Abstract. *Dryopteris crassirhizoma* (DC) has a wide range of pharmacological effects, including antibacterial, anti-influenza virus, anti-tumor, anti-reverse transcriptase and antioxidant effects. However, the inhibitory effect of DC on allergic inflammatory response remains unclear; therefore, the current study used an experimental ovalbumin (OVA)-induced allergic asthma mouse model and phorbol myristate acetate (PMA) and A23187-stimulated HMC-1 cells to reveal the effects of DC in regulating airway inflammation and its possible mechanism. Allergic asthma was initiated in BALB/c mice via exposure to OVA emulsified in aluminum, on days 1 and 14. Thereafter, the mice were treated with DC or dexamethasone (Dex) orally, before being challenged, from days 15 to 26. Subsequently, the mice were challenged with OVA on days 27, 28 and 29. The results of histological analysis indicated that the administration of DC decreased the number of inflammatory cells in the bronchoalveolar lavage fluid (BALF) and suppressed eosinophilic infiltration, mucus production and collagen deposition in the lung tissue. DC treatment increased the level of T helper type 1 (Th1) cytokines (IL-10 and interferon (IFN)-γ) and decreased the levels Th2 cytokines (IL-4, IL-5 and IL-13) and proinflammatory cytokines (IL-6 and TNF-α). Furthermore, DC treatment inhibited the activation of NF-κB signaling (NF-κB, p-NF-κB, IκB and p-IκB), both in BALF and lung homogenates. Serum levels of total IgE and OVA-specific IgE and IgG1 were significantly lower after DC treatment compared with after OVA treatment. However, the anti-inflammatory effect of OVA-specific IgG2a was higher after DC treatment. In addition, DC treatment attenuated the production of proinflammatory cytokines, including IL-6 and TNF-α, and the activation of NF-κB signaling (NF-κB and p-NF-κB), in PMA and calcium ionophore A23187-stimulated HMC-1 cells. In summary, the current study demonstrated that DC acts as a potent anti-allergic and anti-inflammatory drug by modulating the Th1 and Th2 response and reducing the allergic inflammatory reaction in PMA and A23187-stimulated HMC-1 cells via NF-κB signaling in an OVA-induced allergic asthma model.

Introduction

Allergic asthma is a chronic inflammatory disease of the airway that is characterized by general pathological alteration, severe eosinophilia, lymphocyte infiltration, fibrosis deposition, and mucus overproduction (1). The pathophysiology of asthma is related to a Th1/Th2 cell imbalance in the airways (2), and is therefore becoming a focus for asthma treatment. Th1-associated cytokines, IFN-γ and IL-10, were reported to reduce the asthma symptoms in patients with asthma (3).

Nuclear factor-κB (NF-κB) plays a pivotal role in the production of Th2 cytokines and recruitment of inflammatory cells in the airways of murine asthma models (4,5). Recent
studies have suggested that inhibition of NF-xB can help treat allergic asthma (6,7). NF-xB has also been shown to increase levels of cytokines, such as IL-1β, IL-5, and IL-6, in the airway epithelium (8).

Traditional herbal medicines are generally recognized to be safe and exhibit various therapeutic effects (9). Dryopteris crassirhizoma (DC) is a semi-evergreen pteridophyte that is widely distributed in Japan, Korea, and China (10). Several studies have demonstrated that phloroglucinols from DC have a wide range of pharmacological effects, such as antibacterial (11), anti-reverse transcriptase (12), and antioxidant activity (13), which are mediated by active components, such as phloroglucinol derivatives (albaspidin, aspidin, flavaspidic acids, and dryocassin), triterpenes (acylphloroglucinols), and dimethylflavanones (desmethoxymatteucin, matteucinol, and methoxyamateucin) (11,14-16).

The aim of the present study was to investigate the anti-asthmatic action of DC in vivo. In addition, to elucidate the cellular mechanisms underlying the effects of DC on ovalbumin (OVA)-induced allergic asthma, we performed an in vitro study with human mast cells (HMC)-1, stimulated by A23187 and phorbol myristate acetate (A23187/PMA) treatment.

