

Therapeutic Role for TSP-2 Antibody in a Murine Asthma Model

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Keywords

Allergic asthma · Toll-like receptor 2 · Dendritic cells · TSP-2 · Ovalbumin

Abstract

Background: Specific immunotherapy, including agonists for Toll-like receptor 2 (TLR2), have been shown to protect from allergies and to have a high immunomodulatory capacity. **Methods:** A new antibody, TSP-2, reactive against an epitope of the extracellular domain of TLR2, was identified. The effect of the antibody on dendritic cells was assessed by immunohistochemistry, Western blot, and flow cytometric analysis. The effect of TSP-2 in a murine asthma model induced with ovalbumin (OVA) was assessed. The model is a form of airway hyperresponsiveness (AHR) and was analyzed by whole-body plethysmography, the measurement of Th1/Th2 cytokines in bronchial alveolar lavage fluid (BALF) and serum by ELISA, and the CCK-8 assay for lymphocyte proliferation. The effect of TSP-2 on the maturation of bone marrow-derived dendritic cells (BMDCs) was assessed by flow cytometric analysis. **Results:** TSP-2 promoted the maturation of dendritic cells and the proliferation of lymphocyte in vitro and in vivo. The effect of TSP-2 on T helper

1 (Th1)/Th2 cytokine secretion was slightly more powerful than that of Pam₃CSK₄. TSP-2 antibody reduced AHR and OVA-specific IgE levels in allergic asthma. TSP-2 antibody also reduced lung inflammation and decreased leukocyte numbers in an OVA-sensitized and challenged asthma model. TSP-2 antibody increased OVA-stimulated I-A, CD80, CD86, and MHC-II levels on BMDCs. **Conclusions:** This study identifies a novel therapeutic strategy for AHR, which uses antibodies reactive against TLR2. It also provides theoretical evidence for the control of allergic asthma by targeting TLR2.

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Introduction

Asthma is a significant worldwide respiratory disease with ascendant morbidity and mortality. It is estimated that >300 million people currently have asthma, with adolescents being the most vulnerable [1–3]. The World

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Health Organization has designated asthma as a major disease in need of control and prevention due to the significant social, economic, and health costs associated with it. The relatively recent increase in asthma is attributed to the “hygiene hypothesis” of reduced exposure to microorganisms, a result of improved hygiene during infancy and early adulthood [4, 5]. Reduced exposure to microorganisms during these vulnerable periods contributes to the development of lymphocytes with a T helper 2 (Th2) phenotype, i.e., lymphocytes known to promote asthmatic symptoms by producing Th2 cytokines, along with the induced accumulation of leukocytes. Th2 lymphocytes reduce the numbers and function of Th1 lymphocytes, and this reduction in the nonallergic Th1 lymphocyte subset predisposes to allergic-type diseases such as asthma [6, 7].

Dendritic cells (DCs) play an important role in immunity and display Toll-like receptors (TLRs), which are pattern recognition receptors for pathogenic microbes [8–10]. Multiple TLRs participate in allergic inflammation, including TLR2, TLR4, and TLR9. Among these, TLR2 recognizes a wide range of microbial products, and is considered to be a central pattern recognition receptor in innate immunity [11, 12]. Previous studies have demonstrated that activation of TLR2 can initiate both Th1 and Th2 responses, depending on the nature and dose of the microbial ligand. As such, TLR2 could serve as a target for therapeutic treatment of allergic asthma [13–15]. Moreover, the TLR2 agonist Pam₃CSK₄ exhibits unique immunomodulatory properties, characterized by the capacity to induce robust cytokine production by naive CD4⁺ T lymphocytes (both IL-10 and IFN- γ) [16–19]. In a murine asthma model, Pam₃CSK₄ treatment improved allergic hyper-responsiveness within the airways (AHR). Pam₃CSK₄ has been shown to induce a Th1/regulatory T lymphocyte response, and, as such, may be a valid candidate for sublingual adjuvant therapy of allergic disease [20]. TLR1/2, TLR3, TLR4, and TLR7 expressed by human airway smooth-muscle cells can also be activated by Pam₃CSK₄ [21], suggesting that, as a ligand, Pam₃CSK₄ may function via interactions with multiple TLRs. In preliminary experiments, we found that inflammatory reactions during allergic shock and asthma could be inhibited by a specific antibody, TSP-2, with specificity for an extracellular domain of TLR2 (at a concentration of 1.5–3 mg/kg). In this investigation, the effect of TSP-2 on TLR2 activation was evaluated in vivo in a mouse model of asthma, and in vitro for its effects upon DCs.

Materials and Methods

Animals

Female BALB/c mice ($n = 40$), 6–8 weeks of age, weighing 18–20 g, were purchased from the Laboratory Animal Center of Sun Yat-Sen University (Guangzhou, Guangdong, China), and housed under pathogen-free conditions at the animal facility of the School of Basic Medicine of Southern Medical University (Guangzhou, Guangdong, China). The mice were maintained on an ovalbumin (OVA)-free diet. All experimental procedures were approved by the Animal Ethics Committee of Southern Medical University.

Cell Line, Antibodies, and Reagents

Cell line DC2.4 was a gift from Dr. Jia-zhansheng (Infectious Disease Department, Tangdu Hospital of the Fourth Military Medical University, Xi'an, China). RPMI 1640 culture medium, fetal calf serum, and DMSO were purchased from Gibco BRL (Grand Island, NY, USA). Normal rabbit IgG was purchased from Dingguo Biotechnology (Beijing, China). For DC culture, we used the recombinant human cytokine interleukin-4 (IL-4) and GM-CSF, both from Peprotech Inc. (Rocky Hill, NJ, USA). Pam₃CSK₄ was obtained from Tocris Bioscience (Bristol, UK). For flow cytometry, the following antibodies were used: FITC-conjugated anti-mouse CD11c and PE-conjugated anti-mouse MHC class II (HLA-DR), anti-mouse I-A, anti-mouse CD80, and anti-mouse CD86 (all from Pharmingen, San Diego, CA, USA).

