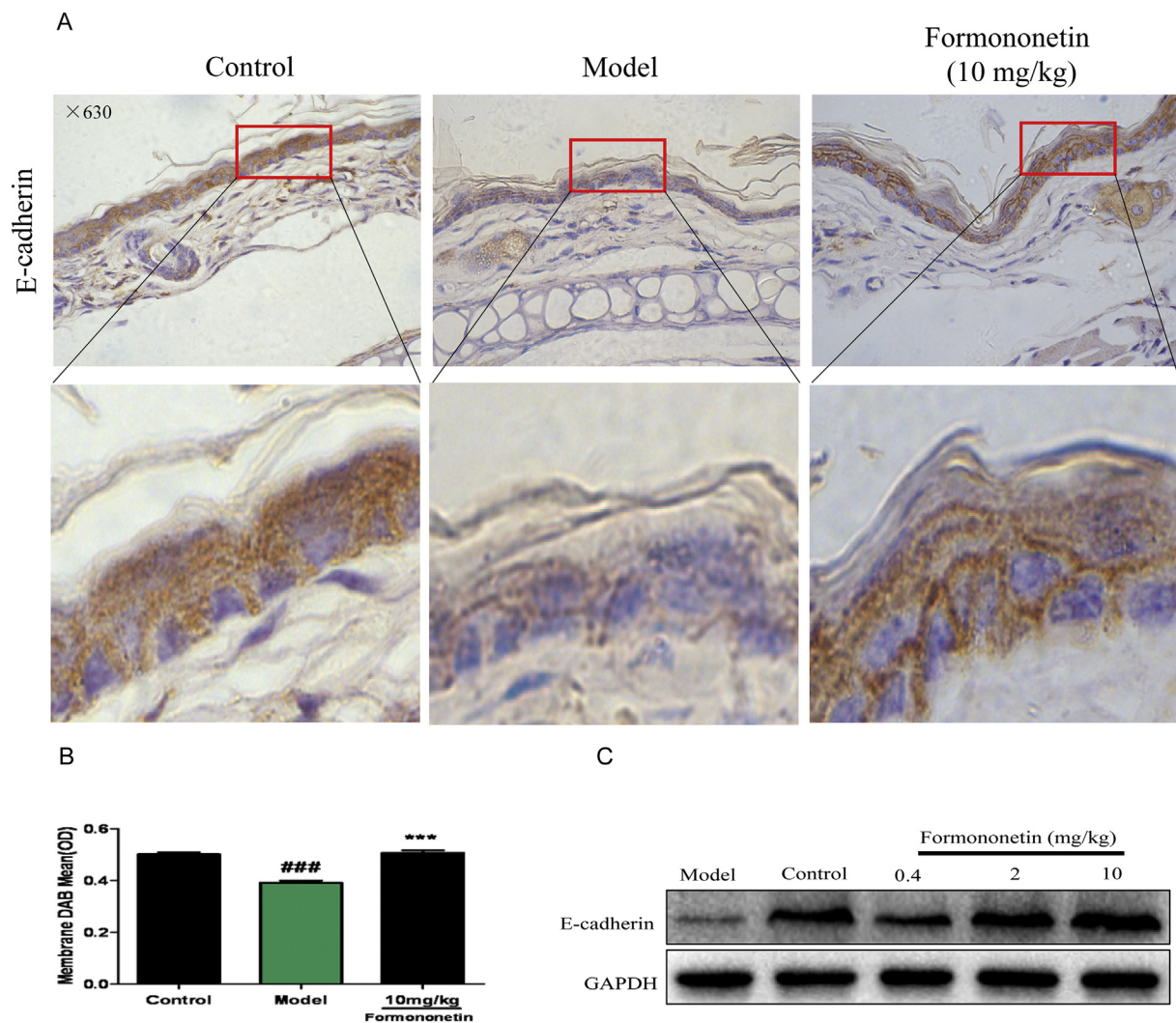


Fig. 4. Effect of formononetin on E-cadherin expression of the ear skin during the initial stage of the atopic contact dermatitis model. The model mice ears were sensitized with 20 μ L 0.6% fluorescein isothiocyanate isomer dissolved in acetone and dibutylphthalate (1:1; vehicle). The control mice were treated with vehicle only with the same volume. Formononetin was given intragastrically during the model process. (A) Effect of formononetin on the histopathology of ear skin sections ($n = 5$) (immunohistochemistry, 630 $\times$). (B) The E-cadherin mean DAB intensity was quantitated using the Mantra Quantitative Pathology Workstation (mean $\pm$ SEM, $n = 5$; ### $P < 0.001$ vs. control; *** $P < 0.001$ vs. model). (C) Protein levels of E-cadherin were determined using western blotting.

3.2. Formononetin restored impaired epithelial barrier function *in vivo* and *in vitro*

3.2.1. Formononetin reduced the gap between epithelial cells during the initial stage of the ACD mouse model

Impaired epithelial barrier function enables the invasion of allergens into the organism, which triggers AD. We investigated the effect of formononetin on epithelial integrity during the initial stage of the ACD model using electron microscopy. The results showed that sensitization of FITC led to disrupted epithelial junctional structures and gaps between epithelial cells, when compared with those in control mice, while administration of formononetin (10 mg/kg) decreased or even repaired the gap between cells (Fig. 3).

3.2.2. Formononetin increased the expression of E-cadherin during the initial stage of the ACD model