Materials and methods

Cell culture. HMC-1 cells were generously provided by Professor Hyun-Ja Jeong (Department of Food Science and Technology and Research Institute for Basic Science, Hoseo University, Republic of Korea). HMC-1 cells were grown in Iscove's modified Dulbecco's medium (IMDM), supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 10 µM monothioglycerol, and 10% heat-inactivated FBS at 37°C, in 5% CO₂ and 95% humidity.

Preparation of DC. DC (KFRI-SL-2021) was provided by the Division of Nutrition and Metabolism Research, Korea Food Research Institute. DC used in this study was purchased from the Kyeong-Dong Oriental Pharmacy Market. DC underwent reflux extraction twice in 95% ethanol. The ethanol extract was dried under vacuum in a rotary evaporator. The concentrated extract was lyophilized, yielding a dried powder that was kept at 4°C until needed and dissolved in saline prior to use.

Ultraperformance liquid chromatography-quadrupole-time of flight (UPLC/Q-TOF) mass spectrometry (MS). To identify the chemical constituents of DC, the ethanolic extracts of DC were analyzed using UPLC/Q-TOF MS (Waters Corp.). The extract was injected into an Acquity UPLC BEH C18 column (2.1x100 mm, 1.7 µm; Waters Corp.) at a column temperature of 40°C. The mobile phase consisted of water with 0.1% formic acid and acetonitrile with 0.1% formic acid, at a flow rate of 0.35 ml/min for 9 min. The capillary voltage was set at 3 or 2.5 kV, for positive or negative mode, respectively, while the sample cone voltage was 40 V. The desolvation flow rate was 900 l/h at 400°C and source temperature was set at 100°C. Leucine enkephalin [(M+H)=m/z 556.2771] was used as a reference for lock mass at a frequency of 10 sec. The MS/MS spectra were obtained using collision energy ramps from 20 to 45 eV. Metabolites were identified by UniFi software using various LC/MS databases.

Animals. Pathogen-free 5-week-old male BALB/c mice, weighing approximately 20 g, were purchased from Damool Science. Five mice were housed per cage in a laminar air-flow cabinet, maintained at 23±2°C at a relative humidity of 55±10%, with a 12 h dark/light cycle, throughout the study period. All animal experiments were performed in compliance with the NIH guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committees of Chonbuk National University Laboratory Animal Center (CBNU 2016-37 and CBNU 2019-071).

Mouse allergic asthma model and treatment. In this study, the mice were randomly divided into four groups (n=6 per group), namely control, OVA, DC, and Dex. The first sensitization was performed on day 1 by intraperitoneally injecting 50 µg of OVA (Grade V, Sigma, St. Louis, MO, USA), emulsified in 1 mg of alum (Imject Alum; Pierce), in a total volume of 200 µl. The control group received saline alone (NaCl 0.9%; B. Braun Medical BV, Oss, The Netherlands). On day 14, the second sensitization was performed by intraperitoneally injecting 50 µg of OVA in saline. From days 15 to 26, mice of the DC group and Dex group received DC (20 mg/ml) and Dex (2.5 mg/ml) by oral gavage. Mice belonging to the control and OVA groups received sham saline. On days 27, 28, and 29, the OVA, Dex, and DC groups were challenged with inhalation of ultrasonically nebulized 5% OVA solution in saline, for 20 min. Animals were sacrificed 24 h after day 29, to evaluate for airway inflammation and the production of allergen-specific cytokines (Fig. 1A).

Analysis of bronchoalveolar lavage fluid (BALF) and lung homogenates. The airway lumina of the sacrificed animals were washed using a tracheal cannula with 1 ml of saline twice. The BALF so obtained, was centrifuged and supernatants were stored at -80°C and subjected to ELISA assay using kits. Total and differential cell numbers were counted double-blind, using a hemocytometer. Cytospin cell preparations were made by placing the cells onto glass slides, centrifuging at 4°C for 10 min at 1,000 x g, and staining with Diff-Quik. Lung tissues were homogenized in saline to a concentration of 100 mg/ml with the complete, Mini, EDTA-free Protease Inhibitor (Roche Applied Science) and the debris-free supernatant was used for cytokine measurement.