Preparation of Antibody TSP-2

We used the online prediction of antigenic peptide software to analyze and predict the sequence of mouse TLR2 (mTLR2) epitopes, synthesized the prevalent B lymphocyte epitope peptide in vitro, and produced a rabbit polyclonal antibody reactive against a single epitope of mTLR2. Antibodies were affinity-purified, and the purified antibody was identified as TSP-2.

Sensitization and Aerosol Challenge with OVA and Pharmacological Intervention

As previously described [22], at the age of 6–7 weeks, mice were sensitized via 2 intraperitoneal injections of 20 μ g OVA (grade VI; Sigma-Aldrich, St Louis, MO, USA) emulsified with 50 μ g alum (Sigma-Aldrich) in PBS and a total volume of 100 μ L. Challenge was on days 0 and 14. Control mice received a saline injection rather than the OVA/alum solution. On days 21–23, the mice were exposed to aerosol challenge with 1% OVA in PBS for 30 min/day. This group of animals is designated as the asthma group. The aerosol challenge was performed in a Plexiglas exposure chamber coupled to a jet nebulizer (Pari IS-2 Jet Nebulizer; PARI Respiratory Equipment, Richmond, VA, USA; particle size 2–3 μ m) driven by compressed air at a flow rate of 6 L/min. In a similar manner, control mice inhaled PBS only.

The control and asthma groups were intravenously injected with saline, and the TSP-2- and IgG-posttreated groups were intravenously injected with TSP-2 and rabbit IgG, respectively (both 2.5 mg/kg, 100 μ L/mouse, 30 min after the aerosol challenge). The TSP-2- and IgG-pretreated groups were intravenously injected via the tail with antibody TSP-2 and rabbit IgG, respectively (both 2.5 mg/kg, 100 μ L per mouse, 2 h prior to the aerosol challenge). Pam₃CSK₄ (InvivoGen, San Diego, CA, USA) was stomach-administered (15 mg/mouse, 2 h after each OVA challenge).

Cell-Counting Kit-8 Assay for Proliferation of Lymphocytes

Cell proliferation was assessed with a cell-counting kit (CCK)-8 assay (Signalway Antibody Co., Maryland, TX, USA) as previously described [23]. Before the assay, spleen lymphocytes from each experimental group were freshly isolated and seeded in 96-well plates at a density of 3×10^4 cells/100 μ L, and then cultured for 24, 48, and 72 h. CCK-8 solution (10 μ L) was added to each well of the plate. After 4 h of incubation at 37°C, the plates were analyzed with a Synergy H1 microplate reader (BioTek Instruments, Bradenton, FL, USA) at 450 nm. All values are reported as the mean $\pm$ standard error of the mean for at least 3 wells and at least 3 separate experiments.

Cell Immunohistochemistry and Immunofluorescence

As described previously for immunochemistry [24], DC2.4 cells (1×10^5 /mL) were cultured on coverslips in 6-well plates for 2 days. The coverslip-attached cells were rinsed gently with PBS, fixed with 4% paraformaldehyde in PBS (pH 7.4 for 15 min), rinsed gently with PBS, and blocked for 10 min with 0.5% BSA in PBS. They were then incubated at 37°C for 40 min in the following manner: DC2.4 + PBS, DC2.4 + rabbit IgG, DC2.4 + Pam₃CSK₄, DC2.4 + TSP-2. The cells were rinsed again with PBS and each of the 4 groups was incubated with secondary antibody conjugated with HRP at 37°C for 40 min, rinsed with PBS, stained with diaminobenzidine for 15 min, hematoxylin for 1 min, rinsed with PBS, dried, and evaluated by light microscopy.

For immunofluorescence analysis, DC2.4 cells were cultured as described above, at a concentration of 1×10^6 /mL, and then incubated with TSP-2, rabbit IgG, PBS, or Pam₃CSK₄ at 4°C for 18 h. Each cell-coated coverslip was rinsed gently with cold PBS, fixed with 4% paraformaldehyde in PBS (pH 7.4 for 15 min at room temperature), blocked with 5% goat serum for 40 min at 37°C, rinsed with PBS, incubated with primary antibody (T2.5, 1 mg/mL) for 40 min at 37°C, rinsed with PBS, incubated with secondary antibody conjugated with FITC for 40 min at 37°C, rinsed with PBS, stained with DAPI at room temperature for 2 min, rinsed with PBS, dried, and then evaluated for the expression of TLR2 by confocal microscopy.

Culture and Generation of Bone Marrow-Derived Dendritic Cells

Bone marrow-derived DCs (BMDCs) were generated from OVA-immunized BALB/c mice treated with PBS, TSP-2, Pam₃CSK₄, or rabbit IgG, and cultured as described previously [25, 26]. The cells were then cultured with recombinant human IL-4 and GM-CSF for 5 days. Flow cytometry demonstrated that $\geq 85\%$ of the cells were CD11c-positive.

Measurement of AHR

Twenty-four hours after the final OVA aerosol challenge, AHR was assessed in conscious and unrestrained mice by whole-body plethysmography as described previously [27]. Each mouse was placed in a plastic chamber and exposed to aerosolized normal saline for 3 min, followed by increasing concentrations of aerosolized methacholine (2.5, 5, 10, and 20 mg/mL; Sigma-Aldrich), each administered for 3 min. Ten seconds after each aerosol exposure, 3-min enhanced pause (Penh) readings were calculated, based on the mean pressure generated in the plethysmography chamber during inspiration and expiration, with the time of each phase recorded and averaged.

