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# The therapeutic effect of 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol on chemically induced atopic dermatitis

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**Atopic dermatitis (AD) is the most common chronic inflammatory skin disease worldwide. However, it is still urgent to develop innovative treatments that can effectively manage refractory patients with unpredictable chronic disease courses. In this study, we evaluated the therapeutic efficacy of 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG) as a novel agent for AD treatment using a human-like mouse model of AD. PLAG significantly improved 2,4-dinitrochlorobenzene (DNCB)-induced AD skin lesions compared to those in mice treated with DNCB alone. PLAG substantially modulated the AD-induced infiltration of monocytes and eosinophils into skin lesions and humoral systemic responses involving immunoglobulin E (IgE), interleukin (IL)-4, and IL-13, restoring them to a normal state. Next, we compared the therapeutic efficacy of PLAG and abrocitinib for severe AD treatment. PLAG exhibited a significant therapeutic effect on AD skin lesions compared to abrocitinib. Unlike abrocitinib, PLAG significantly reduced AD-induced eosinophil infiltration to a level similar to that observed in untreated negative controls. Notably, both PLAG and abrocitinib downregulated IgE, IL-4, and IL-13 in a similar pattern, reaching levels similar to those in the untreated negative controls. Our findings strongly suggest that PLAG may serve as a therapeutic agent for AD with an efficacy comparable to that of abrocitinib.**

**Keywords** Atopic dermatitis, PLAG, Abrocitinib, EASI score, IgE, IL-4, IL-13

**Abbreviations**

| | |
|---|---|
| AD | Atopic dermatitis |
| DNCB | 2,4-Dinitrochlorobenzene |
| EASI | Eczema area and severity index |
| EMA | European Medicines Agency |
| EU | European Union |
| FDA | Food and Drug Administration |
| GBD | Global Burden of Disease |
| IFN-γ | Interferon gamma |
| IgE | Immunoglobulin E |
| IL | Interleukin |
| JAK | Janus kinase |
| LTE | Long-term efficacy |
| PBS | Phosphate-buffered saline |
| PDE4 | Phosphodiesterase 4 |
| PLAG | 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol |
| STAT | Signal transducer and activator of transcription |
| TARC | Thymus and activation-regulated chemokine |
| TB | Tuberculosis |
| TH | T helper |
| TYK | Tyrosine kinase |

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Atopic dermatitis (AD) is the most common chronic inflammatory skin disease[1,2]. Approximately 80% of cases typically begin in infancy or childhood, while the remainder manifest in adulthood[3,4]. According to the Global Burden of Disease (GBD) consortium, at least 171 million people suffered from AD in 2019, which corresponds to 2.23% of the global population when accounting for age-standardized prevalence[5]. AD is characterized by sensitive, dry skin and localized or scattered eczematous lesions that are often accompanied by severe itching[6]. A recent study reported that the average annualized healthcare cost per patient with moderate to severe AD undergoing clinical treatment was $20,722[7].

AD is caused by complex interactions, including environmental factors, genetic predisposition, and abnormal immune responses[8]. The primary treatment approach for AD typically involves local anti-inflammatory treatments, such as topical therapies including corticosteroids, calcineurin inhibitors, and the phosphodiesterase 4 (PDE4) inhibitor crisaborole. In cases of severe AD, therapeutic guidelines recommend systemic therapies, including immunosuppressive agents such as cyclosporin A, methotrexate, azathioprine, and mycophenolate mofetil.

A key immunological feature of AD is the imbalance between T helper 1 (Th1) and T helper 2 (Th2) cells[9]. Interleukin (IL)-4 and IL-13 are classified as Th2 cytokines[10]. IL-4 and IL-13 secreted by immune cells are the major triggers of AD. They mediate the immune response, sustain inflammation, and exacerbate dysfunction in the skin barrier[11–13]. The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved the use of the anti-IL-4/IL-13 antibody dupilumab and the anti-IL-13 antibody tralokinumab as innovative therapeutic approaches for the treatment of moderate to severe AD. These biological therapies specifically target immune signaling and related cytokines, or receptors. The Janus kinase (JAK)1/3 pathway regulates Th1 differentiation and IFN-γ production, while JAK1/2 plays a central role in Th2 differentiation and the release of associated cytokines[14]. Small-molecule JAK inhibitors, such as abrocitinib and upadacitinib, interact with multiple signaling pathways associated with inflammatory cytokine receptors and the immune system, thus providing diverse approaches for mechanistic studies[15]. Despite recent advances, the currently available treatment regimens for patients with AD with various disease progression patterns are limited. Therefore, it is crucial to understand the underlying mechanisms of sequential immune responses from the acute to the chronic disease stages and to develop innovative treatments capable of effectively managing refractory patients experiencing an unpredictable chronic disease course.

1-Palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG), a synthetic small molecule derived from the antlers of sika deer, has demonstrated potential as a Th2 modulating factor that regulates IL-4 expression via the STAT6 (signal transducers and activators of transcription) pathway[16]. In this study, we evaluated the therapeutic effects of PLAG as a novel agent for the treatment of AD using a chemically induced AD mouse model. Further, we compared the efficacy of PLAG with that of abrocitinib, the most recently approved JAK1 inhibitor.

## Results

### DNCB induces a human-like mouse model of AD

Based on the potential of PLAG as a Th2 modulating factor, we investigated its therapeutic efficacy as a novel treatment for AD[16]. Our first objective was to establish a human-like mouse model of AD using DNCB, which is commonly used to induce chronic AD-like skin lesions[17]. The repeated application of DNCB to mouse skin results in different phases of sensitization and challenge, depending on the dosage used. A human-like AD mouse model was successfully established by applying 1% DNCB during the sensitization phase and 0.4% DNCB during the induction phase to the dorsal skin of mice[18–21]. Severe erythema, erosion, and excoriation were observed in the dorsal skin of the DNCB-treated mice (Supplementary Fig. S1b and c).