Epithelial barrier function is primarily determined by the integrity of intercellular junctions, and E-cadherin is an important molecule in the maintenance of epithelial integrity. To determine whether formononetin reduced the gap between cells by regulating intercellular junctions, we investigated the effects of formononetin on E-cadherin

expression using immunohistochemistry and western blotting. The model mice showed significantly lower expression of E-cadherin compared with that in control mice, and formononetin significantly increased the expression of E-cadherin, which was quantitatively measured by the mean E-cadherin DAB intensity (Fig. 4A, B). A similar trend was observed using western blotting (Fig. 4C).

3.2.3. Formononetin improved the redistribution of E-cadherin induced by poly I:C or LPS *in vitro*

To further confirm the effect of formononetin on E-cadherin, we investigated its *in vitro* effect using immunofluorescence and western blotting, which showed that poly I:C or LPS did not significantly affect the protein expression of E-cadherin, and formononetin had no significant effect on the levels of E-cadherin (Fig. 5A, B). However, E-cadherin predominantly localized in the regions of cell-cell contacts (red arrows) in control cells, while this distribution was dramatically altered in poly I:C- or LPS-treated cells, in which E-cadherin tended to accumulate in the cytosolic compartment (yellow arrowheads). Moreover, formononetin improved the redistribution of E-cadherin, which may have mainly been responsible for the barrier dysfunction (Fig. 5C).