Bronchoalveolar Lavage and Leukocyte Cell Counts

Twenty-four hours after the final OVA aerosol challenge, the mice were anaesthetized using 1% pentobarbital sodium. Mouse tracheas were cannulated and lungs were washed twice with 1 mL of sterile PBS to collect BALF as described previously [28]. BALF was centrifuged immediately and the cell-free fluid stored at -80°C for later cytokine analysis. Cell pellets were resuspended in 1 mL of 1% BSA/PBS solution for cell number determination. The number of nucleated cells in each group ($n = 6$) was recorded as leukocytes in BALF.

Lung Histology Assay

For histopathologic assessment, the lungs were fixed overnight in formalin, embedded in paraffin, sectioned to 4 μ m, and stained with hematoxylin and eosin (HE); the degree of lung inflammation was evaluated as described previously [28]. In brief, lung lesions, including alveolar septal infiltrates, perivascular infiltrates, and parabronchial infiltrates were subjectively graded on a numeric scale of 1–4, corresponding to minimal, mild, moderate, and marked severity, by 2 independent pathologists both blinded to the treatments. Overall histopathologic scores were generated from the average of the individual lesion scores for each mouse.

ELISA for the Detection of Th1/Th2 Cytokines in BALF

The concentrations of IL-4, INF- γ , IL-12, and IL-13 in the BALF of each group were measured by ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). All assays were performed in triplicate. The concentration of each cytokine was calculated from a standard curve.

Flow Cytometric Analysis

Cultured BMDCs were treated with PBS, TSP-2, rabbit IgG, or Pam₃CSK₄. Single-cell suspensions were prepared and analyzed using multicolor flow cytometry (Becton Dickinson & Co., Mountain View, CA, USA) as described previously [22, 29]. Cells were suspended in 100 μ L PBS, containing 0.1% sodium azide, Fc block (BD Pharmingen, San Diego, CA, USA), and 1 μ g of various directly conjugated monoclonal antibodies, namely FITC anti-mouse CD11c, PE-anti mouse CD80, and PE-anti mouse CD86. After 30 min of incubation on ice, cells were washed and then analyzed using flow cytometry, gating out the majority of nonviable cells based on low forward-angle light scatter.

Western Blot Analysis

Protein samples were extracted from lung tissues and BALF cell pellets. Cells and tissues were lysed with a 2% solution of SDS, 2 M urea, 10% glycerol, 10 mM Tris-HCl (pH 6.8), 10 mM DTT, and 1 mM PMSF. Proteins were separated using 10% SDS-polyacrylamide gel electrophoresis. After electrophoresis, the proteins were transferred to membranes (Bio-Rad Laboratories, Berkeley, CA, USA) by the wet-transfer method. Each membrane was blocked with TBST (100 mM Tris-HCl pH 7.5, 150 mM NaCl, and 0.05% Tween20) with 5% nonfat dried milk for 1 h at room temperature, and then incubated with primary antibodies overnight on a shaker at 4°C. The appropriate HRP-coupled secondary antibody was then added, and incubation for 1 h took place at room temperature. The membranes were treated with enhanced chemiluminescence Western blot detection reagent (ECL Kit, Amersham Biosciences, Piscataway, NJ, USA), and specific antibody binding was detected by chemiluminescence. β -Actin served as a protein loading control.