Next, a dose range-determination trial was performed to determine the optimal dose for obtaining the desired therapeutic effects in the mouse model. Based on our findings, the effective doses ranged from 150 to 400 mg/kg (Supplementary Fig. S1a)[16,22]. The primary focus was to determine the dose that yielded the most significant improvement in AD symptoms assessed through phenotypic, histopathological, and biological indicators, including EASI scores (Supplementary Fig. S1b-h). It is well established that AD-affected skin exhibits decreased cornification and defects in the epithelial barrier, such as epithelial thickening due to hyperkeratosis and hyperplasia, along with immune dysregulation[23]. H&E staining was performed on the epidermal skin to evaluate histopathological changes (Supplementary Fig. S1c and d). Eosinophils and mast cells are important granulocytes that play a central role in regulating the release of Th2-related cytokines during allergic AD skin inflammation[24,25]. These cells interact with other cells in the skin to regulate both innate and adaptive immune responses. Therefore, eosinophils and mast cells in the skin lesions were quantified using CR and TB staining, and cells in the bloodstream were measured using a CBC analyzer (Supplementary Fig. S1e-g). Additionally, ELISA was used to determine serum IL-4 and IL-13 levels, as they are key regulators of Th1/Th2 imbalance and serum IgE upregulation in chronic AD (Supplementary Fig. S1h)[11–13,26].

In the dose-range determination study, the dorsal skin of mice treated with DNCB alone exhibited moderate to severe human-like AD symptoms and a thicker epithelium compared to that of mice in the negative control group. However, for all PLAG-treated groups of mice (150–400 mg/kg), the dorsal skin showed a significant decrease in EASI score and epidermal thickness compared to that in mice treated with DNCB alone. Moreover, the numbers of eosinophils and mast cells in the lesions and blood of DNCB-treated mice treated with PLAG were low, and the serum levels of IgE, IL-4, and IL-13 were similarly low (Supplementary Fig. S1b-h). Based on these results, PLAG concentrations of 62.5, 125, and 250 mg/kg were selected for the efficacy studies.

### PLAG alleviates AD symptoms by balancing the Th1/Th2 immune responses

In the efficacy study, DNCB-induced AD symptoms were significantly improved in all PLAG-treated mice (62.5–250 mg/kg), as determined by the EASI score and epidermal thickness measurements (Fig. 1a-c). Both

