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Research Article

Epicutaneous Sensitization with Ovalbumin, *Staphylococca*l Enterotoxin B and Vitamin D Analogue Induced Atopic Dermatitis in Mice

Jianyun Lu[1], Meilin Chen[1], Yaping Xiang[1], Qingmei Cheng[2], Yunsheng Liang[3], Jinhua Huang[1], Jian Huang[1], Jing Chen[1], Lihua Gao[1*]

[1]Department of Dermatology, The Third Xiangya Hospital of Central South University, Changsha, Hunan, China

[2]Department of Physiology, School of Basic Medical Sciences, Central South University, Changsha, Hunan, China

[3]Department of Dermatology, The Second Xiangya Hospital of Central South University, Changsha, Hunan, China

[*]**Corresponding author:** Lihua Gao, Department of Dermatology, The Third Xiangya Hospital of Central South University, 138 Tongzipo Road, Changsha, 410013, Hunan, China. Tel: +8673188618930; Fax: +8673188618936; E-mail: 49516596@qq.com

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Abstract

Numerous Atopic Dermatitis(AD)mouse models have been established to better understand this disease. However, the establishment of current available AD mouse models is time consuming and highly cost. In this study, we established a cost-efficient AD mouse model by topical application of Ovalbumin (OVA), superantigen *Staphylococcal* Enterotoxin B (SEB) and Calcipotriene Ointment(CO) on the back of BALB/c mice. Experimental mice were topically treated with (1) OVA/SEB and (2) OVA/SEB/CO, or (3) Non-Treated (NT) control every other day during 15 days of induction. Clinical alterations in the skin area were monitored and measured every other day with Reflectance Confocal Microscopy (RCM) and H&E histological staining. Blood samples and skin biopsies were harvested at different times to assess IL-2, IL-4, IL-31, IFN-γ, TNF-α, pruritus-associated Nerve Growth Factor (NGF) and serum IgE levels. Human AD-like cutaneous local inflammatory reaction was characterized by the accumulation of inflammatory cells, increased epidermal thickness and IgE serum levels as well as Th1 cell-associated cytokines (IFN-γ,TNF-α), Th2 cell-associated cytokines(IL-4, IL-31) and pruritus-associated Nerve Growth Factor(NGF)in the experimental group through comparing with the non-treated control group.

Keywords: Atopic Dermatitis; BALB/c mice; Epidermal Thickness; IgE; Inflammatory Cytokines; Vitamin D Analogue

Introduction

Atopic Dermatitis (AD) is one of the most common chronic skin inflammatory diseases that affect 10-20% of infants and 1-3% of adults worldwide. The main feature of AD is the presence of eczematoid, dry and pruritic skin lesions [1,2]. The pathogenesis of AD is multifactorial and involves interactions between environmental and genetic factors, thus making it difficult to be effectively treated and leading to a huge economic burden [3]. Patients with AD usually have a thicker epidermis and the presence of abnormal inflammatory cells in skin lesion, high levels of IgE and IL-4 in the serum compared to healthy individuals. To investigate the etiology of AD, different AD mouse models have been developed, including (1) application of epicutaneous sensitizers such as house dust mite, superantigen, haptens, Tri Nitro Chloro Benzene (TNCB), etc; (2) transgenic mice with manipulated selected genes, such as SEB; (3) spontaneous AD-like skin lesions developed in mice.

Considering the cost and time to establish various models, application of Epicutaneous (EC) sensitizers, such as Ovalbumin (OVA) and superantigens (such as SEB) to induce AD-like skin lesions in mice is one of the most common approaches [4]. Sepergl et al. described the presence of a cellular infiltration rich in T cells and eosinophils, as well as increased levels of IL-4, IL-5 and IFN-γ mRNA in the local AD-like skin lesion area of mice after the appli-

cation of protein antigen OVA [5]. Laouini et al. also showed that EC exposure to SEB induced acute elevated Th2 cytokine IL-4, but not the Th1 cytokine IFN-γ in an AD-like skin lesion area [6]. New insights into the recent findings of Terhi Savink suggested that exposure to both SEB and OVA/SEB elicited a mixed Th1/Th2-associated cytokine and chemokine expression profile in lesional skin locally [7]. Huang et al.also showed that BALB/c mice subjected to EC application of the recombinant mite induced localized dermatitis characterized by pronounced epidermal hyperplasia and spongiosis, skin infiltration with CD4+/CD8+T cells, and a locally and systematically skewed Th2 response [8].