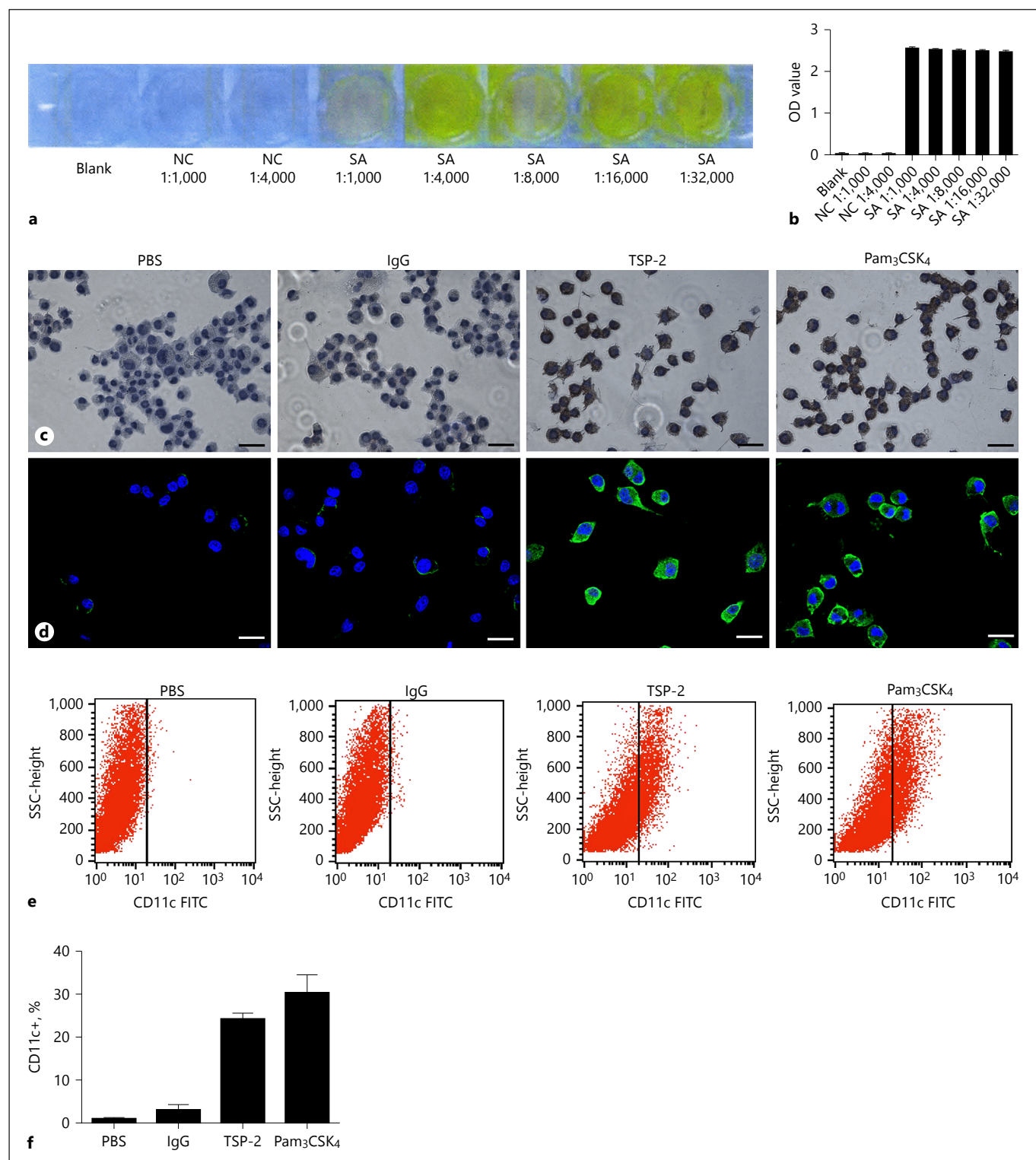


Fig. 1. **a** Photo of ELISA test of antibody TSP-2 at different concentrations. **b** ELISA optical density values at different concentrations of TSP-2 antibody. **c** Immunohistochemistry analysis of DC2.4 with different treatments. Scale bar, 20 μ m. **d** Immunofluorescence analysis of DC2.4 with different treatments. Scale bar, 10

μ m. **e** CD11c flow cytometric analysis of BMDCs with different treatments. Representative data from 1 of 3 replicate experiments are shown. **f** Quantitative analysis of 3 replicate experiments. The results are expressed as the mean $\pm$ SD. * $p < 0.05$, versus control group.

Statistical Analysis

Normally distributed data are presented as means $\pm$ standard deviation (SD) for group comparisons. Nonparametric (nonnormally distributed) data are expressed as medians with interquartile range (25–75%), and were compared using the Wilcoxon rank sum test between groups. All data are expressed as the mean $\pm$ standard error of the mean. For comparisons between groups, the Mann-Whitney U and Kruskal-Wallis tests were used. Statistical significance was defined as $p < 0.05$ or $p < 0.01$.

Results

TSP-2 Antibody Binds TLR2 on Mouse DC2.4 Cells and Promotes DC Maturation

Mouse TLR2 extracellular, single-epitope specific antibody, TSP-2, was prepared as described in Materials and Methods. The purification of TSP-2 antibody was evaluated (Fig. 1a, b) and, based on the results, we concluded that the TSP-2 antibody was successfully made.

Immunohistochemistry was used to assess the specificity of TSP-2 for TLR2 on the murine DC line, DC2.4. TSP-2- and Pam₃CSK₄-treated DC2.4 cells showed positive results (Fig. 1c). PBS and rabbit IgG did not. The activation status of DC2.4 cells pretreated with TSP-2, rabbit IgG, PBS, or Pam₃CSK₄ was assessed by immunofluorescence (Figure 1d). These results were confirmed by flow cytometry of the cultured BMDCs (Fig. 1e, f), in which TSP-2 antibody and Pam₃CSK₄ were shown to promote DC maturation. These data demonstrate that TSP-2 is reactive with murine TLR2 and that TSP-2 promotes DC maturation.

Effect of TSP-2 Antibody on Lymphocyte Proliferation

The CCK-8 assay was used to assess the effect of TSP-2 on lymphocyte proliferation. Significant differences in lymphocyte proliferation across the PBS, TSP-2, rabbit IgG, and Pam₃CSK₄ groups were noted at 24, 48, and 72 h of culture (Fig. 2). TSP-2 and Pam₃CSK₄ promoted greater lymphocyte proliferation than PBS or rabbit IgG ($p < 0.05$). No significant difference was observed for any group based on the period of time in culture. TSP-2 promoted lymphocyte proliferation but rabbit IgG did not.

Effect of TSP-2 Antibody in the OVA-Induced Mouse Model of Asthma

To evaluate the effect of TSP-2 antibody, BALB/c mice were separated into 7 treatment groups: control, asthma, TSP-2 pretreated, TSP-2 posttreated, rabbit IgG pretreated, rabbit IgG posttreated, and Pam₃CSK₄ (each group: $n > 3$). Mice were treated as described in Materials and

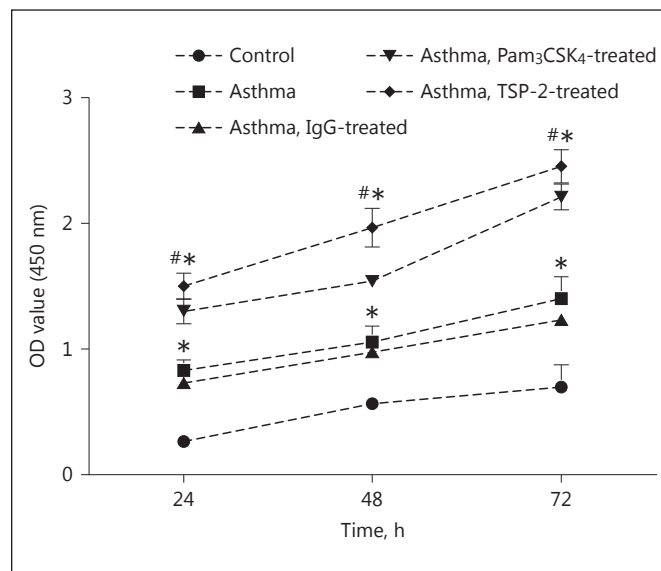


Fig. 2. CCK-8 proliferation assay. Data are expressed as the mean $\pm$ SD. * $p < 0.05$, versus control group; # $p < 0.05$, versus asthma and IgG control groups; $n = 6$.

