

First report in a dog model of atopic dermatitis: expression patterns of protease-activated receptor-2 and thymic stromal lymphopoietin

Ha-Jung Kim*, Kim Ahrens*, Hee-Myung Park† and Rosanna Marsella*

*Department of Dermatology, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, FL, 32615, USA

†Department of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, #1 Whayang-dong, Kwangjin-gu, Seoul, South Korea

Correspondence: Rosanna Marsella, Department of Dermatology, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, FL 32615, USA. E-mail: marsella@ufl.edu

Background – Protease-activated receptor (PAR)-2 plays a crucial role in inflammation and the skin barrier. Protease-activated receptor-2 is activated by proteolytic enzymes of allergens and stimulates thymic stromal lymphopoietin (TSLP), promoting T-helper 2 cytokines. In humans with atopic dermatitis (AD), increased expression of PAR-2 and TSLP has been reported.

Hypothesis/Objectives – To compare the pattern of staining of PAR-2 and TSLP between normal and atopic beagle dogs. The hypothesis tested was that increased expression is present in atopic dog skin compared with healthy control skin.

Animals – Eight atopic and five normal dogs were challenged for 3 days with house dust mites.

Methods – Skin biopsies were taken to measure the intensity, distribution, integrity and cell staining pattern on days 0, 3 and 10, both objectively and subjectively. Clinical signs were scored and compared between groups.

Results – Atopic dogs showed a significant increase in clinical scores on days 3 (peak of challenge) and 10 (resolution) and a significant condensed staining pattern for TSLP in the stratum basale at all times in comparison to normal dogs. They showed a significant patchy pattern for PAR-2 on days 0 and 3 and for TSLP at all times compared with normal dogs. The intensity itself was not significantly increased in atopic dogs compared with normal animals for both PAR-2 and TSLP.

Conclusions and clinical importance – These preliminary findings do not confirm a difference in the amount of expression but rather in its pattern. Studies using PAR-2 or TSLP inhibitors could shed light on their clinical relevance.

Introduction

Atopic dermatitis (AD) is a common allergic skin disease in both humans and dogs, with an incompletely understood pathogenesis.^{1,2} The common allergens, house dust mites (HDMs), cockroach, mould and pollens, contain proteolytic enzymes, and their enzymatic activities may contribute to the pathogenesis of the allergic response.³

Protease-activated receptors (PARs) are G protein-coupled receptors that are activated by proteolytic cleavage of the amino-terminus and thereby act as sensors for extracellular proteases.⁴ Studies have indicated that PAR-2, as a sensor for endogenous as well

as exogenous proteases,^{5,6} plays numerous physiological and pathophysiological roles in the skin; in particular, it has been described to play an important role in AD.⁷

Thymic stromal lymphopoietin (TSLP), a T-helper 2 (Th2)-promoting cytokine mainly produced by epithelial cells, is highly expressed in the epidermis of human patients and mouse models of AD.^{8,9} Thymic stromal lymphopoietin can be released by PAR-2 activation induced by protease, such as that of HDMs, in the respiratory system or skin.^{10,11} Both PAR-2 and TSLP have been reported at increased levels in lesional skin in humans and mice with AD,^{9,12} but there have been only a few studies about PAR-2 and TSLP in dogs, even with AD.^{12–15} In addition, there is increasing evidence that PAR-2 and TSLP play an important role in barrier function in AD.^{7,16,17} In AD, the altered skin has increased expression of PAR-2 and TSLP, which then trigger Th2-mediated inflammation.^{11,14} This leads us to propose that these mediators have important roles in the development of canine AD.

To date, no study has specifically evaluated how allergen exposure affects the pattern of expression of

Accepted 31 December 2014

Sources of Funding: This study was self-funded.

Conflict of Interest: No conflicts of interest have been declared. This study was presented at the North American Veterinary Dermatology Forum and published as an abstract, *Vet Dermatol* 2014; 25: 155 (abstract).