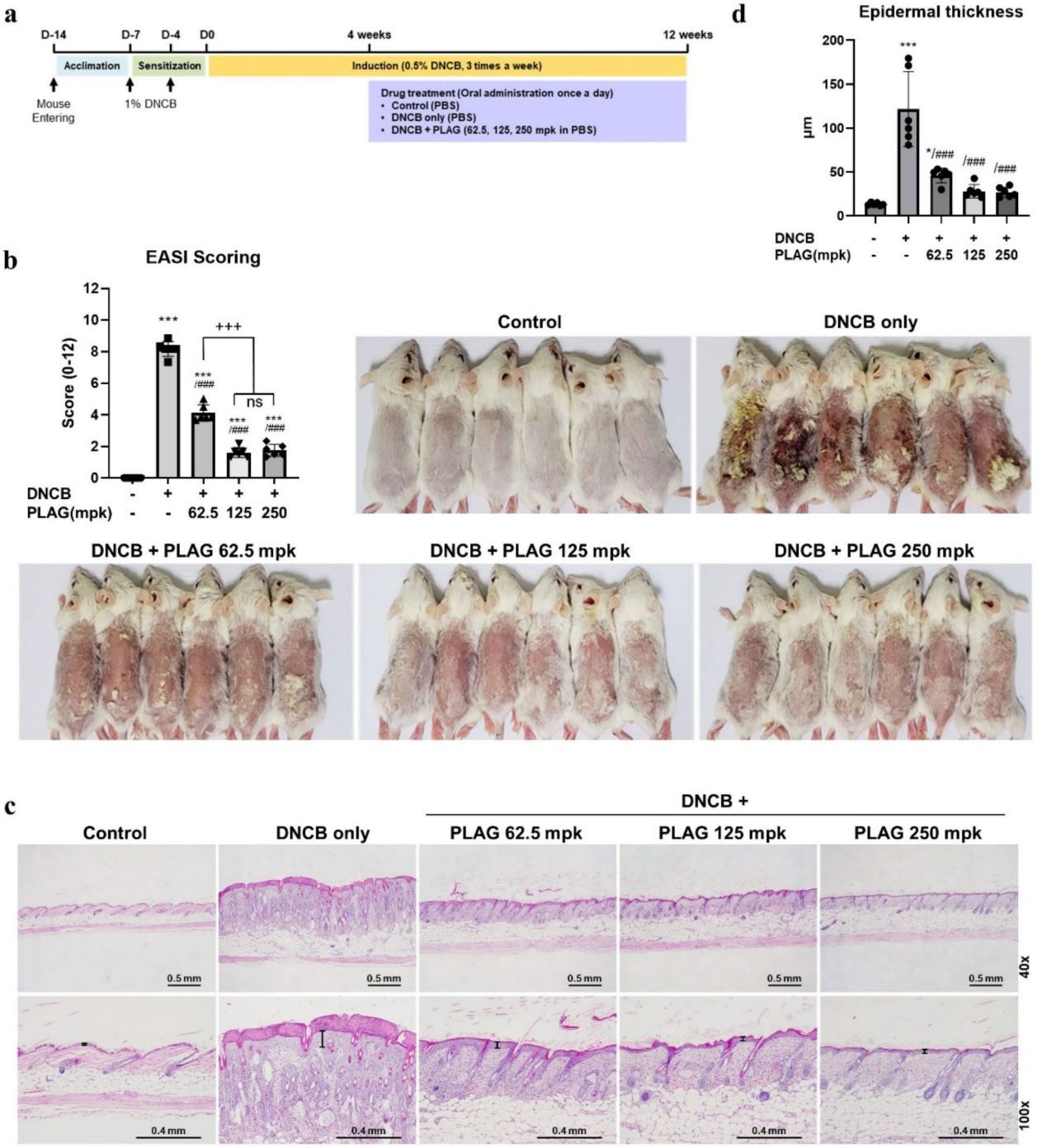


**Fig. 1**. **PLAG alleviates DNCB-induced AD in a mouse model.** **(A)** BALB/c mice were divided into five groups ($n = 6$ per group): the control, 2,4-dinitrochlorobenzene (DNCB)-treated, and DNCB/1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG)-treated groups. The mice were treated with 0.5% DNCB for twelve weeks (three times a week) after being sensitized with a 1% DNCB solution (acetone: olive oil = 3:1). PLAG (62.5, 125, or 250 mg/kg) was administered orally daily from the 4th week. **(B)** All mice were photographed and assessed macroscopically based on the eczema area and severity index (EASI) score. EASI scores were evaluated on the last day of the experiment. **(C)** Skin lesion sections (upper/lower panels: scale bar = 0.5/0.4 mm, 40/×100 magnification of camera lens, and epidermal thickness indicated by I) from sacrificed mice were stained with hematoxylin and eosin (H&E), and **(D)** epidermal thickness was measured. Data are presented as the mean ± SD. $^{*}p < 0.033$, $^{***}p < 0.001$, compared to the control group. $^{\#\#\#}p < 0.001$, compared to the DNCB-alone group, $^{+++}p < 0.001$, compared to the DNCB + PLAG 125, or 250 mg/kg group.

groups treated with 125 or 250 mg/kg of PLAG exhibited greater improvement than mice treated with 62.5 mg/kg of PLAG; however, there was no significant difference in the EASI scores between the two groups (Fig. 1b, upper left panel). This finding suggests that the optimal dose for maximum therapeutic effect is approximately 125 mg/kg.

Next, we investigated whether PLAG had therapeutic effects on AD-induced cellular and molecular immunological responses (Fig. 2 and Supplementary Fig. S1e, f). The number of infiltrated eosinophils and mast cells in the PLAG-treated mice was significantly reduced compared with that in mice treated with DNCB alone (Fig. 2a and b). Remarkably, the numbers of infiltrated and circulating eosinophils in mice treated with 125 or 250 mg/kg of PLAG were statistically similar to those in the untreated negative control (Fig. 2a and c). The levels of circulating monocytes and eosinophils in mice treated with 125 or 250 mg/kg of PLAG were decreased to similar levels to those of the untreated negative control group, indicating that PLAG ameliorates AD-induced cellular infiltration into skin lesions to a normal state (Fig. 2c).

The overexpression of IgE and IL-13 induced by DNCB was significantly downregulated in all PLAG-treated mice compared to those treated with DNCB alone. In contrast to IgE and IL-13 levels, IL-4 levels were reduced only in the two groups treated with 125 and 250 mg/kg of PL AG, except for the group treated with 62.5 mg/kg of PLAG. Similar to the regulation of cellular infiltration, the levels of IgE, IL-4, and IL-13 in the group treated with 250 mg/kg of PLAG decreased to levels comparable to those in the normal control group (Fig. 2d). These results confirmed that PLAG modulates AD-mediated humoral responses to a normal state.

TSLP (thymic stromal lymphopoietin) triggers the production of Th2-attracting chemokines such as TARC (thymus and activation-regulated chemokine) and eotaxin in human[27,28]. Patients with AD exhibit high serum concentrations of TARC (thymus and activation-regulated chemokine) and eotaxin, and their concentrations accurately reflect disease activity[28]. TSLP, TARC and eotaxin levels were significantly reduced in all PLAG-treated mouse groups compared with those treated with DNCB alone, reaching levels similar to those in the untreated negative group. Consistent with the findings of experiments investigating cellular and humoral responses, PLAG modulated the expression of TSLP, TARC and eotaxin in the skin tissue and serum to similar levels to those of the normal group (Fig. 2e).

It is well known that the JAK-STAT pathway plays a pivotal role in regulating multiple immune reactions involved in the pathogenesis of AD[29]. On the basis of the scientific state, we investigated whether PLAG affects the JAK-STAT pathway. Interactions between a large number of cytokines and the JAK-STAT pathway affect immune cell differentiation and development and involve immunomodulatory effects. IL-4 enhances GATA3 gene expression via STAT6[30]. The level of phosphorylated JAK1/STAT6 and GATA3 expression in mice treated with 125 and/or 250 mg/kg of PLAG was significantly reduced compared with that in mice treated with DNCB alone (Fig. 3 and Supplementary Fig. S2). These results suggest that PLAG might modulate DNCB-induced JAK-STAT pathway.

### PLAG exhibits a therapeutic effect comparable to or better than that of abrocitinib on AD

The therapeutic effect of PLAG was compared to that of abrocitinib in a DNCB-induced AD mouse model (Fig. 4a and Supplementary Fig. S3a). Abrocitinib is the latest JAK1-specific inhibitor approved by the FDA and EMA for the treatment of moderate-to-severe AD in adults (Supplementary Table S2)[31]. Based on the efficacy results, a comparative efficacy study between PLAG and abrocitinib was conducted using 125 mg/kg of PLAG, the lowest concentration exhibiting maximal therapeutic effect. For abrocitinib, a dose of 10 or 15 mg/kg was administered mg/kg based on a previous report employing a murine model[32].

To compare the efficacy of PLAG and abrocitinib in the treatment of severe AD, phenotypic and histopathological evaluations were performed by stratifying the EASI scores and quantifying epidermal thickness. The results indicated that PLAG had a statistically significant therapeutic effect on AD compared to abrocitinib (Fig. 4b-d, Supplementary Fig. S3b-d). Unlike abrocitinib, 125 mg/kg of PLAG mg/kg significantly reduced AD-induced eosinophil infiltration to similar levels as those in the untreated negative controls. In contrast, AD-induced mast cell infiltration was similarly reduced by both PLAG and abrocitinib compared to that in groups treated with DNCB alone; however, the levels did not reach those observed in the untreated negative controls (Fig. 5a and b, Supplementary Fig. S3e and f). Notably, both PLAG and abrocitinib downregulated IgE, IL-4, and IL-13 in a similar pattern, reaching levels similar to those in the untreated negative controls (Fig. 5c, Supplementary Fig. S3g).