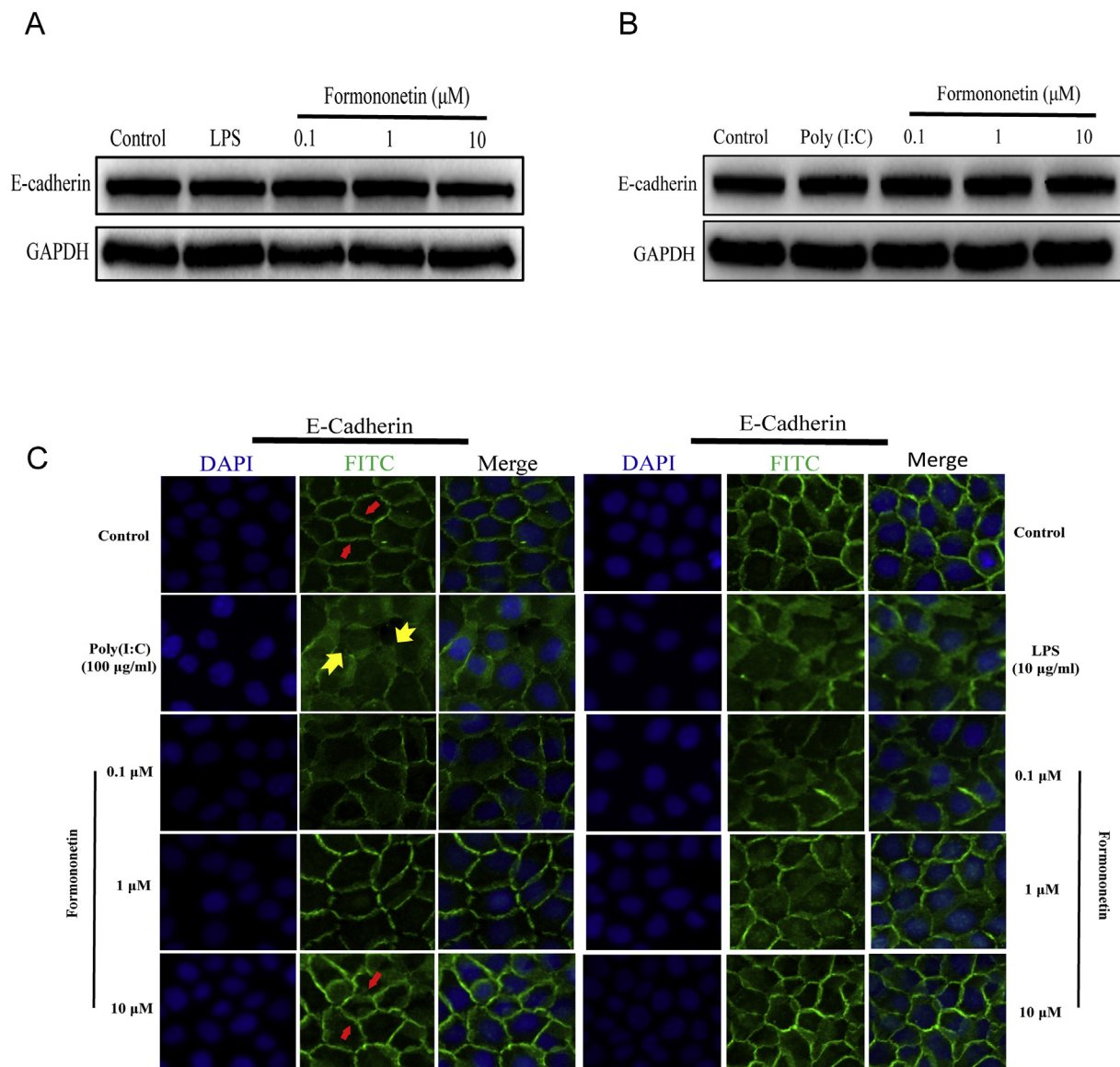


Fig. 5. Formononetin improved the redistribution of E-cadherin in HaCaT cells. (A–B) E-cadherin expressions were analyzed by western blotting in HaCaT cells ($n = 3$). (C) Localization of immunofluorescence of E-cadherin expression. Note the localization of E-cadherin in control cells and formononetin-treated cells (red arrows) and their translocation into the cytosolic compartment after poly I:C or lipopolysaccharide exposure (yellow arrowheads) ($n = 3$, magnification: $400\times$). The data are representatives of three independent experiments (Φορ ιντερπρετατιον οφ τηρ ρεφερειχεσ το χολοουρ ιν τηισ φιγυρε λεγεινδ, τηρ ρεαδερ ισ ρεφερρεδ το τηρ ωεβ περσιον οφ τηισ αρτικλε.).