PAR-2 and TSLP *in vivo* over the course of development of acute AD lesions. These dynamic reactions are best observed using an experimental model where allergen exposure can be controlled and timed. Such a model has been validated.¹⁹ In this model, atopic beagles are challenged with HDMs and observed to develop pruritus and AD lesions. These lesions are clinically, immunologically and ultrastructurally similar to natural, spontaneous AD in both dogs and humans.¹⁸ Before allergen exposure, these dogs are known to have impaired skin barrier function even in nonlesional skin,²⁰ and this skin barrier impairment is aggravated further by allergen exposure. Thus, the purpose of the present study was to investigate the expression of PAR-2 and TSLP in the skin of atopic beagle dogs during the course of allergen challenge and to compare it with normal dogs. To the best of the author's knowledge, this is the first study to attempt to show the changes in expressions of PAR-2 and TSLP during the development of acute AD lesions over time. Our hypothesis was that atopic beagles have increased expression of both PAR-2 and TSLP and that allergen exposure enhances their expression.

Materials and methods

Atopic dogs

The atopic dogs ($n = 8$) had previously been sensitized epicutaneously to the HDM *Dermatophagoides farinae* and were known to develop skin lesions and pruritus upon allergen exposure.¹⁸ These dogs were sensitized to HDM at a young age and showed low-grade AD when not exposed to HDM; they reacted strongly when under allergenic challenge.

Study protocol approval

The dog's care and study protocol were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee.

Experimental design

Atopic beagle dogs ($n = 8$) belonging to a colony that had been validated as a model of AD were used. Healthy, unrelated beagle dogs ($n = 5$) with no history of skin disease and pruritus were included as control animals. Clinical signs were scored blindly using a validated scoring system [Canine Atopic Dermatitis Extent and Severity Index (CADESI)]. The dog's intact skin surface was exposed to 50 mg of *D. farinae* allergen extract (Greer, Lenoir, NC, USA) placed on the bottom of their kennels. All dogs were allowed to stay in the kennels for 3 h/day, for 3 days consecutively.¹⁸ Skin biopsies were taken before (day 0), during (day 3) and after the end of allergen challenge (day 10). Indirect immunofluorescence was performed using rabbit polyclonal anti-PAR-2 antibody (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and a mouse monoclonal anti-TSLP antibody (Imgenex, Littleton, CO, USA). The intensity of staining in slide photographs was analysed objectively using a computer imaging program (ImageJ® 1.41; <http://rsb.info.nih.gov/ij/>) as previously reported.²¹ The intensity, distribution, integrity and cellular staining pattern were also analysed subjectively by four investigators blinded to sample source.

Clinical evaluation

The CADESI used in the present study is a modified version of the validated CADESI-03.² Dogs were scored at baseline [day 0; before allergen exposure (nonlesional skin)], 3 days after the beginning of the challenge [day 3; acute lesional skin (only atopic dogs)] and 7 days after discontinuation of allergen exposure [day 10; postchal-

lenged skin with resolution of the cutaneous lesions (only atopic dogs)].

Indirect immunofluorescence

Skin biopsy specimens taken from the inguinal region were fixed in 10% neutral buffered formalin for 48 h and then transferred into phosphate-buffered saline (PBS) until they were dehydrated and embedded in paraffin. Sections obtained on days 0, 3 and 10 were embedded in the same block for each dog. Sections were cut at 3 µm and placed on Superfrost positively charged slides (Fisher Scientific, Pittsburgh, PA, USA). Slides were deparaffinized, rehydrated to PBS, then placed in citrate buffer, pH 6.0, for 10 min in sealed tubes, which were then boiled in a water bath for 10 min, removed and set at room temperature for 10 min to unmask antigenic sites. After washing in PBS, slides were quenched in 3% hydrogen peroxide in methanol, washed in PBS, then blocked in Power block from the BioGenex HRP kit (BioGenex, San Ramon, CA, USA), postblocked in 10% normal donkey serum for 20 min, and then a rabbit polyclonal anti-PAR-2 antibody for dogs (Santa Cruz Biotechnology, Inc.) and a mouse monoclonal anti-TSLP antibody for humans (Imgenex), diluted at 1:20, were added for 1.5 h. Phosphate-buffered saline was used as a negative control. The sections were washed, followed by addition of a donkey anti-rabbit IgG bound with a green fluorochrome (Alexa Fluor® 488; Invitrogen, Grand Island, NY, USA) at a 1:1000 dilution for PAR-2 and a donkey anti-mouse IgG bound with a red fluorochrome (Alexa Fluor® 594; Invitrogen) at a 1:1000 dilution for TSLP, and then incubated for 45 min at room temperature in the dark. Slides were washed and coverslipped using Vectashield® Mounting Medium with 4',6-diamidino-2-phenylindole (DAPI) for nuclear detection (Vector Laboratories, Burlingame, CA, USA).

