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A Refined Comparative Mouse Model of Acute and Chronic Atopic Dermatitis

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Abstract (up to 350 words)

Canine and human atopic dermatitis (AD) is a complex inflammatory skin disorder with an increasing incidence, characterized by distinct acute and chronic phases with unique histological and immunological profiles. Although research into effective treatment methods has been insufficient, there has been a surge in the exploration of probiotics as a therapeutic strategy for AD. Such probiotics are often originated from the animals, and these are being developed to modulate the immune system and enhance skin barrier function, offering promising new treatment options for AD. To better understand the pathogenesis of both canine and human AD and develop treatments, animal models that accurately replicate the symptoms of both species are indispensable. This study aimed to establish a standardized and cost-effective BALB/c mouse model to more accurately simulate canine and human AD using dinitrochlorobenzene (DNCB) alone and in combination with ovalbumin (OVA). We evaluated histological and immunological changes from acute to chronic stages of AD in the mouse model induced by treatment of DNCB alone and DNCB combined with OVA to determine their similarity to both canine and human AD symptoms. The results showed that the pathological changes observed in the mouse AD model demonstrated significant parallels with both species, including increased mast cell infiltration, epidermal thickening, and elevated cytokine levels such as IL-4 and IFN- γ. Acute phase observations highlighted pronounced epidermal defects such as dryness and skin erosion, while chronic phase findings indicated persistent skin thickening, inflammation, and notable edema. Although both mouse models showed comparable symptoms and immunological responses, the model induced by the combination of DNCB and OVA more accurately represented canine and human AD compared to the model induced by DNCB alone. This combined DNCB and OVA mouse model provides valuable insights into AD pathogenesis and potential therapeutic targets, underscoring its significance in AD research.

Keywords (3 to 6): Animals, Mouse model, Probiotics, Atopic dermatitis.

Introduction

Atopic dermatitis (AD) is an inflammatory skin disorder resulting from a complex interaction between genetic and environmental factors. AD can be classified into acute and chronic stages, each with distinct histological and immunological characteristics. Acute AD is characterized by a predominant T helper 2 (Th2) cytokine response, including IL-4 (1-3). Chronic AD, however, is noted for lichenification due to repeated scratching and features a predominant T helper 1 (Th1) cytokine response, including IL-12 and IFN-γ, along with Th2 cytokines (3-5). One of the prominent immunological features of AD is elevated immunoglobulin E (IgE) levels (5). Cytokines including IL-4 secreted by activated Th2 cells induce and promote IgE synthesis in B cells (6). The IgE produced through this continuous reaction exacerbates skin inflammation and barrier defects in AD, contributing to its chronic progression (7).

AD is a prevalent skin disorder with an increasing incidence in humans. Similarly, AD is also prevalent among companion animals, with dogs showing a reported prevalence of 3% to 15% and cats exhibiting a prevalence rate of around 12.5% (8, 9). Notably, canine atopic dermatitis presents a pathophysiological profile remarkably akin to that of human AD, with overlapping immunological responses and clinical manifestations, making it an important model for comparative studies in atopic dermatitis research (10, 11). But research into effective treatment methods remains insufficient. However, both canine and human AD is a complex inflammatory skin disorder with an increasing incidence, characterized by distinct acute and chronic phases with unique histological and immunological profiles (3). Although research into effective treatment methods has been insufficient, there has been a surge in the exploration of probiotics as a therapeutic strategy for AD (12-15). Such probiotics are often originated from the animals, and these are being developed to modulate the immune system and enhance skin barrier function, offering promising new treatment options for AD (16-19). To better understand the pathogenesis of AD and develop treatments, animal models that closely resemble the symptoms observed in both humans and companion animals are indispensable. Mouse models are predominantly used in AD research due to their ease of handling, cost-effectiveness, and the simplicity of genetic manipulation. Notably, the murine model of atopic dermatitis shares multiple significant features with canine and human AD, most prominently the elevated IgE levels that characterize the immune responses, as well as similar clinical presentations(20). Mouse AD models can be divided into three main categories: 1) inbred models, 2) genetically modified models, and 3) models induced by exogenous substances (1, 21). Inbred and genetically modified models have the disadvantage of being time-consuming and costly to produce. Conversely, models induced by exogenous substances are applicable to various mouse strains and are relatively inexpensive and efficient (22, 23).

Among the exogenous substances used to induce AD, dinitrochlorobenzene (DNCB) is the most common. It induces contact dermatitis by forming haptens, leading to AD-like skin lesions similar to those observed in both dogs and humans (1, 24-26). Another exogenous AD inducer is ovalbumin (OVA), an allergenic protein found in egg whites, used to sensitize the immune response to induce AD (27-29). While DNCB-induced contact dermatitis models effectively replicate human AD-like skin lesions, they lack sufficient antigenic stimulation to produce significant levels of IgE (30). In contrast, OVA-induced models result in the production of OVA-specific IgE but typically cause only mild skin lesions (7). Therefore, developing standardized AD mouse models that combine these exogenous substances is necessary to more accurately mimic AD in both animal and human contexts.

This research utilized the cost-effective and accessible BALB/C mouse to develop a comparative model of acute and chronic AD in both dogs and humans, comparing a group treated with DNCB alone to a group treated with both DNCB and OVA. By evaluating histological and immunological changes from acute to chronic stages in each treatment group, we aimed to verify the similarities between the symptoms in the mouse model and those of canine and human AD, thereby contributing to a better understanding of AD pathogenesis.