Calcipotriene is a Vitamin D analogue approved as an effective drug for the treatment of psoriasis [9]. Surprisingly, Li et al. found that topical application of Vitamin D analogue was able to induce the expression of Thymic Stromal Lymphopoietin (TSLP) in epidermal keratinocytes, by which to lead to an atopic dermatitis-like skin lesion [10]. Furthermore, Turner et al. demonstrated that application of calcipotriene induced an AD-like phenotype in mice as defined by clinical and histological parameters and increased expression of IL-4 transcripts locally [11].

Although these mouse models are effective to study AD-like lesion, they are costly and time consuming. It is well known that topical OVA and SEB exposure induced AD-like skin lesions, and recently vitamin analogues, like Calcipotriene Ointment (CO), have proven to exacerbate AD. To develop more effective AD-like models, we reported an AD-like mouse model by sensitization of mouse skin with SEB, OVA and CO.

Materials and Methods

Sensitization and Treatment Protocol

Animals: 6-8 weeks old female BALB/c mice were obtained from the laboratory animal center of Xiangya School of Medicine at Central South University, which supplies a pathogen-free and humidity controlled environment for mouse housing. Animal related studies were conducted in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United National Institutes of Health. All experimental mouse protocols were approved by the Review Committee for the Use of Human or Animal Subjects of Xiangya School of Medicine in Central South University.

- SEB (Sigma, USA) stock: Prepared at a concentration of 2.5μg/mL in sterile Phosphate-Buffered Saline(PBS). Aliquots were storage at -20^{0}C until use.
- OVA (Sigma, USA) stock: Prepared at a concentration of 1mg/mL in sterile Phosphate-Buffered Saline(PBS). Aliquots were storage at -20^{0}C until use.
- CO: Produced by LEO Laboratories Limited. Approval number: H20100803
- Others: chloral hydrate (liq.), test chambers (used for patch-test), PBS, medical infusion paste, Veet (depilatory cream, France).

Contact Sensitization and Elicitation Procedure

The skin of the dorsal back(1cm2) of 8 weeks old female mice were marked, shaved and depilated under anesthesia with 10% chloral hydrate and sensitized afterwards accordingly to three groups: (1) OVA/SEB only (2) OVA/SEB/CO and (3) PBS (control group). Treated skin patch would be then sensitized 24 hours later. The dose of SEB and OVA used was 1.25μg and 50μg/patch, respectively. The amount of CO solution used was10mg/patch. The marked skin patch was consecutively treated every other day for a total of eight times during a 15-day experimental period (Figure 1). Results from 8miceper treatment group were collected and analyzed.

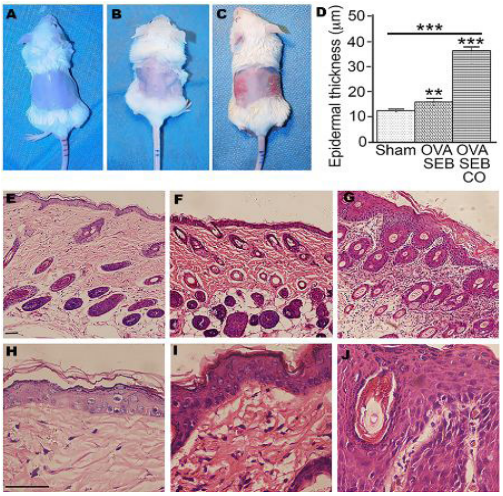

Figure 1: Skin surface feature, epidermal thickness and H&E staining of skin lesion after treatment. Skin surface features after treatment with vehicle **(A)**, OVA/SEB **(B)**, and OVA/SEB/CO **(C)**. (**D)**. Epidermal thickness of mice, measured by RCM. **(E-G)** H&E staining of skin lesion after treatment, vehicle **(E, H)**, OVA/SEB (F, I), and OVA/SEB/CO **(G, H)**, bar size is 20um. OVA/SEB/CO group showed marked hyperplasia of the epidermis, numerous inflammatory cells infiltration, hyperkeratosis and parakeratosis coexist, focal erosions. Note: * P<0.05. # P<0.05.