## Discussion

Fred Wise and Marion Sulzberger first proposed the term "atopic dermatitis (AD)" in 1933[33]. Despite nearly 100 years passing since then, numerous patients continue to suffer from AD. However, significant progress has been made in understanding the pathophysiology of AD over the past two decades. Several researchers have reported that AD encompasses a variety of clinical phenotypes; recently, Bieber introduced a multidimensional AD disease model that highlights a dynamic immune response divided into multiple components for preventive and therapeutic interventions[6]. This improved understanding of the cellular and humoral elements involved in the pathophysiology of AD has expanded the possibility of exploring new therapeutic drugs.

Skin inflammation is associated with various types of immune cells, including Th1, Th2, Th17, and Th22 cells[34]. The overexpression of Th2 cytokines such as IL-4, IL-13, and IL-31 is the most common cause of AD[35]. Among the recently approved treatments for AD by the FDA and EMA, abrocitinib, a JAK1 inhibitor, has demonstrated promise for the treatment of moderate-to-severe AD[36]. JAKs are a family of intracellular nonreceptor tyrosine kinases that activate STATs by transmitting signals from cytokines. IL-4 activates JAK1/JAK3-STAT6; IL-4 and IL-13 activate JAK1/JAK2/TYK2-STAT3 and 6; and IL-13 activates JAK1/JAK2-STAT1, 3, and 5[36].

Abrocitinib attenuates inflammation by reducing cytokine production. A recent meta-analysis of several studies revealed that abrocitinib significantly improved the skin condition and itchiness of patients in an entire

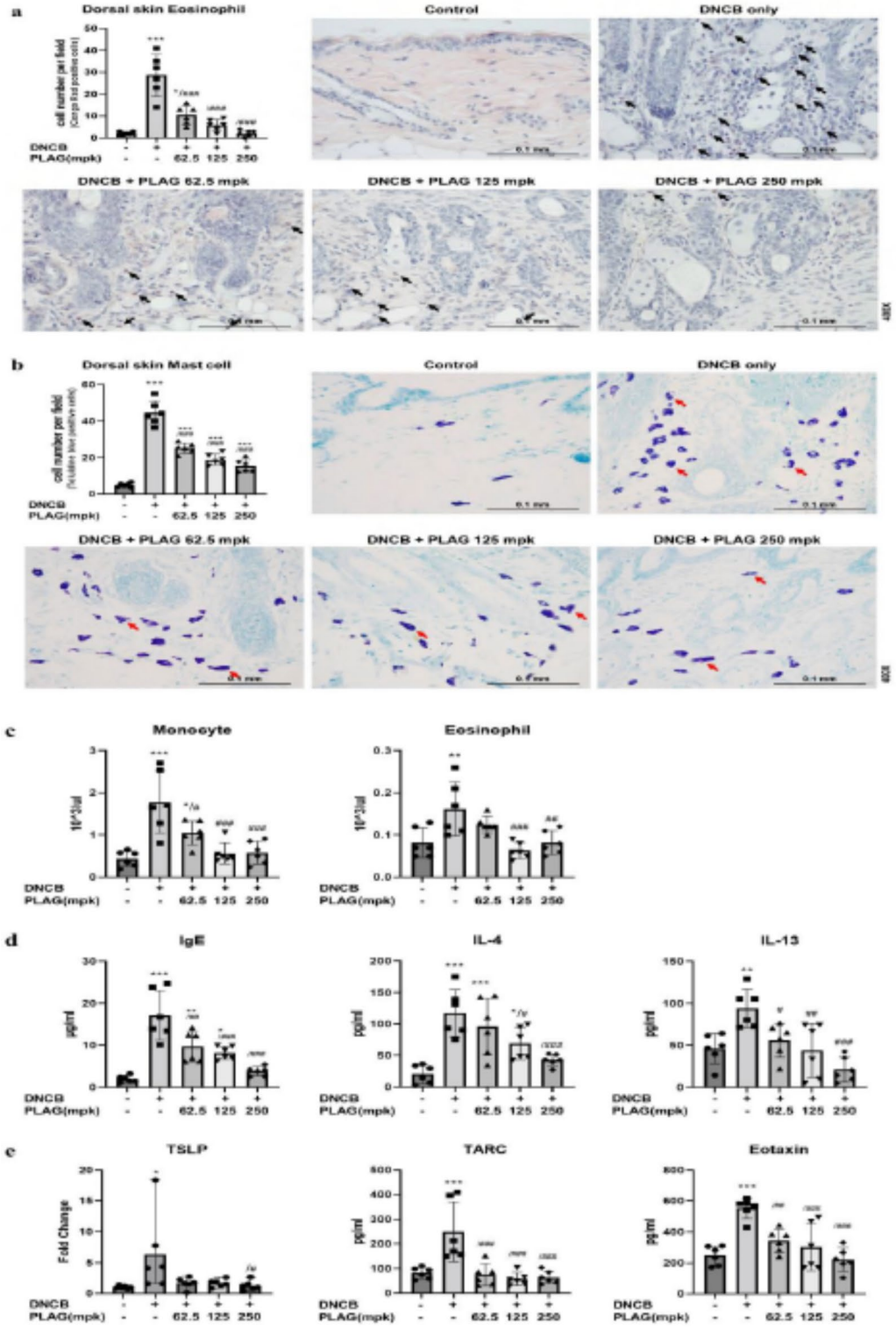


**Fig. 2**. **PLAG exhibits a therapeutic effect on AD-induced cellular and molecular immunological responses.** Skin lesion sections were stained with Congo red **(A)** and toluidine blue **(B)** to measure the infiltration of eosinophils (black arrows) and mast cells (red arrows). Cells were counted under a microscope at ×400 magnification. A complete blood count (CBC) analyzer was used to analyze the number of monocytes and eosinophils in blood samples from mice **(C)**. The quantity of secreted proteins in the skin tissue or plasma was measured using qRT-PCR or ELISA **(D, E)**. $^{*}p < 0.033$, $^{***}p < 0.001$, compared to the control group. $^{\#\#\#}p < 0.001$, compared to the DNCB alone group.