Materials and Methods

1.1 Animals

Thirty female BALB/c mice, each weighing between 20 and 25 grams at six weeks of age, were purchased from RaonBio (Yongin, South Korea) and divided into three treatment groups. They were fed a commercial rodent diet (Cat No. 2018C, Raonbio Inc, Yongin, South Korea) and housed under controlled environmental conditions: a temperature of 23±1℃, humidity of 50±10%, and a 12-hour light/12-hour dark cycle. The animal experimental protocol used in this study was reviewed and approved by the Institutional Animal Care and Use Committee of Dankook University, Cheonan, South Korea (Approval no. DKU-23-057).

1.2 Experimental design

After acclimating to the laboratory conditions for one week, the mice were anesthetized using an intraperitoneal injection of 2,2,2-Tribromoethanol (Avertin, Catalog No. T48402, Sigma-Aldrich, Saint Louis, USA) formulated with 2-Methyl-2-butanol (Catalog No. 152463, Sigma-Aldrich, Saint Louis, USA) at a dosage of 250 mg/kg to prevent any potential injuries during the shaving process. Subsequently, an electric razor was used to carefully remove the dorsal fur of all mice. Twenty-four hours after hair removal, the skin of the mice was

inspected for any cuts or abrasions. In this study, all prepared agents were applied to both the back and ears using a cosmetic brush, which was used to gently rub the solution into the skin.

The study groups were as follows (Figure 1A):

1. Control group (n=10): Mice in this group were treated with only saline. No haptens, allergens, or drugs were administered, serving as the baseline for comparison against the experimental groups.

2. DNCB only group (n=10): This group served as a chemical-induced model for predicting the breakdown of the skin barrier. A 1% solution of DNCB (Chloro-2,4-dinitrobenzene, Catalog No. 237329, Saint Louis, USA) was administered twice during the first week of the experiment. In addition, a 0.5% solution of DNCB was applied the 1% DNCB treatments. Subsequently, a 0.5% solution of DNCB was applied three times per week for three weeks to induce localized dermatitis and impair the skin barrier function, mimicking conditions similar to atopic dermatitis. The DNCB solutions at concentrations of 1% and 0.5% were prepared using a 3:1 mixture of acetone and olive oil (Catalog No. O1514, Sigma-Aldrich, Saint Louis, USA) as the solvent.

3. D+O (DNCB+OVA) mix group (n=10): In addition to DNCB, this group received OVA (OVA, Catalog No. A2512, Sigma-Aldrich, Saint Louis, USA) as an allergen to induce not only irritation and breakdown of the skin barrier but also to simulate allergen-induced itching. DNCB was administered twice a week, and OVA was applied once a week between DNCB treatments to induce a more complex skin condition resembling atopic dermatitis, involving both contact with allergens and chemical irritants. During the first week of experiment, a 1% solution of DNCB and 100ug of OVA were applied to the mice, followed by a 0.5% solution of DNCB and 50 μg of OVA.

The volumes used for each mouse were 100 μL for DNCB and 20 μL for OVA, regardless of concentrations. Euthanasia was performed by cervical dislocation. Five mice from each group were sacrificed on day 14 to evaluate the acute phase response, and the remaining five were sacrificed on day 28 to assess the chronic phase effects (Figure 1A). Blood, spleen and tissue samples, including dorsal back skin and ear tissues, were collected after euthanasia on days 14 and 28. To monitor general health status, body weight was measured and recorded weekly during the experimental period.

1.3 Evaluation of skin gross lesion

For the evaluation of skin gross lesions, four categories were assessed: crusting, erythema, erosion, and edema. Each criterion was graded on a scale from 0 to 4: 0 (clear), 1 (almost clear), 2 (mild), 3 (moderate), and 4 (severe). The evaluations were conducted by three independent observers who were not involved in the experimental procedures, ensuring an unbiased assessment. Each observer scored the severity of the skin lesions

without prior knowledge of the treatment groups. The scores for each category were averaged for each mouse on the day of sacrifice, providing a standardized measure of lesion severity at the endpoint of the study.

1.4 Ear thickness measurement

To assess the changes in skin thickness following agent application, measurements were conducted on the treated areas. Due to difficulties in accurately measuring changes in the thickness of the dorsal back tissue, only the ear tissues were evaluated, as these could be measured precisely. A Vogel Digital Vernier Calipers (BC. 12116, Kevelaer, Germany) was used to measure the thickness of the ear tissues once a week.

1.5 Histological examination

The histological examination of excised dorsal skin and ear tissues from mice treated with the agents was conducted on days 14 and 28, following the euthanasia of the mice. For histological assessment, a 1 cm x 1 cm section was collected from the center of the treated dorsal skin area. To prevent drying, the samples were flattened on aluminum foil and fixed in a 10% normal formalin solution for over 24 hours. Similarly, the middle section of the ear tissues, divided into thirds longitudinally, was fixed in formalin. These samples were then sent to K2O Co. (Siheung, South Korea) for toluidine blue staining. After fixation, the tissues were processed and embedded in paraffin. Toluidine blue staining was performed by K2O Co., and the stained slides were observed under an Olympus CKX53 microscope (Olympus, Tokyo, Japan) to evaluate histological alterations, focusing particularly on the degree of epithelial changes and other tissue responses.