Histopathological examination of lung tissue. After collecting the BALF, the lobes of the lung were removed for histological examination, fixed in 10% paraformaldehyde, and embedded in paraffin, using standard methods. Hematoxylin and eosin staining to assess the general morphological structure of lung tissue. Periodic acid-Schiff (PAS) staining for visualizing goblet cell hyperplasia. PAS staining was performed on the lung tissue sections to visualize the development of goblet cell hyperplasia. Congo red staining for visualizing eosinophilic infiltration of the nasal mucosa. Eosinophils were morphologically defined by the presence of granules in the cytoplasm and a two-lobed nucleus and counted under a microscope. Masson’s trichrome staining

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was used to reveal the sub‐epithelial deposition of collagen in the lung tissue. Positive trichrome‐stained areas to assess the degree of sub‐epithelial fibrosis.

**Measurement of cytokine levels in BALF and lung homogenates.** For assessing the Th1 response, the level of anti‐inflammatory (Th1‐associated) cytokines, such as IFN‐γ and IL‐10, in BALF and/or lung homogenates was assayed using ELISA kits (R&D Systems), following the manufacturer's instructions.

For assessing the Th2 response, we evaluated the secretion of inflammatory (Th2‐associated) cytokines, such as IL‐4, IL‐5, and IL‐13 and proinflammatory cytokines, including IL‐6, in BALF and lung homogenates using ELISA kits (R&D Systems), as per the manufacturer's instructions.

**Measurement of serum levels of total and OVA‐specific IgE and OVA‐specific IgG1 and IgG2a.** Blood was collected from the orbital venous plexus of anesthetized mice 24 h after the last challenge. The samples were centrifuged (1,000 x g, 10 min, 4˚C) to isolate the serum and stored at -80˚C until further analysis. Thereafter, the serum was separated and levels of total IgE and OVA‐specific IgE, IgG1, and IgG2a were measured in each group using ELISA (Chondrex), following the manufacturer's instruction.

**Measurement of NF‐κB, p‐NF‐κB, IκB, and p‐IκB in BALF and lung tissue.** The expression of NF‐κB p65, IκB, phosphorylation of NF‐κB p65 (p‐NF‐κB p65) and p‐IκB in BALF and lung homogenate were examined by using ELISA kits (eBioscience Inc.), according to the manufacturer's instructions. The optical density was measured in 96‐well plates using an ELISA reader, at 450 nm. We evaluated the activation of NF‐κB p65 and p‐NF‐κB p65 and the nuclear translocalization of NF‐κB in lung tissues, using ProteinSimple capillary immunoassay (Wes) method a gel- and blot‐free method requiring less sample, antibody, and time to run than conventional western blot assays.

**Measurement of TNF‐α and IL‐6 production and NF‐κB and p‐NF‐κB activation in HMC‐1 cells.** HMC‐1 cells were pretreated with various concentrations of DC (0.1, 1.0, and 10 mg/ml) for 30 min and stimulated with 10 µM of A23187 and 200 nM of PMA overnight. The samples were centrifuged (1,000 x g, 10 min, 4˚C) and the supernatants were used to evaluate the concentration of cytokines, including IL‐6 and TNF‐α. The activation of NF‐κB and p‐NF‐κB was quantified using ELISA, according to the manufacturer's instructions (R&D Systems).

**Statistical analysis.** Each experiment was repeated three times with six mice per group. Data are expressed as mean ± SEMs. Statistical comparisons were performed using one‐way ANOVA, followed by Fisher's test. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Effect of DC on infiltration of inflammatory cells in BALF of OVA‐induced asthma mouse model.** To examine the anti‐inflammatory effect of DC on allergic asthma, we used the OVA‐induced allergic asthma mouse model (Fig. 1A). The Cytospin cell preparations showed that the number of inflammatory cells, especially eosinophils, was markedly increased in the OVA‐challenged mice compared with the control group. However, the DC and Dex treatment groups showed few cells—mostly macrophages (Fig. 1B). We measured the number of total and differential cells, including macrophages, neutrophils, and eosinophils, in BALF. The OVA‐challenged group showed a significant increase in the number of total cells and inflammatory cells, including eosinophils and neutrophils, compared with the control group (Fig. 1C). However, treatment with DC markedly reduced the number of total cells and inflammatory cells compared with the OVA‐challenged group.