Methods, and the protocol for OVA-induced allergic asthma is depicted in Figure 3a. AHR for each group is shown in Figure 3b. At 20 mg/mL methacholine, the Penh value for the asthma group was the highest. The mice in the TSP-2 pre- and posttreated groups and in the Pam₃CSK₄-treated group had lower levels than the asthma group. OVA-specific IgE levels in serum were assessed for each group. The IgE level in the asthma group was the highest. The rabbit IgG pre- or posttreated groups had higher levels than the TSP-2 pre- and posttreated groups and the Pam₃CSK₄-treated group. The TSP-2 pre- or posttreated groups had IgE levels nearly equivalent to those in the Pam₃CSK₄ group (Fig. 3c). Inflammatory responses were assessed in situ by the qualitative histologic evaluation of lung tissue sections, and assessed ex vivo by leukocyte counts of cells retrieved from BALF. Histologic analysis of lung tissue sections revealed negligible inflammation in the saline-treated mice. Prominent inflammatory cell influx, increased goblet cells and mucus production, smooth-muscle hyperplasia, and fibrosis were observed in the OVA-sensitized and challenged groups. The IgG pre- or posttreated groups both showed a similar inflammatory infiltrate to that observed in the asthma group (Fig. 3d). Importantly, a modest inflammatory infiltrate was present in the TSP-2 pre- or posttreated groups. The Pam₃CSK₄-treated group had an inflammatory reaction comparable to that in the TSP-2-treated