1.6 RNA isolation and quantitative real-time PCR for the evaluation of tissue cytokine gene expression

To extract total RNA from the dorsal skin and ear tissues, 0.2g of the sample was finely cut using a blade. The samples were then homogenized using a bead beater to ensure thorough tissue disruption. The homogenized samples were processed using the NucleoSpin RNA isolation kit (MACHEREY-NAGEL, Cat No. 740955, Dueren, Germany) in a clean bench, following the provided instructions. Extracted RNA was quantified using a Colibri Microvolume Spectrometer (TITERTEK BERTHOLD, Pforzheim, Germany), and its purity was confirmed with A260/A280>2.0 and A260/A230>2.1 ratios. The isolated RNA was then synthesized into cDNA using the AccuPower RT Premix kit (BIONEER, K-2041, Daejeon, Republic of Korea). Quantitative real-time PCR was performed on a CFX Connect™ Real-Time System (BIO-RAD, Hercules, United States) with the following conditions: initial denaturation at 95°C for 30 seconds, followed by 40 cycles of 95°C for 10 seconds, and annealing at either 60°C for 10 seconds or 58.5°C. The reaction was finalized with 65°C for 5 seconds and a final step at 95°C. The target primer information is provided in Supplement Table 1. Gene-expression levels

were presented relative to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and compared to the nontreated group.

1.7 Enzyme-linked immunosorbent assay(ELISA) for serum IgE measurement

Blood samples were collected on days 14 and 28, and the concentrations of IgE in the mouse serum were measured using the Mouse IgE ELISA kit (Colorimetric, Catalog No. NBP3-18786, Novus Biologicals, CO, USA) following the manufacturer's instructions. The kit instructions recommend diluting the serum samples before use, thus mouse serum was diluted at a ratio of 1:20. The samples were assayed in duplicate, and absorbance was measured at 450 nm.

1.8 Statistical analysis

The values from each individual animal were measured and used for statistical analysis. All statistical analyses were conducted using GraphPad Prism 8.0 software (GraphPad Software, Inc., San Diego, USA). Significant differences between groups were determined based on ANOVA. Statistical significance was defined as $p < 0.05$. Significance levels were denoted as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Results

1.9 Body weight and changes in spleen weight

To monitor general health status, body weight was measured and recorded weekly during the experimental period. In this study, the body weight of all groups generally increased over the 2-week and 4-week periods and no significant differences were observed between groups. Spleen weight was measured as an indicator of immune response (Figure 1B). After 2 weeks of the experimental period, a significant increase in spleen weight was observed in the DNCB+OVA-treated group compared to the NC group ($P<0.05$). Although the DNCB-only group did not show statistically significant results, there was a trend towards increased spleen weight. After 4 weeks of the experimental period, both the DNCB-treated group and the DNCB+OVA-treated group showed significant increases in spleen weight and size compared to the NC group (Figure 1B and 1C) ($P<0.05$).

1.10 Evaluation of skin gross lesion

To evaluate the effects of DNCB and DNCB+OVA treatments on atopic dermatitis, skin lesion scores were assessed in both dorsal and ear skin of mice during the acute (on day 14) and chronic (on day 28) phases. The

combined dermatitis scores for the dorsal skin showed significant differences between the reagent-treated groups and the NC group (Figure 2A). During the acute phase, which included 1 week of high concentration exposure followed by 1 week of low concentration exposure, both treatment groups exhibited severe symptoms. By the sacrifice on day 28, the severity had decreased to moderate levels (Figure 2A). When examining the individual dermatitis indices, dryness and crusting were prominent during the acute period, with the DNCB group showing notable crusting and erythema (Figure 2B and 2C). In the chronic period, dryness was significantly higher, and the dermatitis scores for the DNCB-only group were generally higher compared to the DNCB+OVA group (Figure 2B and 2C). For the ear skin, the combined dermatitis scores indicated that the DNCB-only group maintained a moderate level of dermatitis without significant changes over the 2-week and 4-week periods (Figure 2D). In contrast, the DNCB+OVA group showed a decrease from severe levels at 2 weeks to moderate levels at 4 weeks (Figure 2D). When evaluating the individual dermatitis indices for ear skin, the DNCB-treated group displayed high levels of edema during both the acute and chronic periods (Figure 2E and 2F). For the DNCB+OVA group, dryness and crusting scores were high during the acute period, while edema was more prominent in the chronic period (Figure 2E and 2F).

1.11 Changes in cytokine expression in target tissues

Atopic dermatitis is characterized by skin barrier dysfunction accompanied by dysregulation of immune cell responses. Disruption of the epidermal layer by DNCB and DNCB+OVA treatment leads to the activation of keratinocytes, which subsequently produce various pro-inflammatory cytokines. To investigate the inflammatory response in local tissues induced by DNCB and DNCB+OVA, we measured the mRNA expression levels of key cytokines (IL-12, IFN-γ, and IL-4) in dorsal and ear skin tissues during the acute (Day 14) and chronic (Day 28) phases (Figure 3A, 3B and 3C).