Skin sections were examined using an upright fluorescence microscope (Leica Microsystems, Buffalo Grove, IL, USA). The images were analysed using MagnaFire Software version 070121-01A (Optonics, Goleta, CA, USA).

Subjective evaluation

Six photographs of the slides were evaluated blindly by four investigators and scored for intensity on a scale of 0–3 (0 = absent, 1 = mild, 2 = moderate and 3 = severe). The score was calculated and the mean value used for statistical analysis. For the distribution, the epithelium was divided into four layers (stratum corneum, granulosum, spinosum and basale) and evaluated for staining characteristics. If there were evident staining objects in the dermis, it was also noted. The epidermal staining pattern was evaluated as 'homogeneous' or 'patchy', and cellular staining pattern was scored as 'cytoplasmic' or 'nuclear'. They were calculated by percentage for statistical analysis.

Statistical analysis

The data were tested for normality using the Kolmogorov–Smirnov test. The data were then analysed using Student's unpaired *t*-test to evaluate the differences between the two groups (normal versus atopic) at each time point (between-groups effect). Repeated-measures ANOVA with subsequent Tukey's multiple comparison test were also performed to analyse the over time effect (within the group). A *P*-value of <0.05 was considered statistically significant. GraphPad Prism 5.0 statistical software (GraphPad, San Diego, CA, USA) was used for the analysis.

Results

Clinical evaluation

The CADESI scores increased significantly in atopic dogs on days 3 and 10 compared with normal dogs and with their own baseline ($P = 0.0028$ and $P = 0.0005$, respectively; Figure 1a).