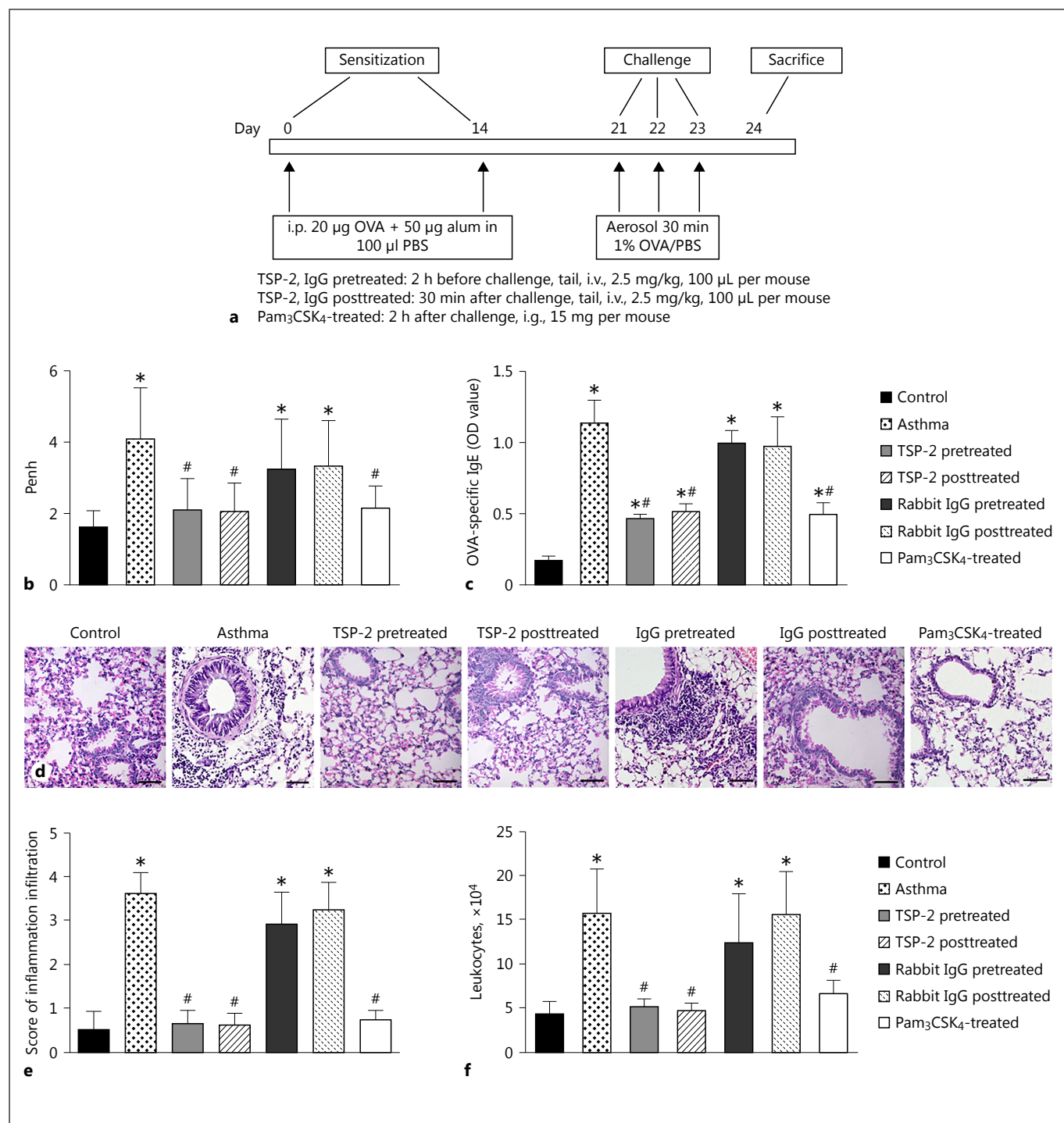


Fig. 3. **a** Time-line representation of the asthma model with pharmacological intervention. **b** Measurement of airway hyperresponsiveness: Penh value of each group at the methacholine concentration of 20 mg/mL. The results are expressed as mean ± SD. * $p < 0.05$, versus control group; # $p < 0.05$, versus asthma and IgG control groups; $n = 6$. **c** OVA-specific IgE antibody levels in plasma of each group as assessed by ELISA. **d** Assessment of inflammatory cell infiltrate. Representative lung tissue sections from mice

treated with saline, OVA, TSP-2 (pre- and posttreated), rabbit IgG (pre- and posttreated), and Pam₃CSK₄. HE. Scale bar, 50 µm. **e** Morphometric analysis of inflammatory infiltrate for each group. **f** Quantitative analysis of leukocytes in bronchial alveolar lavage fluid of each group. The results are expressed as the mean ± SD. * $p < 0.05$, versus control group; # $p < 0.05$, versus asthma and IgG control groups; $n = 6$.

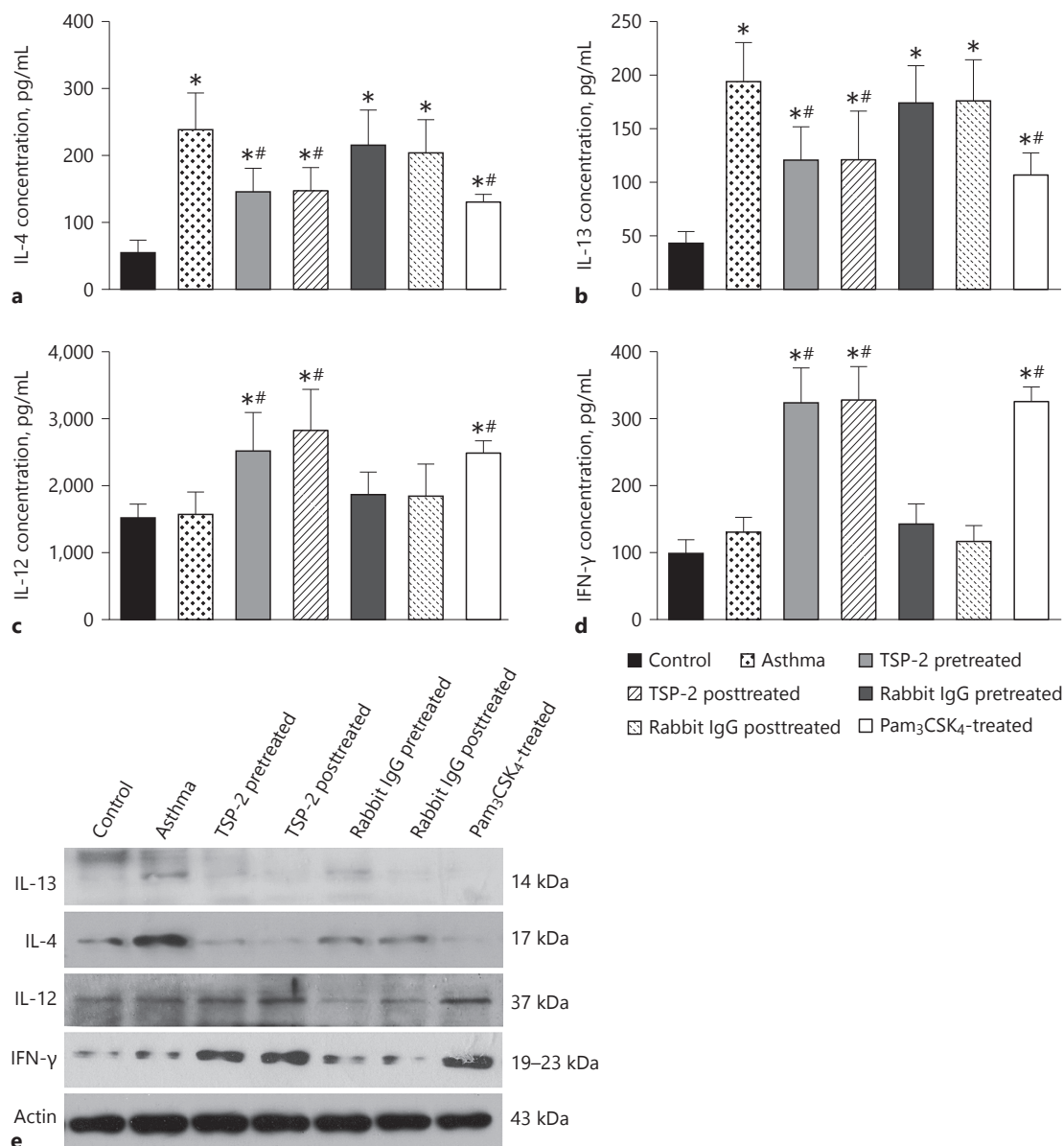


Fig. 4. ELISA analysis of cytokine levels in bronchial alveolar lavage fluid (BALF) of each group. **a** IL-4 levels in BALF. **b** IL-13 levels in BALF. **c** IL-12 levels in BALF. **d** INF-γ levels in BALF. The results are expressed as mean $\pm$ SD. * $p < 0.05$, versus control group; # $p < 0.05$, versus asthma and control IgG groups. **e** Western blot analysis of lung tissues from each group. β -Actin was used as an internal reference.

group. Morphometric analysis is shown in Figure 3e. A difference was exhibited in the numbers of leukocytes in BALF (Fig. 3f).