IL-12 expression in dorsal skin at Day 14 showed an increase in the DNCB+OVA-treated group compared to the NC group. By Day 28, both reagent-treated groups exhibited decreased IL-12 expression (Figure 3A). In ear skin, IL-12 expression levels were higher at day 28 compared to day 14, with the DNCB+OVA group showing significantly elevated levels (Figure 3A). For IFN-γ expression, dorsal skin showed a notable decrease in the DNCB+OVA group at day 14, followed by an increase at day 28 (Figure 3B). In ear skin, the DNCB-treated group displayed high IFN-γ expression at day 14, which decreased by day 28, whereas the DNCB+OVA group showed increased expression at day 28 (Figure 3B).

IL-4 expression was elevated in all reagent-treated groups compared to the NC group across both time points. The DNCB+OVA group in dorsal skin showed an increasing trend in IL-4 expression over time (Figure 3C). In ear skin, IL-4 expression was consistently higher in the DNCB-treated group initially but decreased over time,

while the DNCB+OVA group maintained similar levels throughout the treatment period (Figure 3C). To evaluate the Th2/Th1 cytokine pattern, the IL-4/IFN-γ ratio was analyzed. Both the DNCB and DNCB+OVA groups exhibited higher IL-4/IFN-γ ratios in both dorsal and ear tissues during the acute phase, which decreased during the chronic phase (Figure 3D and 3E). Notably, the DNCB+OVA-treated group displayed a pronounced difference in the dorsal skin (Figure 3E).

1.12 Measurement of Serum IgE levels

To assess the systemic allergic response, serum IgE levels were measured. On day 14, the DNCB+OVA group (7384.384 ± 655.447 ng/mL) exhibited significantly elevated serum IgE levels compared to the NC group (6165.502 ± 249.265 ng/mL), while the DNCB-only group (6462.182 ± 166.282 ng/mL) showed higher levels that were not statistically significant (Figure 3F). By day 28, serum IgE levels remained significantly elevated in the DNCB+OVA group (7473.286 ± 521.920 ng/mL) compared to both the NC (6331.920 ± 211.991 ng/mL) and DNCB groups (6996.555 ± 171.920 ng/mL) (Figure 3F). These data suggest that DNCB and DNCB+OVA treatments induce significant inflammatory responses in both dorsal and ear skin, as demonstrated by the increased expression of the cytokine such like IL-4 (Figure 3C).

1.13 Mast cell count and epidermal changes using toluidine blue staining

To evaluate the effects of DNCB and OVA treatments on mast cell infiltration and epidermal changes, toluidine blue staining was performed on skin samples from the dorsal and ear regions at days 14 and 28. As shown in the graphs (Figure 4A), mast cell counts in the dorsal skin were significantly higher in both the DNCB and DNCB+OVA groups compared to the NC group at both day 14 and day 28. This increase is clearly evident in the toluidine blue-stained images, which show numerous mast cells infiltrating the dermal layer (Figure 4B). Additionally, the measurement of epidermal thickness revealed a significant increase in both the DNCB and DNCB+OVA groups compared to the NC group (Figure 4A).

In the ear skin, mast cell counts were elevated in both the DNCB and DNCB+OVA groups compared to the NC group at days 14 and 28 (Figure 4C). Mast cell infiltration was particularly pronounced at Day 28. Toluidine blue staining of the ear skin demonstrated similar epidermal changes, with increased thickness and cellular infiltration in the DNCB and DNCB+OVA groups compared to the NC group (Figure 4D). These results indicate that DNCB and DNCB+OVA treatments lead to increased mast cell infiltration and significant epidermal changes in both dorsal and ear skin.

Discussion (optional)

Using mouse models in AD research is crucial due to their ability to replicate the complex genetic and environmental interactions observed in canine and human AD. These models are essential for understanding disease mechanisms and testing potential therapeutic interventions(31, 32) . Mouse models induced by exogenous substances, such as DNCB and OVA, are particularly valuable as they effectively replicate key symptoms of both canine and human AD, including skin barrier dysfunction, Th2 cytokine responses, and elevated IgE levels. These characteristics are crucial for studying the pathogenesis of AD and evaluating new therapeutic interventions(6, 33, 34) . Elevated IgE levels are a hallmark clinical feature of canine atopic dermatitis and closely mirror the immunological response observed in human AD, highlighting the similarity in disease manifestation between the two species (20, 35, 36). In this study, we utilized a BALB/c mouse model to examine the effects of DNCB and DNCB+OVA on the development and progression of AD in both acute and chronic stages.

Throughout the experimental period, the body weights of all groups were gradually increased, with no statistically significant differences observed between the groups. However, spleen weight measurements taken on the sacrifice days revealed that both the DNCB and DNCB+OVA groups exhibited increased spleen weights compared to the NC group. The group treated with both DNCB and OVA showed a particularly significant increase (Figure 1E). These results suggest that the increase in spleen weight, or splenomegaly, is due to factors such as inflammation and immune cell infiltration, indicating an inflammatory response consistent with the inflammatory nature of AD(37, 38) .