Skin Histology

After the eighth sensitization, treated skin patches were harvested, fixed in 10% buffered formalin and embedded in paraffin for routine histopathological examination using4μm sections. Images were obtained under the microscope (The Product Mode: OLYMPUS CX41).

Skin Thickness Analysis

The thickness of the skin in each group was measured by Reflectance Confocal Microscopy (RCM, Viva Scope 1500, LUCID NIC) before the mice were sacrificed.

Quantitative Real-time PCR Analysis

Skin biopsies obtained from treated patch areas were snap frozen in liquid nitrogen and stored in -80°C until use. RNA extraction was performed after skin tissue had been grounded into a powder [12]. RNA quantity was determined by measuring optical density at 260/280nm. cDNA was synthesized using 0.5μg of total RNA in total volume of 20μL reaction mixture with Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). The cDNA was then used for Quantitative Real-time PCR (qRT-PCR, ABI Prism 7700; Applied Biosystems, USA) as previously described [13]. The expression of specific genes was analyzed by delta-delta Ct method, with β-actin as an internal control. Three mice from each group were analyzed. The primers used were listed in (Table 1).

Primer Name		Sequence (5'-3')
IL-4	UP	GCCATATCCACGGATGCGACAA
	DN	GGTGTTCTTCGTTGCTGTGAGG
IL-31	UP	CATCTCGGTCATCATAGCACATC
	DN	TTCATCATATTTCCAGGCACAG
IL-2	UP	AGATGAACTTGGACCTCTGCG
	DN	ACTCATCATCGAATTGGCACTC
TNF-α	UP	GGAACTGGCAGAAGAGGCACTC
	DN	GTAGACAGAAGAGCGTGGTGGC
IFN-γ	UP	CTCAAGTGGCATAGATGTGGAAG
	DN	TGCTGATGGCCTGATTGTCT
NGF	UP	ATAAAGGTTTTGCCAAGGACG
	DN	AGTGGGCTTCAGGGACAGAG
β-actin	UP	TTGCAGCTCCTTCGTTGCC
	DN	GACCCATTCCCACCATCACA

Table 1: Primers Sequences for the qPCR.

Plasma Cytokines Analysis

On the 16th day post induction, mice were sacrificed and plasma was collected under centrifugation at 2000 rpmfor 10 minutes, and immediately frozen in -80°C.The samples were complied with Bio-Plex Pro™ kit (BIO-RAD, USA) to examine the levels of cytokines (IL-2, IL-4, IL-31, IFN-γ, TNF-α and NGF), results were analyzed by Bio-Plex system.

Statistical Analysis

Non-parametric Mann-Whitney test and ANOVA were used to compare difference among mice groups, and a two-tailed $p<0.05$ was considered statistically significant for all comparisons.

Results

Topical Exposure to OVA/SEB/CO induces Skin Inflammation and Exacerbates Allergic Dermatitis

Topical exposure to OVA/SEB/CO induced local erythema and epidermal thickening. With continuous stimulation, remarkable local skin changes, such as erythema, oozing, bleeding and crusting were further developed. OVA/SEB sensitized mice only showed mild redness and scaling. The PBS-treated control mice were normal (Figure 1A-C). Histologic analysis was consistent with the skin clinical changes. Topical OVA/SEB/CO exposure significantly induced skin thickening and increased accumulation of inflammatory cells in the dermis compared to mice treated with OVA/SEB only, which mainly induced hyperplasia (increased collagen in the dermis) with a mild inflammatory response(a small amount of inflammatory cells under tested patches), while vehicle (PBS)-sensitized skin patch was normal (Figure 1E-G is×100, H-J is×400). Additionally, as measured by RCM, the epidermal thickness was greatly increased in OVA/SEB/CO-treated skin compared with either PBS and OVA/SEB treated groups (Figure 1D).

OVA/SEB/CO Stimulates the Expression of Th1/Th2-type Cytokines and Pruritus-related Cytokines

Th1-type cytokines, including IFN-γ and TNF-α were increased in the OVA/SEB/CO-treated skin in comparison to the PBS-treated control. The expression level of IL-2 is higher in the OVA/SEB-treated group than the OVA/SEB/CO-treated group (Figure 2). Th2-type cytokines (IL-4, IL-31) expression were locally elevated in the OVA/SEB/CO group when compared to the control group, while IL-4 was only slightly increased in the OVA/SEB group (Figure 2). Additionally, pruritus-related cytokine NGF was significantly increased in the OVA/SEB/CO group compared to OVA/SEB and control groups. The expression of NGF in OVA/SEB and control groups was similar (Figure 2).