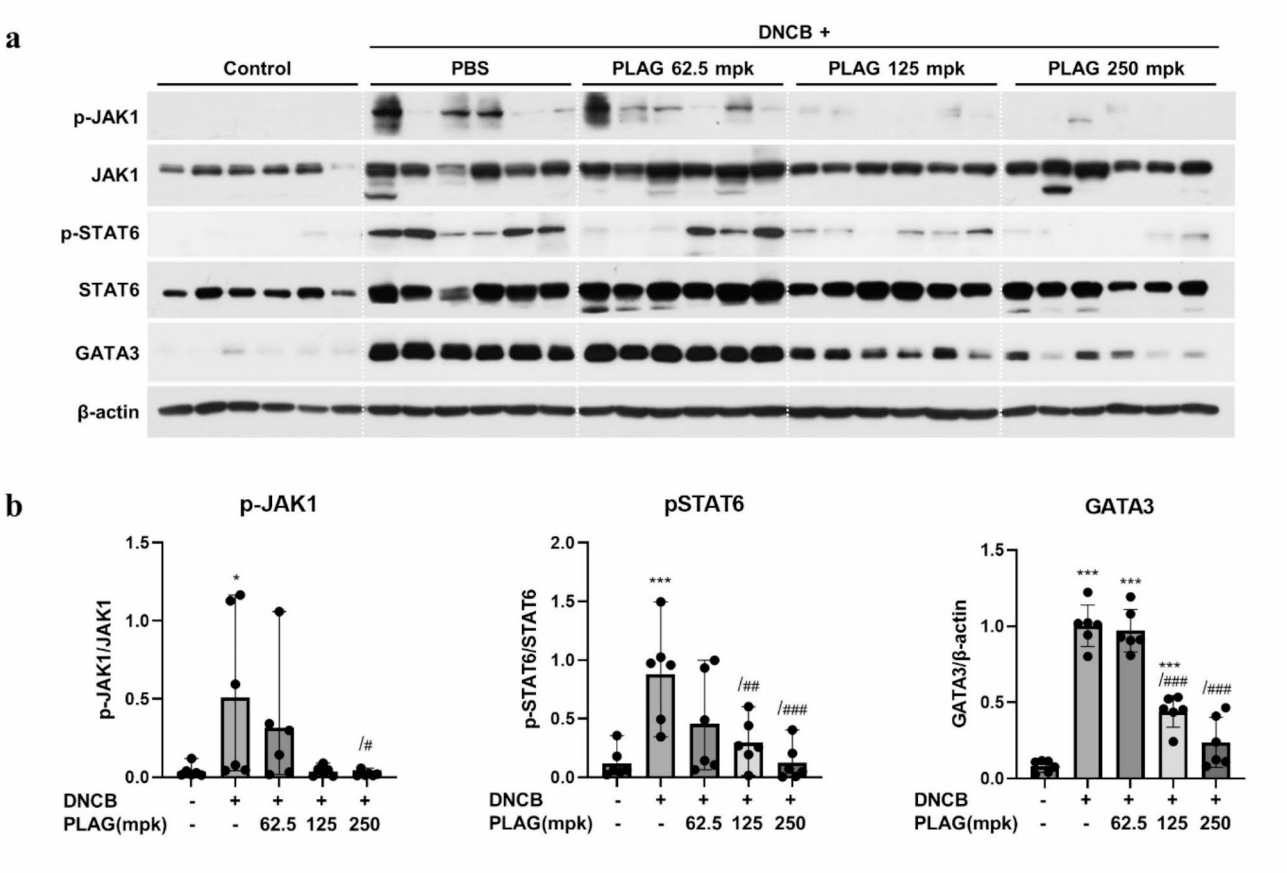


**Fig. 3**. **PLAG downregulates the JAK-STAT pathway. Frozen tissues from skin lesion were extracted using the buffer.** The extracts were treated with anti-p-JAK1, anti-JAK1, anti-p-STAT6, anti-STAT6, anti-GATA3, and β-Actin antibodies **(A)** and graphs were showed corresponding density to above blots **(B)**. Data are presented as the mean ± SD. $^{*}p < 0.033$, $^{***}p < 0.001$, compared to the control group. $^{\#\#\#}p < 0.001$, compared to the DNCB-alone group, $^{+++}p < 0.001$, compared to the DNCB + PLAG 125, or 250 mg/kg group.

long-term efficacy (LTE) population treated for ≥ 36 and ≥ 48 weeks with abrocitinib[37]. However, some patients experienced adverse reactions, such as serious infections (herpes simplex, herpes zoster, and pneumonia), malignancy, thrombosis, and major adverse cardiovascular events[38]. Additionally, other JAK inhibitors, such as baricitinib (a JAK1/JAK2 inhibitor) and upadacitinib (a JAK1 inhibitor), may also cause serious infections (tuberculosis, shingles, and others caused by bacteria, fungi, or viruses) and increase the risk of lymphoma and other cancers. Furthermore, both drugs may increase the risk of death in individuals aged > 50 years with one or more cardiovascular risk factors[38]. Despite the proven efficacy of JAK inhibitors, treatment of AD remains challenging.

Because the incomplete resolution of inflammation is considered the underlying cause of numerous diseases, including AD, atherosclerosis, asthma, and arthritis, among other inflammatory diseases, achieving complete resolution is crucial for restoring immune homeostasis and maintaining overall health. Current treatment strategies mainly focus on providing temporary symptom relief by inhibiting pro-inflammatory mediators. However, they do not effectively eliminate foreign invaders or danger signals to achieve complete resolution. Moreover, these treatment methods can lead to unwanted side effects such as immunosuppression, which increases the risk of opportunistic infections. PLAG employed in the present study enhances the resolution of inflammation and strengthens the immune defense system by promoting natural endogenous resolution processes[39–41]. Unlike conventional treatments, PLAG provides a fundamental approach that does not induce immunosuppression or secondary infections. Previous studies have demonstrated that PLAG effectively improves allergies and acute gouty inflammation in mouse models by resolving excessive inflammatory responses[22,42].