The evaluation of the gross legions in the dorsal and ear skin exhibited significant differences between the NC group and the treatment groups. During the acute phase, both the DNCB and DNCB+OVA treatment groups displayed severe symptoms, which were reduced to moderate levels in the chronic phase (Figure 2A and 2D). These observations are consistent with prior studies using the DNCB-induced atopic dermatitis model, which demonstrated a pattern where dermatitis severity initially peaked and then gradually decreased over time(30, 39, 40) . DNCB acts as an incomplete hapten that can covalently bond with soluble portions of epithelial proteins, forming a complete antigen that stimulates the production of sensitized lymphocytes (41). Exposure of sensitized lymphocytes to reintroduced DNCB induces a delayed-type hypersensitivity reaction, characterized by localized erythema and induration. This reaction closely mirrors the cutaneous symptoms observed in patients with AD(42, 43) .

OVA is a protein allergen commonly utilized to sensitize immune responses. While previous studies have typically induced immune responses through intraperitoneal injections, this research applied OVA topically to

target tissues(39, 44) . Our experimental results demonstrated that during the acute phase, tissue defects in the superficial skin layers, such as dryness and erosion, were predominant. In the chronic phase, although noticeable tissue defects decreased, there was a significant increase in skin thickness and persistent edema (Figure 2A-2F). These findings closely resemble the clinical manifestations observed in patients with AD during both acute and chronic phases (45).

The different symptoms observed during the two phases can be explained by the changes in cytokine expression patterns. IL-4, a cytokine secreted by Th2 cells, plays a significant role in AD by persistently activating mast cells, which in turn produce more IgE (Figure 3). The DNCB+OVA group showed a significant increase in IL-4 expression over time in dorsal skin, indicating a sustained Th2 response (Figure 3C). Additionally, the IL-4/IFN-γ ratio was higher during the acute phase and decreased in the chronic phase, highlighting dynamic changes in Th1/Th2 balance (Figure 3D and 3E). Aberrant immune responses involving Th1/Th2 cells have been proposed as crucial in the pathogenesis of AD (46, 47). IL-4, secreted by Th2 cells, is closely associated with the biological functions of AD, as it continuously activates mast cells, leading to increased IgE production (48). Consistent with the IL-4 cytokine levels in dorsal tissue, serum IgE levels in the DNCB+OVA group were significantly higher compared to the control group (Figure 3C and 3F). Additionally, prolonged exposure to OVA was associated with an increasing trend in IFN-γ expression (Figure 3B). This cytokine has been implicated in contributing to epidermal barrier dysfunction by reducing the expression levels of ceramides and long-chain fatty acids (49, 50). The mast cell counts and epidermal changes evaluated using toluidine blue staining corroborate these findings (51, 52). Both DNCB and DNCB+OVA treatments resulted in a significant increase in mast cell infiltration and epidermal thickening in both dorsal and ear skin compared to the NC group. These changes were particularly evident during the chronic phase, suggesting prolonged immune activation and skin remodeling (Figures 4A-4D). Increased mast cell infiltration and epidermal changes are hallmarks of AD, further validating the relevance of this model in mimicking canine and human AD pathology (53-55).

The use of OVA, a protein allergen, in combination with DNCB provided a robust model for studying AD. Unlike traditional models that typically utilize intraperitoneal injections to induce immune responses, our approach involved topical application, which closely mimics natural exposure routes in humans. The acute phase primarily exhibited epidermal defects such as dryness and erosion, whereas the chronic phase showed reduced overt tissue damage but persistent thickening and edema (Figures 2A-2F).

Although there are animal models for studying the clinical symptoms of AD, this research specifically focuses on a chemical-induced model. This approach provides valuable insights into AD pathogenesis and potential therapeutic targets, underscoring its significance in AD research.

The interaction between the skin barrier and host microbiota represents an emerging area of research in the pathogenesis and treatment of AD. Dysregulation of immune responses due to microbial interference and allergen-inducing metabolites has led to the development of various therapeutic interventions. Probiotic-based interventions have emerged, with dietary supplementation products enhancing immune modulation and topical formulations such as shampoos and skincare products designed to provide symptomatic relief. According to recent research on probiotic-based therapy for atopic dermatitis, the application of a heat-treated probiotic mixture on the skin of dogs with AD resulted in notable clinical improvements. This intervention provided direct therapeutic benefits without the potential drawbacks of dysbiosis in the skin microbiota, indicating its potential as a safe and effective treatment option(56). There is evidence suggesting that oral administration of a combined formulation of *Lacticaseibacillus paracasei* and kestose reduced pruritus in dogs suffering from atopic dermatitis(57). Recent investigations into the gut-skin microbial relationship have uncovered clear differences in gut microbiota profiles between dogs with atopic dermatitis (AD) and healthy counterparts. These studies show that chronic AD in dogs is associated with marked dysbiosis in the gut microbiome, alongside shifts in skin microbial communities(58, 59). Probiotics have been proven to play a role in mitigating AD symptoms by modulating the immune response and enhancing skin barrier function. Through their ability to decrease inflammation, adjust the Th1/Th2 cytokine ratio, and strengthen gut-skin axis interactions, probiotic interventions have demonstrated promising efficacy in preclinical and clinical trials for AD treatment (60-62). Probiotic application in this mouse model may offer a pathway to innovative treatment strategies for both human AD patients and dogs suffering from AD, given the shared pathophysiological mechanisms. This approach emphasizes the model's value in facilitating the development of therapies applicable across species.