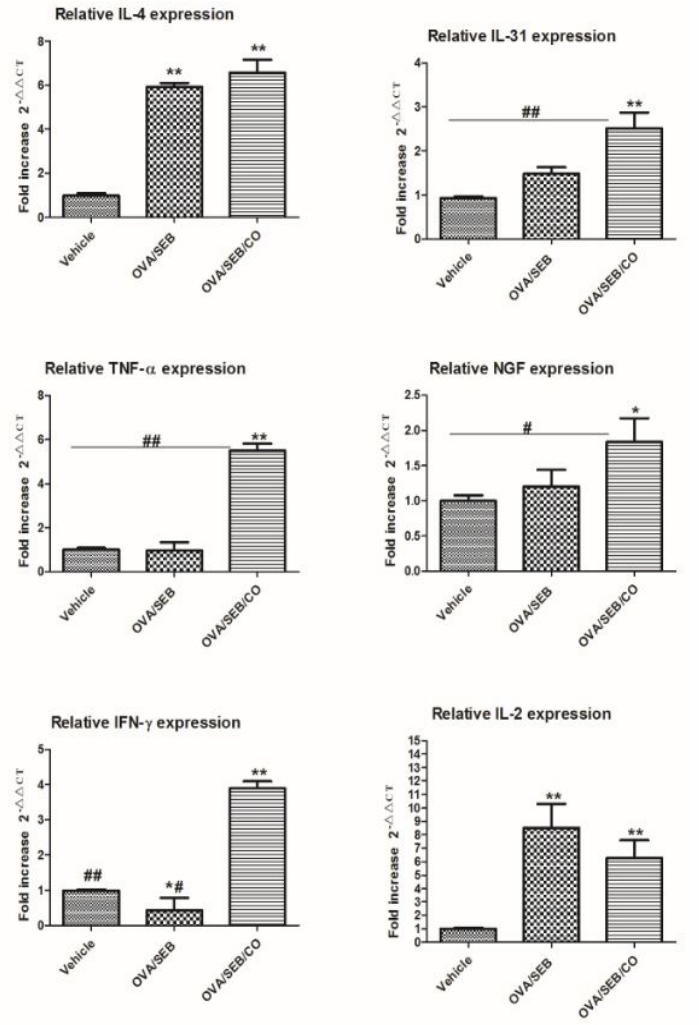

Figure 2: mRNA expression of IL-4, IL-2, IL-31, TNF-α, IFN-γ and NGF in mice skin lesion. Data are presented as mean±standard deviation of normalized relative quantification index. Statistical analysis by ANOVA revealed no significant difference. Note: *Compared with Vehicle group, # Compared with OVA/SEB/CO group.

* P<0.05. **P<0.01. ***P<0.001. # P<0.05. ##P<0.01.

Increased IgE Serum Levels, Th1/Th2-type Cytokines and Pruritus-related NGF after Sensitization with OVA/SEB/CO

As shown in (Figure 3), IgE serum levels were increased in the OVA/SEB/CO group compared to the control group. Th1-type cytokines (TNF-α, IL-2) and Th2-type cytokines (IL-4, IL-31) were increased in both OVA/SEB and OVA/SEB/CO groups. The efficiency of OVA/SEB/CO for AD induction was stronger than OVA/SEB. Surprisingly, IFN-γ plasma levels were same among the three groups. Pruritus-related cytokine NGF levels were significantly increased in the OVA/SEB/CO group compared to the OVA/SEB and control groups, and the NGF levels did not change in the OVA/SEB group (Figure 3).