In the present study, we aimed to investigate the effects of PLAG in reducing DNCB-induced AD. Our results confirmed that the oral administration of PLAG effectively reduced AD symptoms by balancing the Th1/Th2 immune response, suppressing the recruitment of eosinophils and mast cells, and ameliorating the overexpression of IL-4, IL-13, IgE, TSLP, TARC, and eotaxin. Moreover, we found that PLAG downregulates the activated JAK-STAT pathway by induction of DNCB. Notably, the efficacy of PLAG was comparable to that of abrocitinib, a drug commonly used to treat moderate-to-severe AD.

PLAG demonstrated superior improvement compared to abrocitinib in the phenotypic and histopathological evaluations. However, there was no significant difference between PLAG and abrocitinib in terms of the

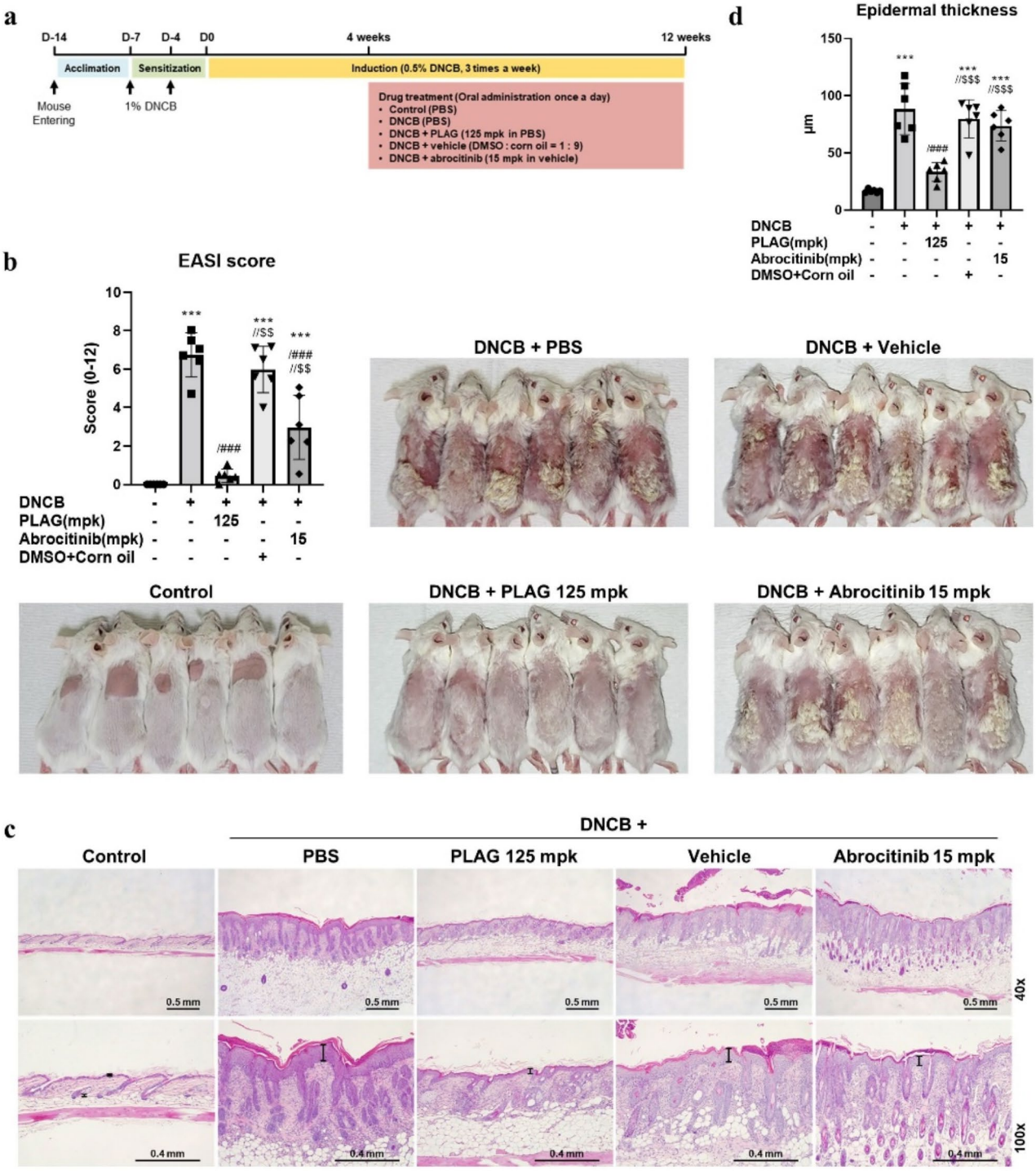


**Fig. 4. PLAG alleviates DNCB-induced AD more effectively than abrocitinib in a mouse model. (A)** BALB/c mice were divided into five groups ($n = 6$ per group): the control, phosphate-buffered saline (PBS) or vehicle (DMSO: corn oil = 1:9) with DNCB, and PLAG- (125 mg/kg, PBS) or abrocitinib-treated (15 mg/kg, DMSO: corn oil = 1:9) with DNCB groups. The mice were treated with 0.5% DNCB for 12 weeks (three times a week) after being sensitized with a 1% DNCB solution (acetone: olive oil = 3:1). PBS, vehicle, PLAG, or abrocitinib were administered orally daily from the 4th week. **(B)** All mice were photographed and assessed macroscopically based on the EASI score. EASI scores were evaluated on the last day of the experiment. **(C, D)** Skin lesion sections from sacrificed mice were stained with H&E (upper/lower panels: scale bar = 0.5/0.4 mm, 40/×100 magnification of camera lens, and epidermal thickness indicated by I), and epidermal thickness was measured. Data are presented as the mean ± SD. $^{***}p < 0.001$, compared to the control group. $^{\#\#\#}p < 0.001$, compared to the DNCB + PBS group, $^{\$\$}p < 0.002$, $^{\$\$\$}p < 0.001$, compared to the DNCB + PLAG group.