This research demonstrated that DNCB and DNCB+OVA treatments in BALB/c mice effectively induced the acute and chronic phases of AD, highlighting significant similarities with canine and human AD pathology. The experimental groups exhibited increased mast cell infiltration, epidermal thickening, and elevated cytokine levels, such as IL-4 and IFN- γ, validating the utility of this model for studying AD. The acute phase was characterized by pronounced epidermal defects, while the chronic phase revealed persistent skin thickening and inflammation. Notably, dorsal skin cytokine expression patterns indicated a shift in immune responses over time, aligning with the histopathological findings. Although both mouse models showed comparable symptoms and immunological responses.

Acknowledgments

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Tables and Figures

Table 1: List of the qPCR primers used in the study.

Gene		Forward	Reverse	Tm(℃)
House keeping	GAPDH	CTC CCA CTC TTC CAC CTT CG	GCC TCT CTT GCT CAG TGT CC	60
Th1	IL-12	AAC TTT GGC ATT GTG GAA GG	ACA CAT TGG GGG TAG GAA CA	60
Th1	IFN-γ	CGG CAT TGC AAG TTG CTG TA	TCT GTC TGC AGT GGG GAA AC	60
Th2	IL-4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT	58.5

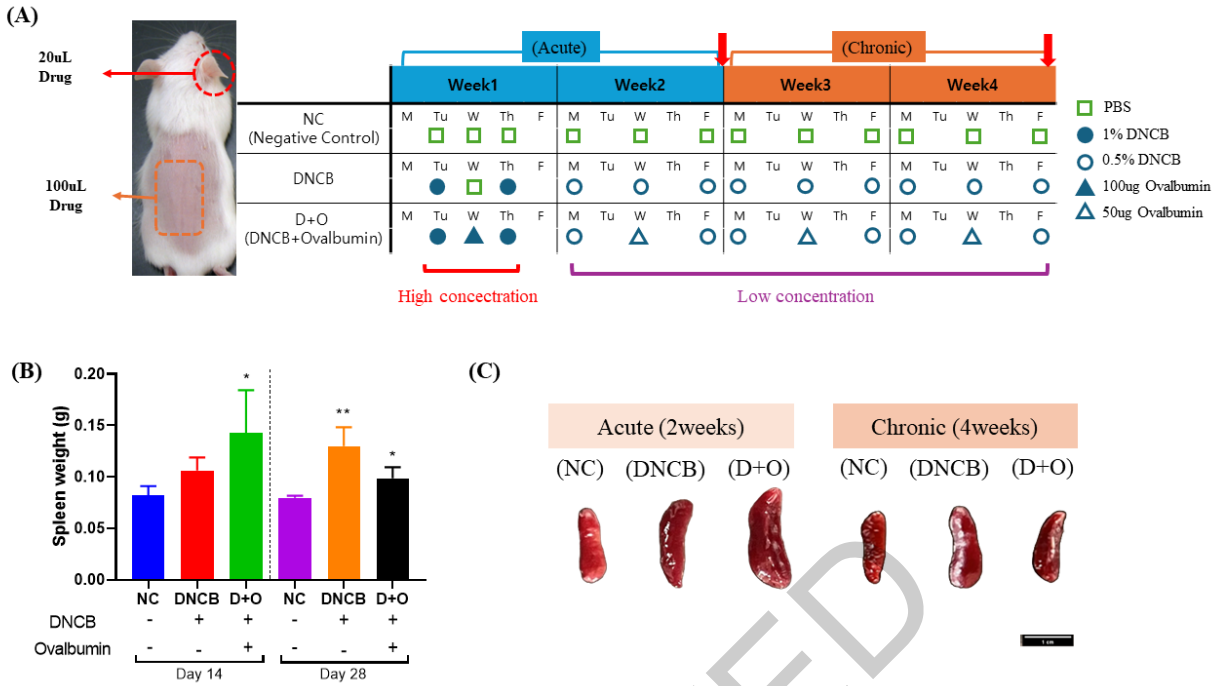

Figure 1: **Experimental design and changes in spleen weight**

(A) Experimental schedule for DNCB and OVA treatment in BALB/c mice (B) Spleen weight on the day of sacrifice. Data are presented as means ± SEM (n = 5 per group). *p < 0.05, **P < 0.01 comparison versus respective time control (C) Representative images showing the differences in spleen appearance based on treatment at the time of sacrifice. Black scale bar represents 1cm.