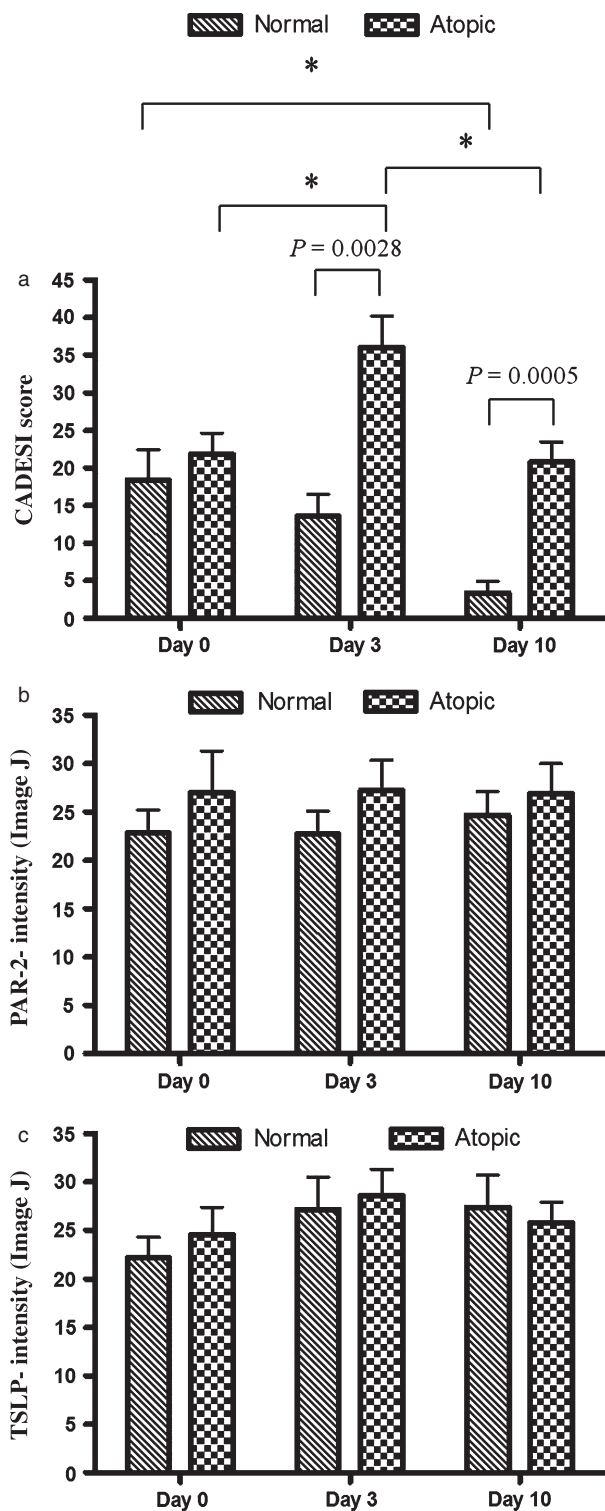


Figure 1. Evaluation of clinical scores and immunofluorescence intensity of protease-activated receptor (PAR)-2 and thymic stromal lymphopoietin (TSLP) in a dog model of atopic dermatitis. (a) Canine Atopic Dermatitis Extent and Severity Index (CADESI) scores during the course of 3 day allergen challenge. Significant differences were found between normal and atopic dogs on days 3 and 10. $*P < 0.05$ unpaired *t*-test. Objective intensity analyses of PAR-2 (b) and TSLP (c) using the ImageJ program. Day 0, before allergen exposure; day 3, acute allergen-challenged skin; and day 10, postchallenged skin with resolution of the cutaneous lesions. No significant differences were observed between samples from atopic and normal dogs.

Intensity of staining of the skin samples

For intensity of PAR-2 and TSLP, there were no significant effects of group, time and group $\times$ time interaction in objective and subjective scores (Figure 1b,c and Figures S1–S3 in Supporting information).

Distribution, staining homogeneity and cellular staining pattern of the skin samples

The distribution of PAR-2 was mostly limited to the stratum granulosum and spinosum (Figures 2a, 3 and 4a), while TSLP was present in all layers of the epidermis in both groups (Figure 3). Interestingly, in atopic dogs, TSLP was significantly condensed in distribution to the stratum basale in comparison to normal dogs at all time points and showed even greater expression after challenge (day 3) compared with baseline (Figures 2b and 5b).

The staining homogeneity for PAR-2 was significantly patchier in atopic dog samples on days 3 and 10 (Figure 2c), while TSLP was patchier in atopic than normal dogs at all time points (Figure 2d). An additional, remarkable finding was that TSLP staining was also noted in some inflammatory cells in the dermis (Figure 4b).

With regard to the cellular staining pattern, most slides showed cytoplasmic staining for PAR-2 and TSLP (Figure 5).

Discussion

This study is the first to describe the relationship between HDM challenge and the pattern of expression of PAR-2 and TSLP *in vivo* using a canine experimental model of AD. The most interesting finding of this study is that the patterns of expression of PAR-2 and TSLP were significantly different in atopic dogs compared with normal dogs even before exposure to HDMs.

The protease activities produced by HDMs promote a Th2 response and increase PAR-2 and TSLP.^{10,11,22,23} The proteases from allergens are associated with various homeostatic responses in skin disease, such as inflammation, immune responses, host defense, chemotaxis, cytokine expression, vascular function, tissue repair and apoptosis.⁵ Serine proteases, such as trypsin or mast cell tryptase, can activate PAR-2 signals.¹³ The present AD model, which was developed by HDM sensitization, could present some unique changes of the homeostatic responses.