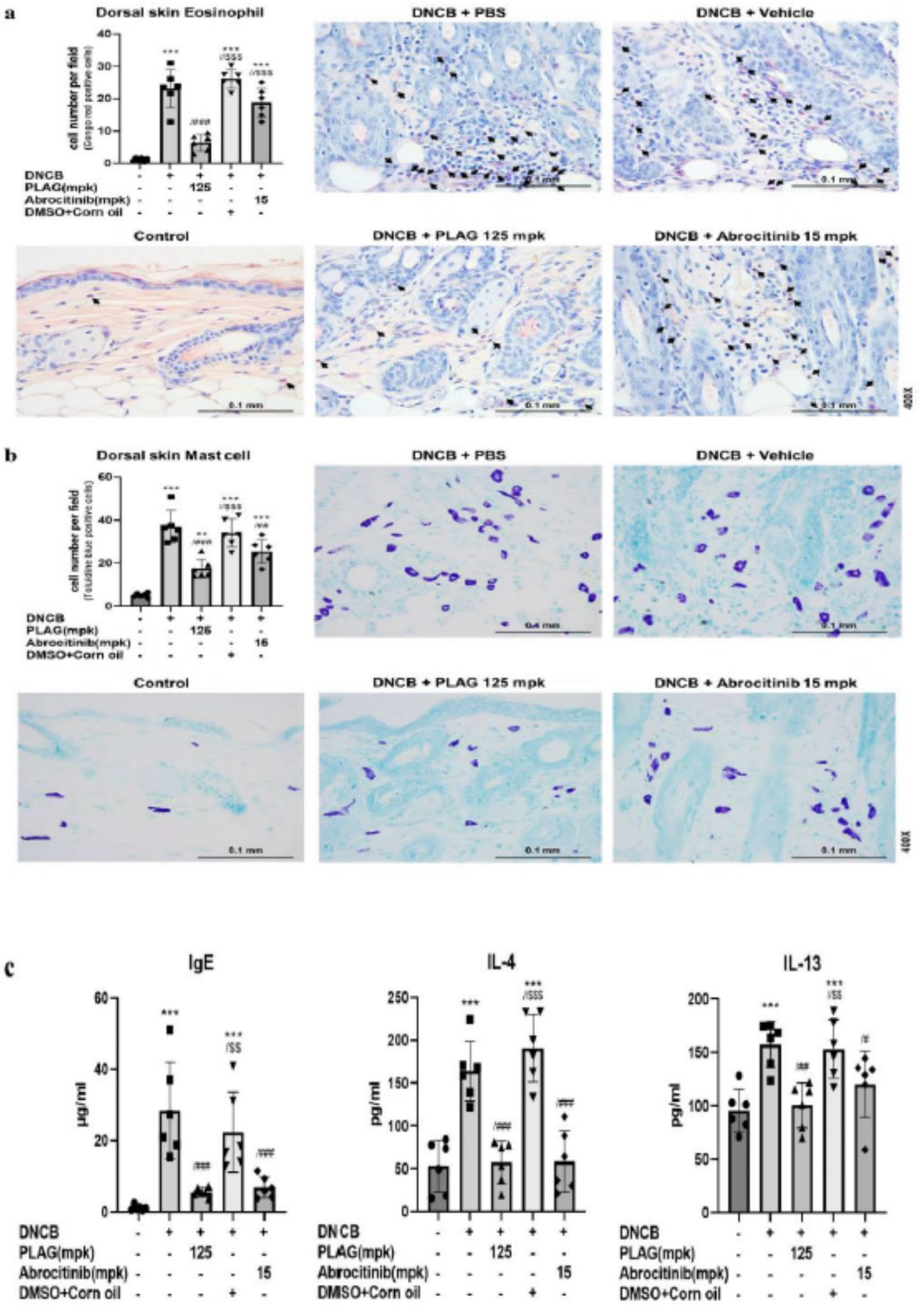


**Fig. 5**. **PLAG exhibits better therapeutic efficacy on DNCB-induced inflammation than abrocitinib.** Skin lesion sections were stained using Congo red **(A)** and toluidine blue **(B)** to measure the infiltration of eosinophils (black arrows) and mast cells (red arrows). Cells were counted under a microscope at ×400 magnification. The quantity of secreted proteins in the plasma was measured using ELISA **(C)**. $^{**}p < 0.002$, $^{***}p < 0.001$, compared to the control group. $^{\#}p < 0.033$, $^{\#\#}p < 0.002$, $^{\#\#\#}p < 0.001$, compared to the DNCB + PBS group. $^{\$\$}p < 0.002$, $^{\$\$\$}p < 0.001$, compared to the DNCB + PLAG group.

downregulation of IgE, IL-4, and IL-13 (Figs. 3 and 4). These results suggest that PLAG would modulate immune pathways distinct from those of abrocitinib and could potentially serve as a therapeutic agent for the effective management of refractory patients with AD who experience an unpredictable chronic disease course. Therefore, to better understand the mechanisms underlying the regulation of AD by PLAG, further studies should be conducted to explore its biochemical and functional effects at the molecular level.

## Methods

### PLAG

PLAG was synthesized by the patented process (Korean Patent Nos. 10-1278874 [2013.60.20] and 10-2333606 [2021.11.26]), provided by a GMP facility of Enzychem Lifesciences Corporation (Jecheon-si, South Korea), and used based on the information provided by the manufacturer.

### In vivo mouse model of AD

Animal experiments were conducted using 6-week-old female BALB/c mice (Koatech Co., Pyongtaek, South Korea). The mice were housed in standard environments with a 12-h dark/light cycle and provided with food and water in a timely manner. The mice were randomly divided into five groups, each containing six mice. The groups were organized according to the following experimental characteristics: (1) Evaluation of PLAG efficacy: control group, 2,4-dinitrochlorobenzene (DNCB) group, and PLAG group (dose range determination: 150, 250, and 400 mg/kg and efficacy evaluation: 62.5, 125, and 250 mg/kg) along with DNCB. (2) Comparison of efficacy between PLAG and abrocitinib: control group, group administered phosphate-buffered saline (PBS) or vehicle (DMSO: corn oil = 1:9) along with DNCB, PLAG group (125 mg/kg, PBS), or abrocitinib group (10 or 15 mg/kg, DMSO: corn oil = 1:9) along with DNCB. All mice in the DNCB groups were sensitized by applying 150 µL of 1% DNCB solution (acetone: olive oil = 3:1) topically on their dorsal skin, and this was repeated on day 4. Starting on day 7, the mice were treated with 0.5% DNCB three times a week for 12 weeks. PLAG and abrocitinib were administered orally daily starting in the 4th week. We confirmed all methods were performed in accordance with the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology's guidelines and regulations (approval number: KRIBB-AEC-22020). The methods of euthanasia performed on the mice and the agents used for euthanasia as follows: (1) Place the mouse in the euthanasia chamber and close the lid. (2) Open the valve to allow the chamber to fill with CO2. (3) Close the valve and wait for about 5–10 min. (4) Open the chamber lid and take out the dead subject.