3.3. TSLP levels increased significantly after E-cadherin silencing, but increased TSLP levels resulted in no detectable changes in E-cadherin expression

To examine the relationship between impaired epithelial barrier function and the expression of TSLP, siRNA oligonucleotides with an E-cadherin-specific target sequence and recombinant TSLP were used. A decrease in immunofluorescence was observed with E-cadherin after transfection with E-cadherin-specific siRNA in HaCaT cells (Fig. 6A). In addition, transfection of E-cadherin-specific siRNA resulted in a marked downregulation of total E-cadherin protein expression (Fig. 6B). Furthermore, silencing of E-cadherin resulted in a significant increase of TSLP expression, and the inhibitory effect of formononetin on TSLP expression was no longer observed after silencing of E-cadherin (Fig. 6C). In contrast, exposure of HaCaT cells to TSLP at 20 ng/mL resulted in no detectable changes neither in the delocalization nor protein expression of E-cadherin (Fig. 6D, E). Taken together, these results indicated that E-cadherin regulated expression of TSLP, and

formononetin inhibited this epithelial-derived initiation via regulating expression of E-cadherin.

4. Discussion

AD is one of the most prevalent diseases worldwide, but its mechanism of action is poorly understood. The main issue for clinical treatment is recurrence. It is therefore urgent to understand the pathophysiology and find effective therapeutic treatments to prevent recurrence of this disorder.

“Yu-Ping-Feng-San (YPFS),” which is a traditional Chinese medicinal formulation, has been widely used to treat AD. It has been reported to reduce the recurrence of AD. High levels of formononetin were found in the serum of YPFS-treated mice, in our previous study [4]. Formononetin alleviated inflammation in ACD mice, as demonstrated by reduced swelling of the ears, reduced inflammatory cell infiltration, and improved local tissue congestion [4]. In addition, Lv et al. in 2017 reported that formononetin inhibited histamine release in a dose-