**Effect of DC on pulmonary inflammation in OVA‐induced allergic asthma mouse model.** To evaluate histological changes after DC treatment, in the OVA‐induced asthma model, the levels of general pathological changes, eosinophil infiltration, mucus production, goblet cell hyperplasia, and bronchial subepithelial fibrosis were examined in the lung tissue. Pulmonary histopathology was found to be normal in the control group. Mice from the OVA‐challenged group exhibited a severe infiltration of inflammatory cells around the respiratory tract and blood vessels (Fig. 2) compared with mice of the DC group, which showed few or no inflammatory cells around the airway, and the difference between the groups was statistically significant. Dex treatment mitigated inflammation around the airway wall (bronchi). Mucus overproduction and goblet cell hyperplasia was observed in the bronchi and eosinophil infiltration increased in the peri‐bronchial epithelium in the OVA group compared with the control group. In contrast, the DC group showed a significant reduction in goblet cell and eosinophil numbers. Masson's trichrome staining revealed prominent collagen deposition and tissue fibrosis around the bronchial epithelia and blood vessels in the OVA group. In contrast, the DC and Dex groups displayed reduced collagen deposition. Histological examination of lung tissues showed similar numbers of eosinophils as that in the BALF. Collectively, these results suggest that cellular responses other than eosinophilic infiltration may play a critical role in the development of allergic inflammation, in the mouse model used in this study.

**Effect of DC on the serum levels of total and OVA‐specific IgE, IgG1, and IgG2a.** To investigate the therapeutic impact of DC on OVA‐specific immune responses, we determined the serum immunoglobulin levels. As shown in Fig. 3, the levels of total and OVA‐specific IgE and IgG1 were markedly increased in the OVA group compared with the control group. However, the DC treatment group demonstrated substantially decreased levels of total and OVA‐specific IgE and IgG1 compared with the OVA group. Moreover, the level of OVA‐specific IgG2a was upregulated by DC treatment. These results indicated that DC might facilitate anti‐allergic activity by suppressing the production of allergic mediators.

**Effect of DC on the levels of Th1 and Th2 cytokines in BALF and lung homogenates.** To elucidate the anti‐allergic mechanism of DC on the Th1/Th2‐mediated allergic response in the OVA‐induced asthma mice models, the levels of secreted...
cytokines in BALF and lung homogenates were examined. First, we examined the secretion of the Th2 cytokines and proinflammatory cytokines, including IL-4, IL-5, IL-6, and IL-13, using ELISA. The OVA group showed increased levels of both in BALF and lung homogenates compared with the control group. However, DC treatment significantly decreased...
the levels of cytokines, including IL-4, IL-5, IL-6, and IL-13. In addition, the levels of anti-inflammatory cytokines (Th1 cytokines), such as IFN-γ and IL-10, were assayed. The DC-treated group showed a significant increase in the levels of IL-10 in BALF and IFN-γ in lung homogenates, and a decrease in IL-10 in lung homogenates and IFN-γ in BALF, as compared to the OVA group; however, no statistically significant differences were observed (Fig. 4). These results were consistent with the reduced infiltration of inflammatory cells in BALF and the lung tissue of DC-treated mice, via modulation of the level of Th1/Th2 cytokines. In this way, DC can reduce bronchial inflammation and immune reaction in patients with asthma.

Effect of DC on the OVA-induced activation of the NF-κB signaling pathway. To understand the inhibitory effect of DC on allergic asthma, activation of NF-κB signaling—that regulates the expression Th2 cytokines—was measured in OVA-challenged mice. The levels of NF-κB p65 and p-NF-κB p65 activation in the lung tissue were assessed via western blot analysis. As presented in Fig. 5A, the activation of NF-κB p65 and p-NF-κB p65 was significantly upregulated in the OVA group compared with the control group. However, this increase was effectively blocked by DC treatment.

Next, we determined whether DC suppressed nuclear translocalization of NF-κB in the lung tissue. As shown in Fig. 5A, the OVA group showed translocalization of p65 into the nucleus. In contrast, DC inhibited translocalization of p65. These findings suggest a significant anti-inflammatory role of DC.