There have been previous studies that reported an increase of PAR-2 and TSLP expression in lesional skin with AD in humans, mice and dogs.^{9,12,15} In the present study, however, the intensity of PAR-2 and TSLP in immunofluorescence was not significantly increased in atopic dogs compared with normal animals. Another study also reported that the mRNA level of PAR-2 was not significantly different between atopic dogs (lesional skin) and normal animals; it concluded that the level of PAR-2 mRNA transcription may not be associated with development of canine AD.¹³

The reported level of TSLP mRNA was significantly increased in the lesional and nonlesional skin samples of dogs with AD compared with normal dogs, but there was no significant difference between lesional and nonlesional skin for the expression.¹⁵ While the previous studies

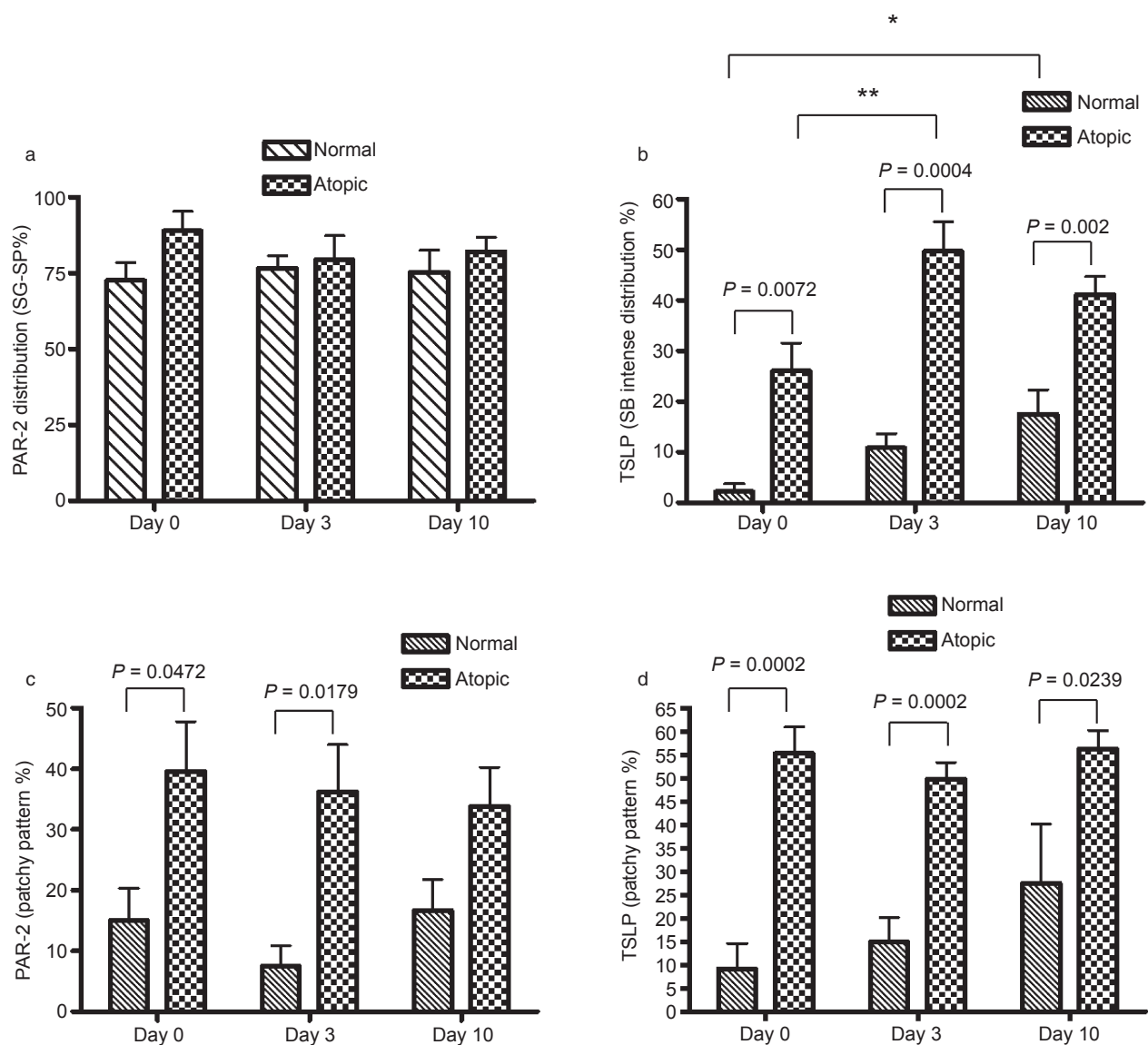


Figure 2. Evaluation of staining distribution and pattern on indirect immunofluorescence examination for PAR-2 and TSLP in the dog model of atopic dermatitis versus normal dogs. (a) No significant differences were seen for the percentage of PAR-2 distribution from stratum granulosum to stratum spinosum. (b) Significant differences were seen for the percentage of intense TSLP in the stratum basale between normal and atopic dogs on days 0, 3 and 10. (c) Significant differences were found for the percentage of patchy pattern of PAR-2 on days 0 and 3. (d) Significant differences were seen for the percentage of patchy staining of the skin for TSLP between normal and atopic dogs on days 0, 3 and 10. Abbreviations: SB, stratum basale; SG, stratum granulosum; and SP, stratum spinosum.

evaluated mRNA levels, in our study we investigated protein expression in a semiquantitative manner. The results in our study showed no differences among lesional (day 3; acute challenge), nonlesional (day 0; baseline) and resolution skin samples (day 10; chronic lesion) in the atopic beagles and between the atopic model and normal skin biopsies at all time points for both PAR-2 and TSLP. Possible reasons may be that the issue is not a matter of quantity but a matter of activation. For example, they may not be activated in the normal skin. In fact, their staining patterns in the atopic skin were very different from normal skin in the present study, which could be an indication of activation signals in the skin.