In conclusion, these data show that TSP-2 antibody can reduce AHR and OVA-specific IgE levels in allergic

asthma. TSP-2 antibody reduced lung inflammation and decreased leukocyte numbers in an OVA-sensitized and challenged asthma model. Furthermore, by comparison, the TLR2 agonist Pam₃CSK₄ was similar in potency to the TSP-2 antibody.

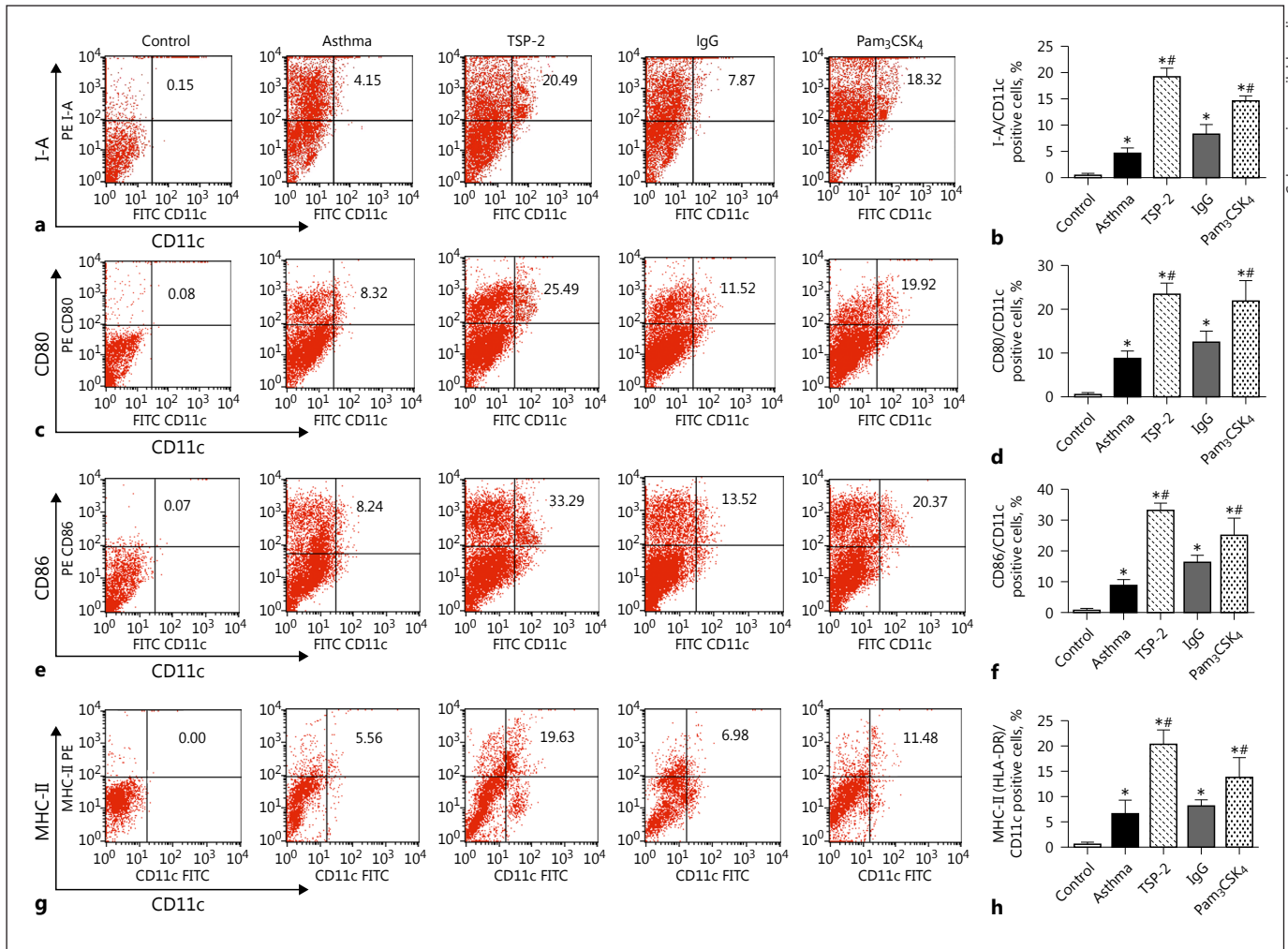


Fig. 5. Expression levels of I-A, CD80, CD86, and MHC-II in CD11c+ BMDCs from each group as determined using flow cytometry. In each group, representative data from 1 of 4 replicate experiments are shown. **a** Expression levels of I-A on CD11c+ BMDC expression in each group. **b** Corresponding quantitative analysis. **c** Expression levels of CD80 in CD11c+ BMDCs from each group. **d** Corresponding quantitative analysis. **e** Expression

levels of CD86 in CD11c+ BMDCs from each group. **f** Corresponding quantitative analysis. **g** Expression levels of MHC-II in CD11c+ BMDCs from each group. **h** Corresponding quantitative analysis. Data are expressed as the mean $\pm$ SD ($n = 4$). * $p < 0.05$, versus control group; # $p < 0.05$, versus asthma and control IgG groups.