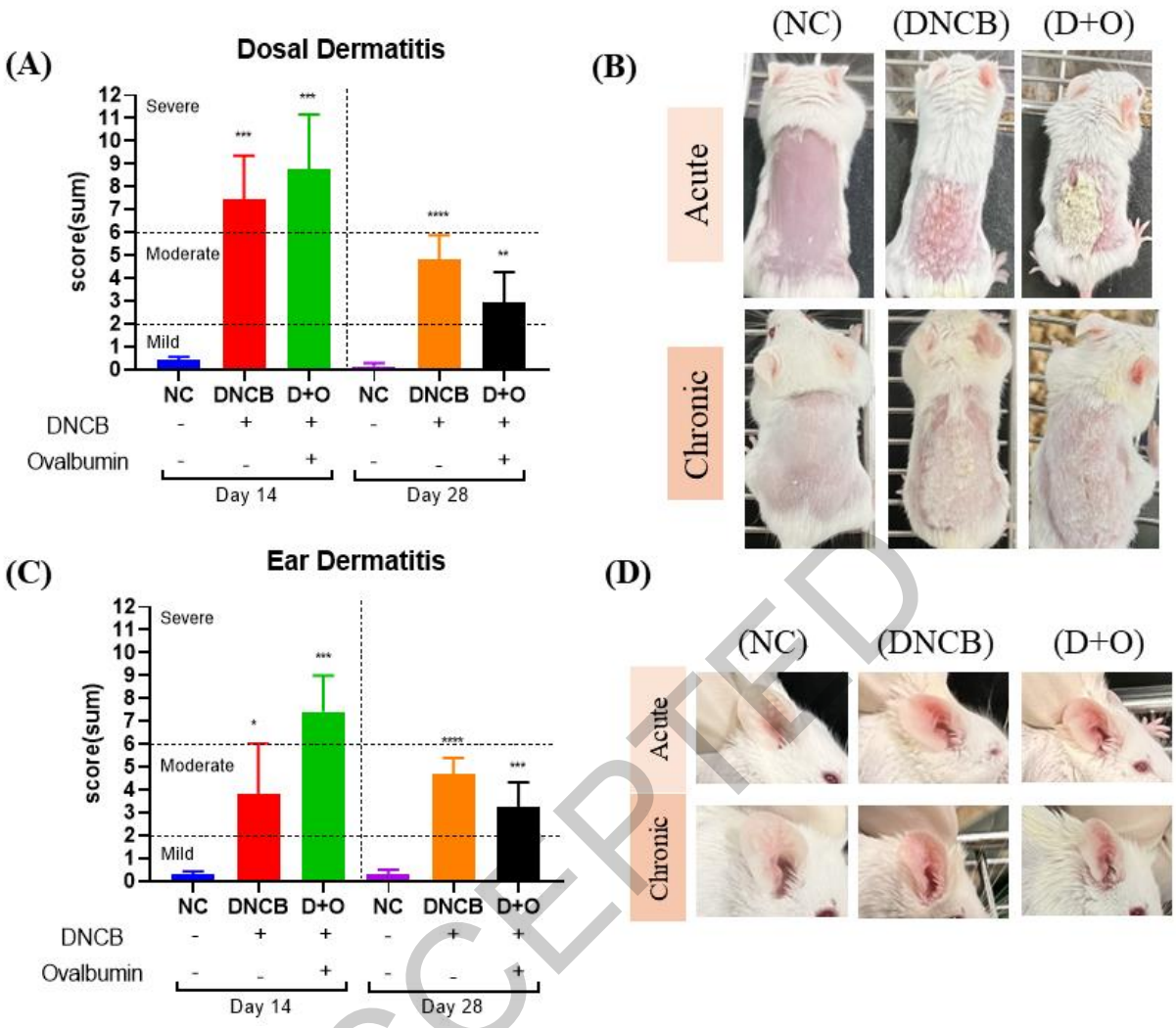

Figure 2: **Evaluation of skin gross lesion**

(A) Combined values of individual dermatitis indices of dorsal skin at both acute and chronic phases (B) Representative images of the dorsal skin at both acute and chronic phases (C) Combined values of individual dermatitis indices of ear (D) Representative images of the ear at both acute and chronic phases. All results are from the final day of sacrifice after 2 weeks and 4 weeks of treatment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