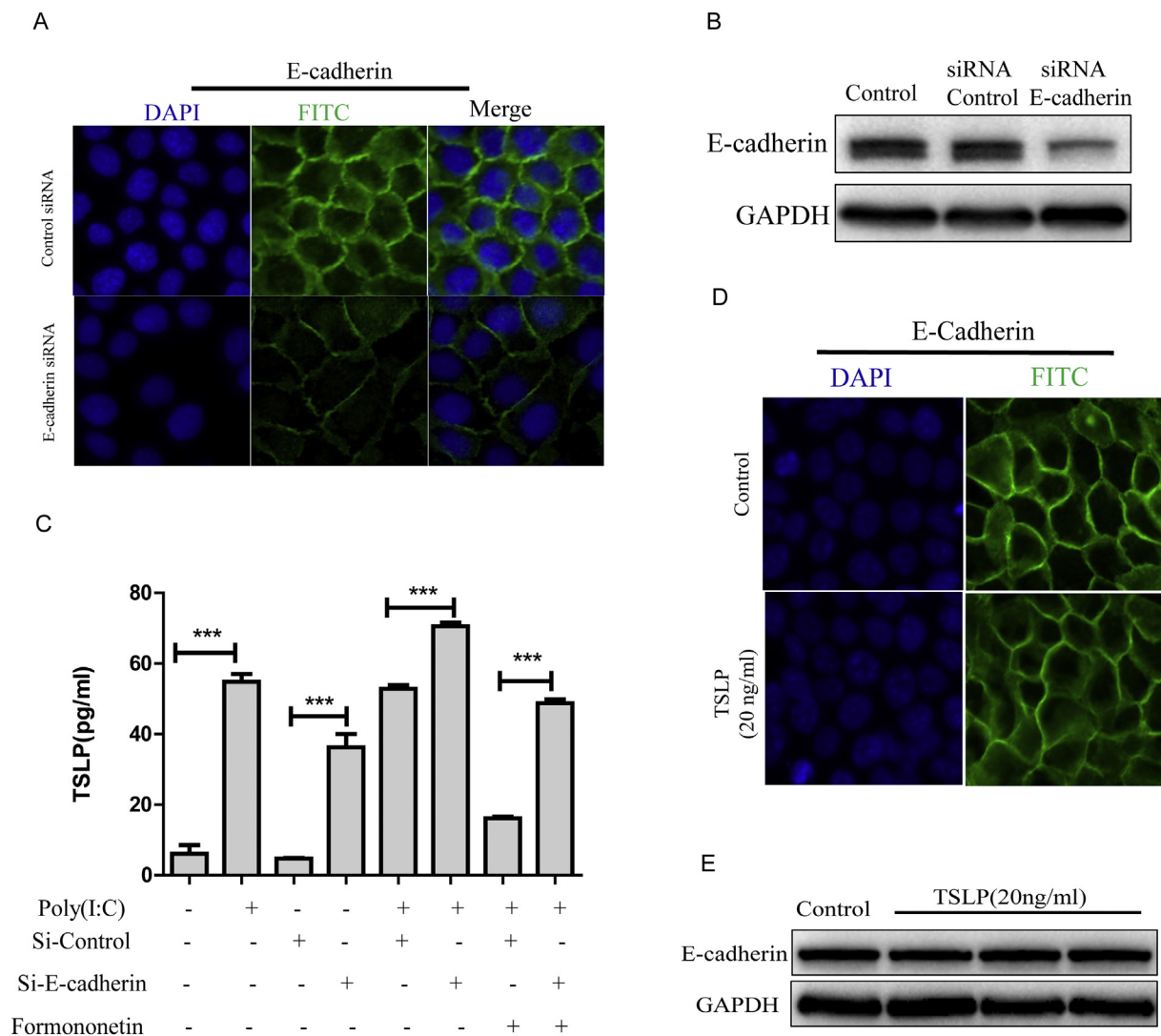


Fig. 6. Thymic stromal lymphopoietin (TSLP) increased significantly after E-cadherin silencing, but overexpression of TSLP resulted in a change in E-cadherin expression. HaCaT cells were transfected with non-targeting control siRNA or siRNA specific for E-cadherin (50 nM) for 6 h. (A) After incubation in fresh nutrient medium for 48 h, E-cadherin was detected by immunofluorescent staining. (B) Protein expression of E-cadherin was measured by western blotting. (C) Expression of TSLP in HaCaT cells was analyzed by ELISA. (D-E) HaCaT cells were treated with TSLP at 20 ng/mL for 24 h, and E-cadherin expression was analyzed by immunofluorescent staining and western blotting (mean $\pm$ SEM, $n = 3$; * $P < 0.05$; *** $P < 0.001$ vs. the corresponding group as indicated in the figure).

dependent manner from 1 μ M to 100 μ M in LAD2 cells [20]. These findings indicated that formononetin inhibited AD. However, the underlying mechanism of this inhibition is not definitively known.

One of the main characteristics of AD is overexpression of Th2 cytokines such as IL-4, IL-5, IL-9, and IL-13. Previous studies have reported that the EC-derived TSLP triggered DC-mediated inflammatory Th2 responses, and that IL-33 activated ILC2s to drive type 2 inflammation, suggesting they play a dominant role in the initial phase of ADs. In the present study, we showed that formononetin decreased expressions of TSLP and IL-33 during the initial stage of the ACD mouse model and in HaCaT cells, indicating that downregulation of TSLP and IL-33 might be the underlying mechanism of the effects of formononetin on AD.

Epithelial barrier dysfunction has been recognized as a critical factor in the initiation and exacerbation of allergic inflammation. The physical barrier function of the epithelium depends on tight junctions (TJs) and adherens junctions (AJs). AJs are important for the maintenance of epithelial adhesions and are thought to be essential for TJ structure and function, especially the adhesion connections of E-cadherin [15, 21]. Our results extended those of de Boer et al., who

reported that expression of E-cadherin was lower in airway biopsies from asthmatic subjects when compared with normal subjects [13]. Consistent with this observation, we found that E-cadherin was decreased during the initial stage of the ACD model. In HaCaT cells, we found poly I:C- or LPS-induced disruption of E-cadherin did not affect the expression of E-cadherin proteins, which was consistent with the results of Rezaee et al. [22]. Overall, our results suggested that formononetin improved epithelial integrity and increased E-cadherin expression during the initial stage of the ACD model.