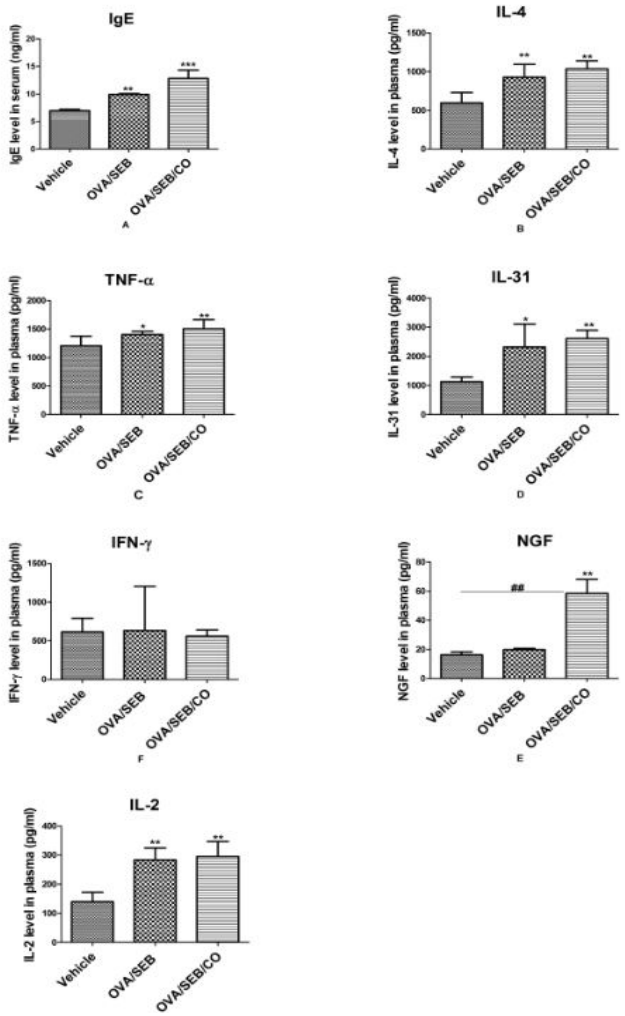

Figure 3: Serum level of IgE and cytokine (IL-2, IL-4, IL-31, TNF-α, IFN-γ and NGF) in mice. (n=5~10). Note: *Compared with Vehicle group, # Compared with OVA/SEB/CO group. * P<0.05. **P<0.01. ***P<0.001. ##P<0.01.

Discussion

We report that topical application of sensitizers, OVA/SEB/CO, on the dorsal skin of mouse back induced a human AD-like animal model with features including skin inflammation, severe allergic dermatitis, thickened epidermis and the presence of inflammatory cells in affected areas as well as increased serum levels of IgE and IL-4. This human AD-like mouse model is easy to establish and convenient to use, thus provides an effective tool to study AD etiology and pathogenesis, as well as develop future therapeutic alternatives for human AD.

Worth noting that EC exposure to OVA/SEB/CO elicited an intense local cutaneous inflammatory response compared to OVA/SEB and control groups. This inflammatory reaction was characterized by erythema, oozing, bleeding and crusting (Figure 1C), increased epidermal thickness with infiltration of numerous inflammatory cells (Figure 1G). Results were verified by RCT, as shown in Figure 1D. RCM, adopted by Confocal Laser Scanning Microscopy (CLSM), is a novel tool that provides skin images in horizontal plane at a level of resolution that allows the view of microanatomic structures [14]. Compared to pathologic examination, RCM is a noninvasive, real-time, dynamic skin diagnostic imaging technique that is used to track the normal morphology and physiological functions of live cells and provides information of the tissue microstructure and metabolic process without the need to sacrifice the mice. RCM was initially used for the diagnosis of skin tumors, hemangiomas, and infectious skin diseases and is useful in the skin examination of patients with AD.

In the acute stage of AD, Th2 cytokines IL-4, IL-5, and IL-13 are predominant, whereas Th1 cytokine IFN-γ is expressed at late stages of the disease [15]. Our study shows a higher expression of Th2-type cytokines IL-4, IL-31 and Th1-type cytokines IFN-γ, IL-2 and TNF-α in the group exposed to OVA/SEB/CO sensitization. These results are consistent with previous studies from Savinko who et al. who suggested that SEB and OVA/SEB exposure elicited a mixed Th1/Th2-associated cytokine expression profile within the skin [6,7].

Additionally, IL-31 and NGF were highly expressed in the OVA/SEB/CO group as shown in (Figure 2 and Figure 3). IL-31 is over expressed in pruritic skin diseases like AD compared with non-pruritic skin inflammation like psoriasis. In the study of patients with AD, activated leukocytes expressed significantly higher levels of IL-31 compared to control groups [16]. NGF levels in patients with AD were also higher compared to a healthy group, and were related to the severity of pruritus, erythema, and inflammatory cells [17].The relationship between NGF and pruritic diseases like AD is still not well understood. Current results suggest that both IL-31and NGF may serve as novel targets for developing antipruritic drugs in the future.