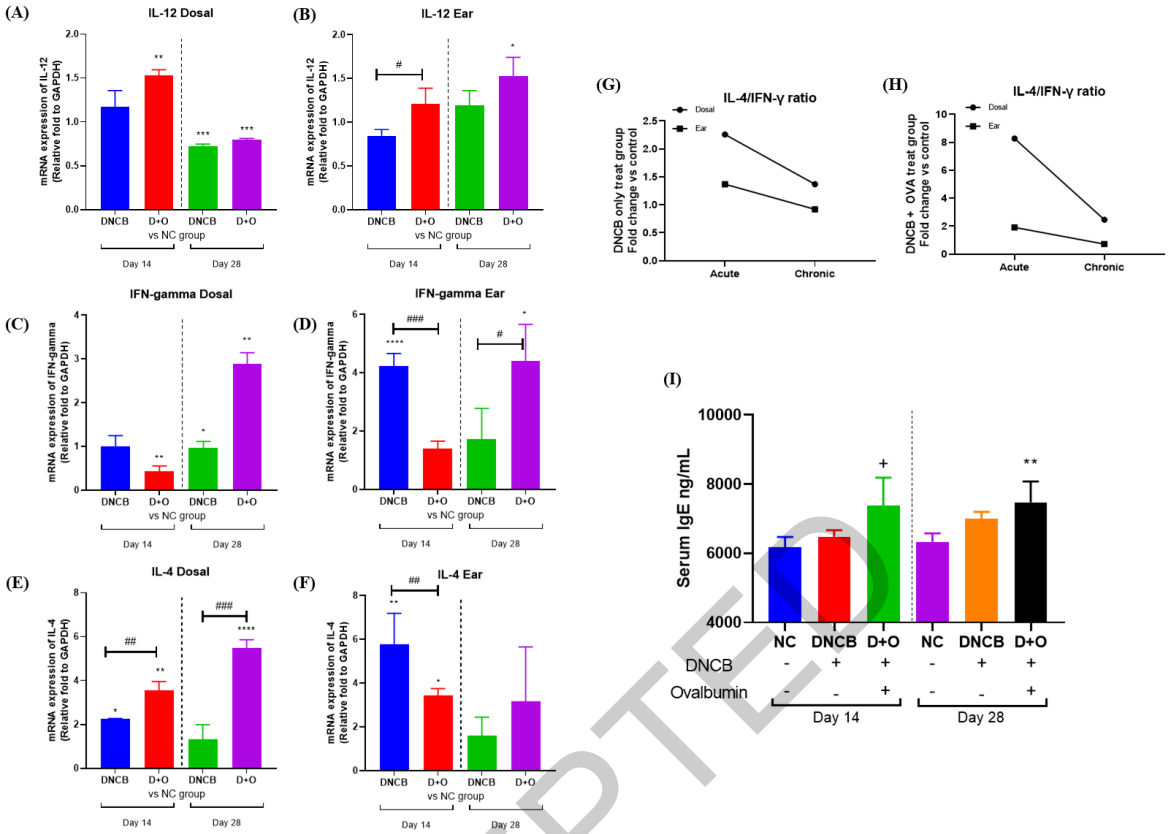

Figure 3: **Evaluation of tissue cytokine gene expression and serum IgE levels**

mRNA expression levels of IL-12 in dorsal (A) and ear (B) skin tissues at days 14 and 28.(B) mRNA expression levels of IFN- γ in dorsal (C) and ear (D) skin tissues at days 14 and 28. mRNA expression levels of IL-4 in dorsal (E) and ear (F) skin tissues at days 14 and 28. IL-4/IFN- γ ratio in dorsal and ear skin tissues of DNCB only treated group at days 14 and 28 (G). IL-4/IFN- γ ratio in dorsal and ear skin tissues of DNCB and OVA treated group at days 14 and 28 (H). Serum IgE levels at days 14 and 28 (I). Data are presented as mean ± SEM (n=5 per group). *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC group.

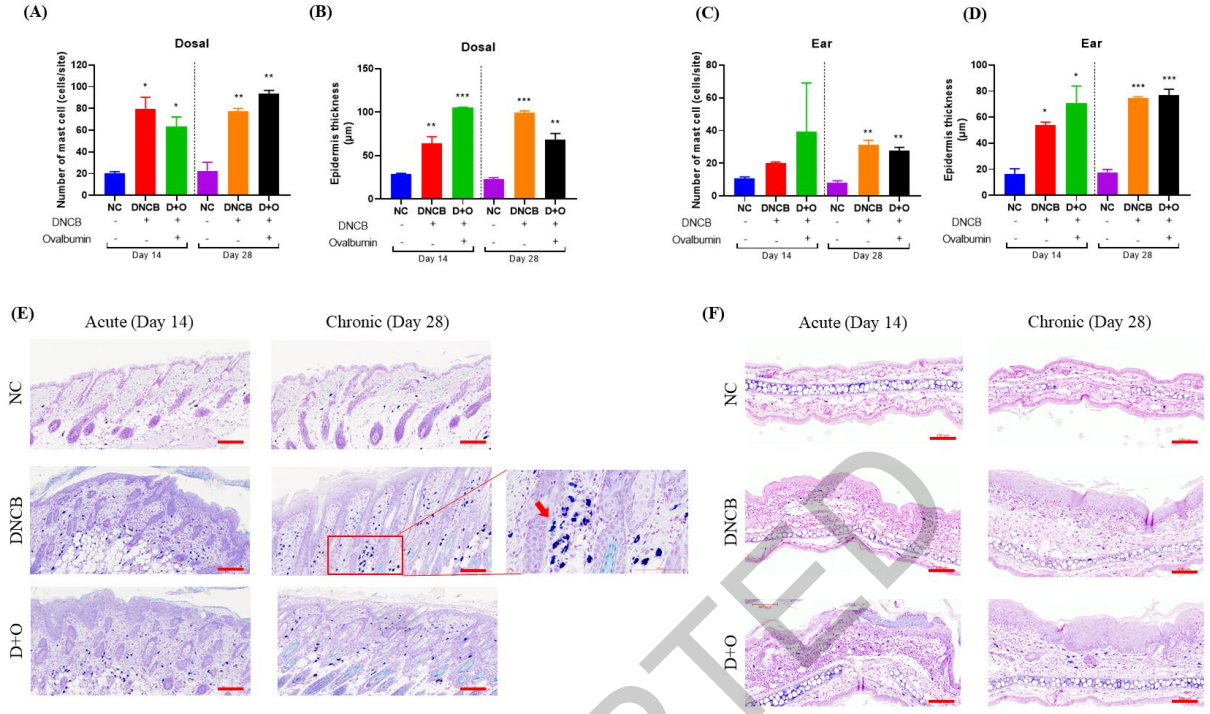

Figure 4: **Mast cell infiltration and epidermal changes in dorsal and ear skin**

Graphs showing the mast cell count (A) and epidermal thickness (B) in the dorsal skin at days 14 and 28. Representative Toluidine Blue-stained images of dorsal skin sections at days 14 and 28 (C). Graphs showing the mast cell count (D) and epidermal thickness (E) in the ear skin at days 14 and 28. Representative toluidine blue-stained images of ear skin sections at days 14 and 28 (F). Data are presented as mean ± SEM, with n=5 per group. Images are at 30% magnification, with a red line indicating a 100 μm scale bar. Red arrow indicate mast cells.