TSP-2 Antibody Mediated Th2 and Th1 Cytokine Secretion

To determine whether TSP-2 antibody influences cytokine levels in BALF, IL-4, INF- γ , IL-13, and IL-12 were measured by ELISA 24 h after the final OVA challenge. With IL-4 (Fig. 4a) and IL-13 (Fig. 4b), the OVA-challenged group had a significant increase in BALF cytokine levels when compared to the control group ($p < 0.01$); the IgG pre- and posttreated groups also showed an increase, but it was less than in the OVA group. Remarkably, cytokine levels in TSP-2 pre- and posttreated groups were sig-

nificantly suppressed and similar to in the Pam₃CSK₄-treated group. As for IL-12 (Fig. 4c) and INF- γ (Fig. 4d), OVA challenge triggered a significant decrease in cytokine levels in the BALF compared with the control group ($p < 0.05$). In OVA-sensitized and challenged mice, TSP-2 pre- or posttreated, the rise in these cytokine levels was significant when compared to the OVA-challenged group, and similar to in the Pam₃CSK₄ group. To confirm the cytokine levels in BALF, we analyzed the protein levels in the lungs of each group. The protein levels of IL-4 and IL-13 were similar and the trends were the same as

was determined by ELISA (Fig. 4e). Likewise, the levels of IL-12 and INF- γ were similar.

All in all, TSP-2 influenced Th1/Th2 cytokine levels in an OVA-sensitized and challenged asthma model. The effect of TSP-2 on Th1/Th2 cytokine secretion was slightly more powerful than that of Pam₃CSK₄.

Effect of TSP-2 Antibody on Maturation of BMDCs in Asthmatic Mice

I-A, CD80, CD86, and MHC-II are important surface markers highly expressed in mature DCs. So we assessed the effect of TSP-2 on the maturation of BMDCs. The levels of I-A on BMDCs were found to be downregulated in the asthma and IgG control groups compared to the PBS-treated group (Fig. 5a, b). The Pam₃CSK₄-treated group showed a significantly increased level of I-A when compared to the asthma and IgG groups. TSP-2-treated DCs had a level of I-A similar to the Pam₃CSK₄ group. Consistent with this, the levels of CD80 (Fig. 5c, d), CD86 (Fig. 5e, f), and MHC-II (Fig. 5g, h) on the BMDCs were similar to the trend in I-A levels. The asthma and IgG groups showed decreased levels, while the TSP-2-treated group showed a marked increase similar to the Pam₃CSK₄-treated group.

Therefore, treatment with TSP-2 antibody increased the levels of OVA-stimulated I-A, CD80, CD86, and MHC-II on BMDCs. The effect of TSP-2 on DC maturation was similar to that of the TLR2 agonist Pam₃CSK₄.

Discussion

Allergic asthma is a type I immediate-hypersensitivity reaction, characterized by an augmented Th2 response and an attenuated Th1 response [6, 30, 31]. Since abundant Th2 cytokines contribute to disease, therapeutic treatment that reduces the Th2 response and increases the Th1 response may be beneficial. Previous work has demonstrated that some Th1 cytokines such as IL-12 and INF- γ can reverse AHR and inflammation in mouse models of Th2-driven allergic inflammatory disease [32, 33]. In human asthma, eosinophils are decreased in blood and sputum with IL-12 treatment, without an obvious effect on AHR [34].

Pam₃CSK₄, a TLR1 and TLR2 ligand, is a synthetic tri-palmitoylated-lipohexapeptide analog of the immunologically active N-terminal portion of bacterial lipoprotein. It activates monocytes and macrophages, and is also a potent activator of proinflammatory transcription factor NF- κ B [20, 21, 35–37]. In our study of an OVA-in-

duced allergic asthma model, animals treated with the antibody TSP-2 had improved cytokine outcomes when compared to animals treated with PBS. Varying doses of TSP-2 (0.5, 1, 3, and 5 mg) were initially assessed. A dose of 3 mg of TSP-2 exerted an effect similar to that from Pam₃CSK₄. Lung inflammation induced by OVA was partially inhibited by TSP-2. We showed that TSP-2 was more effective and more specific than Pam₃CSK₄ in alleviating OVA-induced asthma. TSP-2 may be a therapeutic approach for the treatment of allergic asthma. Since an abnormal immunological reaction is a common clinical manifestation of asthma, it is most important to prevent and block the abnormality with new therapeutic approaches, such as administering TSP-2.

At present, drug treatment and special immunotherapies are employed to treat allergic asthma [38–40]. These have side effects and are not always effective, making treatments that have better safety and fewer side effects essential. The use of the humanized monoclonal antibody omalizumab (Xolair) is an excellent example. It displays high efficiency and safety, but its distribution is narrow and large doses are required. It is also expensive due to a complicated production process, making it less than optimal.

Our results demonstrated that antibody TSP-2 reduced airway methacholine reactivity in an allergic asthma model, decreased the expression of the IL-4 and IL-13 cytokines while increasing the expression of INF- γ and IL-12, reduced allergic asthma lung inflammation, improved the integrity of the bronchial structure, and promoted BMDC activation. Mechanism analysis suggested that the activation of the transcriptional factor NF- κ B may be responsible for these events. Taken together, TSP-2 can induce DC activation and decrease airway inflammation in the lung, which could serve as a therapeutic approach to improve care for the allergic patient.

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Disclosure Statement

The authors have no conflict of interest to declare.

Author Contributions

Kai Li and Cui Lan Yang wrote the main text; Kai Lai, En Ping Huang, and Cui Lan Yang prepared the mouse model and per-

formed the experiments; Kai Li, En Ping Huang, Jin Su, Ping Zhu, and Jun Lin helped prepare the figures and finished the ELISA and WB analysis. Shen Qiu Luo and Cui Lan Yang guided the experiments. All authors reviewed the manuscript.

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