Thymic stromal lymphopoietin is associated with activation of Langerhans cells in the skin. It was stained together with either langerin (the Langerhans cell marker) or dendritic cell-lysosome-associated membrane protein in dou-

ble immunohistology experiments in atopic human skin.²⁴ Protease-activated receptor-2 also promotes dendritic cell antigen transport and T-cell activation in animal models of inflammatory and autoimmune disease;²⁵ therefore, PAR-2 and TSLP could be crucial components in the development of allergic inflammation in atopic skin by interaction with immune cells, including dendritic cells and T cells. Those interactions could lead to the nonhomogeneous staining patterns in the skin tissue. Further functional studies are needed in this model to investigate the mechanism of action.

Another reason for the lack of significant difference may be the small size of the groups. Replicated studies using more dogs are needed to confirm these preliminary results.

It is well known that the skin barrier function is damaged in human AD patients, both in acute eczematous lesions and in clinically unaffected skin.²⁵ The present AD

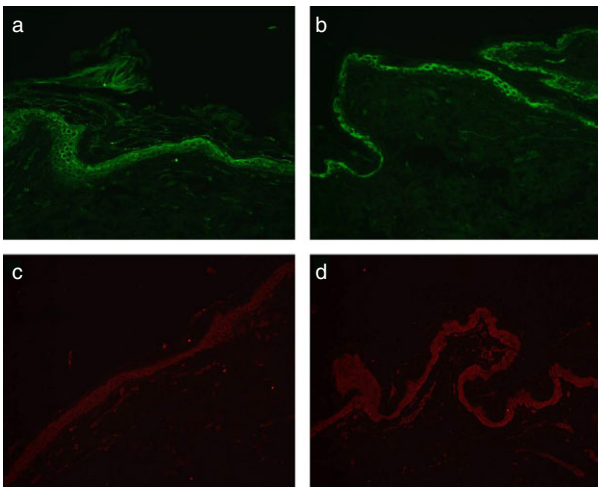


Figure 3. Indirect immunofluorescences of PAR-2 (green; a,b) and TSLP (red; c,d) in normal (a,c) and atopic skin (b,d) on day 3 ($\times 200$ magnification).

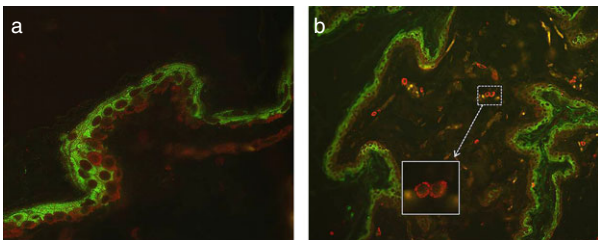


Figure 4. Double indirect immunofluorescences of PAR-2 (green) and TSLP (red) in atopic skin on day 3. (a) Double staining of PAR-2 and TSLP in atopic skin on day 3 ($\times 400$ magnification). (b) Stained inflammatory cells most consistent with mast cells ($\times 400$ magnification in the white square box) were common in atopic skin on day 3 ($\times 200$ magnification).