### Diagnosis of AD

We assessed the severity of dermatitis macroscopically using the eczema area and severity index (EASI) scoring system. This system rates symptoms on a scale as either 0 (none), 1 (mild), 2 (moderate), or 3 (severe), based on erythema, edema, papulation, excoriation, and lichenification. The EASI score integrates these individual scores into a final composite score (Supplementary Table 1)[43]. All mice were photographed weekly, and the total score for the skin lesions was calculated by summing the individual scores. After the final evaluation, the mice were sacrificed, and serum and both lesion and non-lesion skin tissues were collected.

### Histological analysis

Tissue specimens from the mouse model were fixed in 10% formaldehyde, embedded in paraffin, and sectioned into 5-µm slices. Epithelial thickness was visualized by staining using hematoxylin and eosin (H&E) solution (Dako, CA, USA) and examined at 20 randomly selected sites on tissue slides using the NIS-Elements BR Ver4 tool (Nikon, Tokyo, Japan). The infiltration of eosinophils and mast cells into the lesions was visualized by staining with Congo red (CR)(Sigma-Aldrich, MO, USA) and 1% toluidine blue (TB)(Sigma-Aldrich), respectively. The stained areas were analyzed using ImageJ software (National Institutes of Health, Maryland, USA).

### Complete blood count analysis

Hematopoietic cell analysis of the test mice was performed using a complete blood count (CBC) analyzer (Mindray, Shenzhen, China). Whole blood was used to detect cardiac hemorrhage and stored in a coated cube prior to analysis. Secreted proteins in the blood or serum were separated by centrifugation at 6,000 rpm for 10 min at 4 °C.

### Quantitative real time polymerase chain reaction

Total RNA was extracted from a dorsal skin tissue using RiboEx (GeneAll Biotechnology, Seoul, South Korea) according to the instructions of manufacturer. cDNA was generated using the ReverseAids cDNA synthesis kit (Thermo Scientific, MA, USA). RT-PCR was performed with the following temperature profile: pre-denaturation for 10 min at 95 °C; 35 cycles of 95 °C for 30 s, annealing temperature for 30 s, and 72 °C for 30 s; and a final exposure to 72 °C for 10 min. The following TSLP and GAPDH primer sets were used: mouse TSLP forward, 5′-CCCCTGCACACACCTTACTC-3′, mouse TSLP reverse, 5′-GCAGTTTCTCCCAGATTCCA-3′, mouse GAPDH forward, 5′-CCATCACCATCTTCCAGGAG-3′, mouse GAPDH reverse, 5′-ACAGTCTTCTGGGTG GCAGT-3′. The fold change of TSLP was calculated based on GAPDH.

### Western blot analysis

Dorsal skin tissues were lysed using 1 x RIPA lysis buffer containing phosphatase inhibitor (Thermo Fisher Scientific Inc., MA, USA) and protease inhibitor (Roche, Basel, Switzerland) on ice for 30 min. The proteins in each sample were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 8% and 10% gel electrophoresis and transferred to a polyvinylidene fluoride (PVDF) membranes (Millipore Corporation, Germany). The membrane was blocked with 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS)

containing 0.05% Tween-20 for 1 h. It was then incubated with anti-phospho-STAT6 (Cell Signaling Technology, MA, USA), GRK2 (Cell Signaling Technology, MA, USA), phospho-JAK1 (Cell Signaling Technology, MA, USA), JAK1 (Cell Signaling Technology, MA, USA), GATA3 (Cell Signaling Technology, MA, USA), β-actin (Cell Signaling Technology, MA, USA), at 4 ℃ overnight. After washing with PBST, the membrane was stained with peroxidase-conjugated goat anti-rabbit IgG (Enzo LifeSciences, NY, USA) for 1 h at room temperature. Target proteins were detected using Immobilon Western Chemiluminescent HRP Substrate (Merck Millipore, MA, USA). For quantification of protein expression levels, we used Image J software to calculate density of the western blot bands.

### Enzyme-linked immunosorbent assay

The levels of secreted proteins in the plasma of mice were analyzed using factor-specific enzyme-linked immunosorbent assays (ELISAs) following the manufacturer's protocol. The proteins evaluated were eotaxin, thymus and activation-regulated chemokine (TARC), IL-13 (R&D Systems, MN, USA), IL-4, and immunoglobulin E (IgE) (BD Bioscience, New Jersey, USA). Absorbance was measured at 450 nm using an EMax Endpoint ELISA microplate reader (Molecular Devices Corporation, CA, USA).

### Statistical analysis

The results were presented as mean ± standard deviation (SD) values. The significance level was set at a 95% confidence limit or greater ($p < 0.05$) and determined using the Student's t-test. Statistical differences between groups were analyzed using a one-way analysis of variance (ANOVA; Prism 9, GraphPad Software, CA, USA).

## Data availability

The datasets generated and/or analyzed during the current study could be provided by the corresponding authors on reasonable request.

Received: 30 April 2024; Accepted: 23 September 2024
Published online: 08 October 2024

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Conception and design: SHS, GTK, and JWK / Analysis and interpretation: SHS, YJK, GTK, JWK, and JSL / Data Collection: SHS, HL, EYK, and SHL / Data Discussion: KYS / Writing the article: SHS, YJK, and SJK / Final approval of the article: JSL.

## Funding

This work was supported by Grants (IGM0402111 and IGM0382211) from Enzychem Lifesciences and the KRIBB Research Initiative Program (KGM5502423).

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-73951-2.

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