We assessed the levels of total NF-κB and IκB, as markers of NF-κB activation, degradation levels of p-NF-κB, and p-IκB activation. NF-κB, p-NF-κB, IκB, and p-IκB levels were higher in the OVA group compared with the control group, both in BALF and lung homogenates. However, we found that DC treatment downregulated the levels of NF-κB, p-NF-κB, IκB, and p-IκB in BALF and p-NF-κB, IκB, and p-IκB in lung homogenates, compared with the OVA group. DC treatment had a tendency to decrease the levels of NF-κB signaling components in lung homogenates. In addition, the Dex group also showed inhibitory effects on NF-κB signaling (Fig. 5B and C). These findings suggest a potential role of the NF-κB pathway, in the suppression of inflammatory mediators by DC, in allergic asthma.
These data combined with those presented in Fig. 5 suggest that the protective effects of DC on allergic airway inflammation are mediated by downregulating NF-κB activation.

**Effect of DC on TNF-α and IL-6 expression in HMC-1 cells**

Effect of DC on TNF-α and IL-6 expression in HMC-1 cells stimulated by a combination of PMA and A23187. Mast cells play a major role in allergic inflammation. Pro-inflammatory cytokines, such as TNF-α and IL-6, are potent multifunctional cytokines that play an important role in the pathogenesis of allergic disease (17). We examined whether DC could inhibit the expression of TNF-α and IL-6 in HMC-1 cells using ELISA. As shown in Fig. 6A, HMC-1 cells stimulated by a combination of PMA and A23187, showed an increase in TNF-α and IL-6 levels compared with untreated control cells.
Treatment of HMC-I cells with DC significantly attenuated the expression of TNF-α and IL-6 levels, in a dose-dependent manner. Thus, DC treatment showed anti-allergic effect by inhibiting the expression of allergic and inflammatory mediators, in HMC-1 cells stimulated by PMA and A23187.

Effect of DC on infiltration of mast cells in lung tissues of OVA-induced allergic asthma. Activated mast cells play a key role in allergy by releasing numerous mediators, such as cytokines and leukotrienes, via degranulation (18). Therefore, we evaluated local infiltration by mast cells and determined the inhibitory effect of DC on mast cell infiltration in the OVA-induced allergic asthma mouse model. Giemsa staining of the lung tissue was done for determining local infiltration by mast cells and assessing the inhibitory effect of DC on allergic asthma. As shown in Fig. 6B, the number of mast cells in the lung tissue of the OVA-induced allergic asthma mice was significantly higher than that in the control mice (red arrows). In the DC treated mice, OVA-induced infiltration of mast cells was markedly decreased. Thus, these findings indicate that DC treatment can efficiently inhibit inflammatory cells, including eosinophils, goblet cells, and mast cells in lung tissues.

Effect of DC on the activation of NF-κB in HMC-1 cells stimulated by a combination of PMA and A23187. To further investigate whether the NF-κB pathway plays an important role in the DC-mediated regulation of allergic inflammation, we analyzed the activation of NF-κB and p-NF-κB in HMC-1 cells. In HMC-1 cells, NF-κB activation was inhibited dose-dependently by DC treatment compared to PMA and A23187 treatment alone (Fig. 6A). However, stimulation with DC had no effect on the expression of PMA and A23187 induced activation of p-NF-κB (Fig. 6B). Thus, these results further confirmed that the role of DC in mediating anti-allergic and anti-inflammatory effects.

Active components of DC. To investigate the main component present in the ethanolic extract of DC, UPLC/Q-TOF MS was used. The results showed four negative and eight positive modes (Fig. 7), which were identified as follows: Caffeic acid 3-glucoside, chlorogenic acid, isoquercetin, pinellic acid, procyanidin B, quercetagetin dehydrate, asterric acid, skim/ming/protexin, pravastatin, 4-methylumbelliferyl-hexoside, and gabexate/5-[(6,7,8-trimethoxyquinazolin-4-yl) amino] pentan-1-ol.

Discussion

The aim of this study was to investigate the anti-allergic and anti-inflammatory effects of DC using the OVA-induced allergic asthma mouse model. OVA-challenged mice showed an increased number of inflammatory cells in BALF; elevated total IgE, anti-OVA IgG1 and IgE in the serum; increased Th2 cytokines, including IL-4, IL-5, IL-6, and IL-13 in BALF; goblet cell hyperplasia with excessive airway mucus and collagen deposition; eosinophil and mast cell infiltration in lung tissues; activated NF-κB signaling in BALF and lung homogenates; and NF-κB activation in PMA and A23187-stimulated...
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HMC-1 cells. However, DC treatment increased the level of Th1 cytokines and decreased the Th2 cytokines; inhibited NF-κB signaling activation in BALF, lung homogenates, and PMA and A23187-stimulated HMC-1 cells; inhibited total IgE, anti-OVA IgG1, and IgE production in the serum; and lowered the number of inflammatory cells in BALF. The administration of DC also suppressed the infiltration of eosinophils and mast cells, mucus overproduction, and collagen deposition. In addition, DC treatment attenuated TNF-α and IL-6 in PMA and A23187-stimulated HMC-1 cells. Thus, we demonstrated that DC has anti-allergic and anti-inflammatory effects on an OVA-induced asthma mouse model.