The above results indicate that formononetin inhibited TSLP and IL-33 expression and regulated E-cadherin at the same time. This prompted us to examine the possible correlation between TSLP and E-cadherin levels. We found that TSLP increased significantly when E-cadherin was silenced with specific siRNA in HaCaT cells, which was consistent with the report by Heijink [16]. Moreover, the inhibitory effect of formononetin on TSLP was dramatically decreased after silencing of E-cadherin. To further address the function of TSLP in the expression of E-cadherin, we treated HaCaT cells with TSLP at 20 ng/mL. In a previous study, Hu et al. reported that exposure of 16HBE to TSLP at 20 ng/mL resulted in redistribution of E-cadherin [23], but our

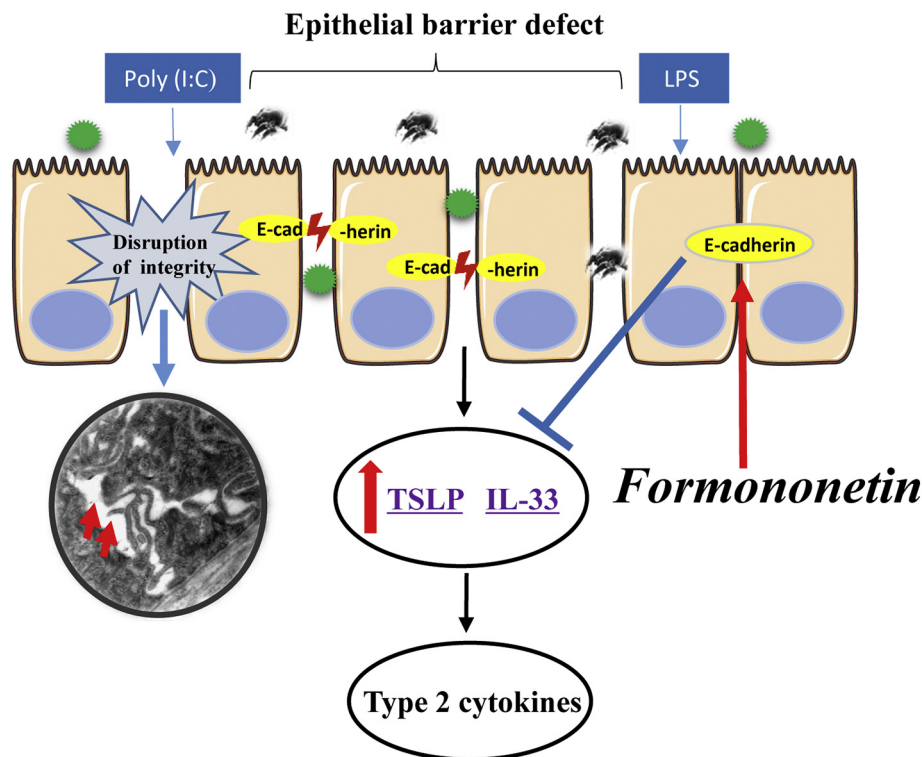


Fig. 7. Formononetin inhibited allergic disease by reducing epithelial-derived initiation via E-cadherin regulation. The mechanism of action of formononetin on allergic disease involves improved barrier function by formononetin, which decreases thymic stromal lymphopoietin expression.

data indicated that TSLP resulted in no detectable change in either delocalization or protein expression of E-cadherin. Our results were consistent with the findings reported by Bahman et al. that TSLP at 50 ng/mL did not disrupt barrier function in 16HBE cells as determined by transepithelial electrical resistance measurements [24]. However, the role of TSLP in the expression of E-cadherin needs additional study.

Our results, which are summarized in Fig. 7, show that formononetin restored epithelial barrier function, which resulted in inhibition of the EC-derived cytokines and TSLP production, and eventually affected allergic inflammation. Most importantly, this study suggested that regulating E-cadherin and EC-derived cytokines might be a therapeutic strategy to effectively treat allergic diseases.

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Conflicts of interest

The authors confirm that there are no conflicts of interest.

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