In conclusion, our study indicates that the combination of clinical features, histopathology and cytokine expression panel obtained from our mouse model simulated human AD skin features. Considering that topically application of OVA, SEB, or both to establish an AD mouse model usually requires 7 or more weeks, and CO-treated models require transgenic mice, our current model is in advantage because of the lower cost and time saving, which are crucial for efficient study for this challenging skin disorder, where time, costs and quality of life of patient are key to seek a relief in the cardinal symptom of this pruritus condition. Although the relationship between CO and AD has not been completely understood, data from multiple clinical researches indicated that CO could induce or worsen the condition. To our knowledge, this is the first study that demonstrated the addition of CO to a basic OVA/SEB treated mice could elicit a perfect AD model on normal mice skin, moreover, this model has a very similar skin lesions seen in human patients with AD.

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References

1. Lifschitz C (2015) The impact of atopic dermatitis on quality of life. Ann Nutr Metab 66: 34-40.
2. Leung DY, Jain N, Leo HL (2003) New concepts in the pathogenesis of atopic dermatitis. Curr Opin Immunol 15: 634-638.
3. Boguniewicz M and Leung DYM (2011) Atopic dermatitis: a disease of altered skin barrier and immune dysregulation: Immune response in atopic dermatitis. Immunol Rev 242: 233-246.
4. Jin H, He R, Oyoshi M, Geha RS (2009) Animal Models of Atopic Dermatitis. J Invest Dermatol 129: 31-40.
5. Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, et al. (1998) Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. J Clin Invest 101: 1614-1622.
6. Laouini D, Kawamoto S, Yalcindag A, Bryce P, Mizoguchi E, et al. (2003) Epicutaneous sensitization with superantigen induces allergic skin inflammation. J Allergy Clin Immunol 112: 981-987.
7. Savinko T, Lauerma A, Lehtimäki S, Gombert M, Majuri ML, et al. (2005) Topical Superantigen Exposure Induces Epidermal Accumulation of CD8+ T Cells, a Mixed Th1/Th2-Type Dermatitis and Vigorous Production of IgE Antibodies in the Murine Model of Atopic Dermatitis. J Immunol 175: 8320-8326.
8. Huang CH, Kuo IC, Xu H, Lee YS, Chua KY (2003) Mite Allergen Induces Allergic Dermatitis with Concomitant Neurogenic Inflammation in Mouse. J Invest Dermatol 121: 289-293.

9. Kang S, Yi S, Griffiths CE, Fancher L, Hamilton TA, et al. (1998) Calcipotriene-induced improvement in psoriasis is associated with reduced interleukin-8 and increased interleukin-10 levels within lesions. Br J Dermatol 138: 77-83.

10. Li M, Hener P, Zhang Z, Kato S, Metzger D, et al. (2006) Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. PNAS 103: 11736-11741.

11. Turner MJ, DaSilva-Arnold SC, Yi Q, Mehrotra P, Kaplan MH, et al. (2013) Topical Application of a Vitamin D Analogue Exacerbates Atopic Dermatitis and Induces the Atopic Dermatitis-like Phenotype in Stat6VT Mice. Pediatr Dermatol 30: 574-578.

12. Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156-159.

13. Bouaboula M, Legoux P, Pessegue B, Delpech B, Dumont X, et al. (1992) Standardization of mRNA titration using a polymerase chain reaction method involving co-amplification with a multispecific internal control. J Biol Chem 267: 21830-21838.

14. Gruber MJ, Wackernagel A, Richtig E, Koller S, Kerl H, et al. (2005) Digital image enhancement for *in vivo* laser scanning microscopy. Skin Res Technol 11: 248-253.

15. Leung DY (2000) Atopic dermatitis: New insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 105: 860-876.

16. Olivry T, Mayhew D, Paps JS, Linder KE, Peredo C, et al. (2016) Early Activation of Th2/Th22 Inflammatory and Pruritogenic Pathways in Acute Canine Atopic Dermatitis Skin Lesions. J Invest Dermatol 136: 1961-1969.

17. Mollanazar NK, Smith PK, Yosipovitch G (2016) Mediators of Chronic Pruritus in Atopic Dermatitis: Getting the Itch Out? Clin Rev Allergy Immunol 51: 263-292.