Medicinal herbs have been used in traditional medicines in Asian countries, such as Korea and China, since ancient times (19). DC is used as a traditional herbal remedy for various diseases, especially to treat tapeworm infestation, cold, and cancer (20). Isoquercetin was identified as a positive mode in DC. Isoquercetin exhibits a variety of medicinal properties, which include oxidative stress attenuation (21) and anti-inflammatory properties (22). Statins, hydroxymethylglutaryl-coenzyme A reductase inhibitors, currently used as lipid-lowering agents, have pleiotropic anti-inflammatory and immune-modulating effects. The benefits of statins in airway inflammation have been shown in patients with allergic asthma and smoking-related asthma (23). To evaluate the anti-inflammatory and anti-asthmatic effects and possible mechanism of DC, we performed an in vivo study using an OVA-induced asthma mouse model.

Asthma is a multifactorial and complex disease in which repetitive allergen challenge leads to permanent airway
remodeling (24). Various medicinal herbs have been shown to downregulate the secretion of Th2 cytokines, IL-4, IL-5, and IL-13 (25). Indeed, treatment with DC decreased the expression of IL-4, IL-5, IL-6, and IL-13 and enhanced the production of IL-10 and IFN-γ in BALF. A reduction in the expression of IL4, IL-5, and IL-13 by DC, is a key indicator of attenuation of allergic asthma symptoms, indicating inhibition of Th2 dominant responses and regulation of Th1/Th2 immune balance by DC.

Previous studies have shown that NF-κB plays an important role in cytokine production (26). NF-κB signaling pathway has been shown to play important roles in the development of inflammatory diseases, including asthma (27,28). To explore the protective mechanism of DC, the effects of DC on OVA-induced NF-κB activation and in PMA and A23187-stimulated HMC-1 cells were measured. Here, we showed that the DC treated group had significantly inhibited NF-κB activation. These findings indicate that DC inhibited Th2 cytokine production may be related to the suppression of NF-κB activation.

A23187 and PMA induced an increase in cytosolic calcium concentration, leading to mast cell degranulation (29). HMC-1 have been widely used to investigate the beneficial effects of anti-allergic drugs (30). Thus, in the present study, A23187 and PMA stimulated HMC-1 were used to investigate the anti-allergic effects of DC in vitro. Our data showed that DC decreased the production of TNF-α and IL-6 in HMC-1 by suppressing degranulation. Therefore, our results suggest that DC exerts a significant anti-allergic effect by reducing TNF-α and IL-6 production.

In summary, our data demonstrated that DC exerts a pivotal role in OVA-induced airway inflammation, both in vivo and in vitro, by reducing the infiltration of inflammatory cells, particularly eosinophils, mucus overproduction, NF-κB expression, and modulating Th1/Th2 cytokines. Serum levels of total and OVA-specific IgE and IgG1 were significantly lower and OVA-specific IgG2a was higher upon DC treatment compared with OVA treatment. Furthermore, DC treatment inhibited production of inflammatory cytokines, such as TNF-α and IL-6, and NF-κB activation in PMA and A23187-stimulated HMC-1 cells. Collectively, these findings suggest that DC exerts potent anti-asthmatic and anti-inflammatory effects in the treatment of allergic asthma.
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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

OHC designed the experiments. CHP, TTB, TVN and YF performed the experiments. CHP and HSS analyzed the data and performed the biological analysis. CHS and HTK collected and analyzed the data. CHP, YF and TVN sacrificed the mice and performed the ELISA assay. DS took samples and performed western blot analysis. SYL also checked the quality of DC and performed western blot analysis. The manuscript was written by CHP and OHC. All authors read and approved the manuscript.

All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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