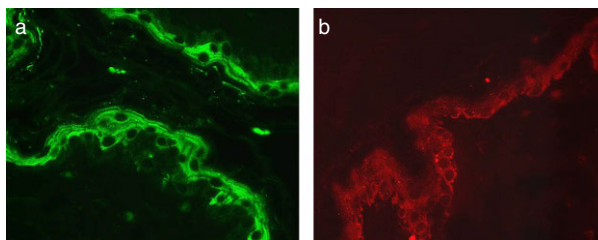


Figure 5. Evaluation of cellular staining patterns of PAR-2 (a) and TSLP (b) in the dog model of atopic dermatitis on day 3 ($\times 400$ magnification). Note the intense cytoplasmic staining for PAR-2 (a) and TSLP (b). Thymic stromal lymphopoietin showed a condensed staining in the stratum basale with patchy distribution on day 3.

model previously confirmed altered filaggrin mRNA expression and protein distribution, which represent a defect of the skin barrier.²⁶ Protease-activated receptor-2 and TSLP are closely related to skin inflammation and barrier function in AD,^{7,16} because they are increased in lesional skin of AD patients.^{18,21} In the present study, the staining homogeneity of PAR-2 and TSLP was much more patchy in the atopic model compared with normal skin at most time points and was not changed by allergen challenge, suggesting a primary defect. This model was developed by sensitization with HDMs at an early age, and the

dogs maintained a low-grade of AD in between allergen exposure. There is evidence that early life exposure to protease-containing allergens leads to allergic sensitization to the allergens, which has been shown to have a strong correlation with the incidence of allergic diseases and their exacerbations.^{27–30} Activation of PAR-2 by protease activity mediates allergic sensitization and inflammation.³¹ Protease-activated receptor-2 may therefore have been activated at an early age by allergen exposure in the present AD model and could have affected the inflammation and barrier function of the skin during the study period.

Clinical scores were significantly increased after allergen challenge in atopic dogs. The change in clinical score was not correlated with the intensity of staining. Instead, TSLP was significantly condensed in the stratum basale layer in the atopic group compared with normal skin at all time points and particularly after challenge, suggesting a role for HDMs in the distribution of this cytokine. Langerhans cells are mainly distributed in the stratum basale, and TSLP activates and initiates Langerhans cells to promote Th2-mediated inflammation in the AD skin.³² We therefore speculate that in this model TSLP is activated in the stratum basale after allergen sensitization and challenge.

Protease-activated receptor-2 and TSLP respond to immune and inflammatory cells, such as dendritic cells, T cells, B cells, mast cells, basophils and eosinophils.^{7,8} Interestingly, the present study showed that only TSLP and not PAR-2 was distributed in some inflammatory cells (mast cell similar-shaped cells) in the dermis. This could mean that TSLP may play a role in dermal inflammation in the skin of the model. Thymic stromal lymphopoietin, a Th2-associated cytokine produced by inflammatory cells and epithelial cells, is known to induce Th2 cell recruitment and allergic inflammation via dendritic cell stimulation in response to allergen challenge, microbial infections and inflammation.³³

Proteases in HDMs can activate PAR-2, leading to increased TSLP and then the Th2-mediated immune response could be induced.^{10,11} Our study did not investigate the mutual relationship between them. A previously published study¹⁵ found that in cultured primary canine normal keratinocytes, TSLP expression increased after stimulation with HDM allergen extract. Further studies are needed in this experimental model to evaluate the response of TSLP *in vivo* after HDM challenges using keratinocytes from atopic dogs.

In conclusion, the present study showed changes of expression pattern in the development of AD over time using an experimental AD model in dogs. The impaired skin barrier and activated inflammatory signals may have an impact on the phenotypes of PAR-2 and TSLP expression in this study, and it can represent a significant activation of allergic reaction by allergen exposure in the AD skin. Further mechanistic studies of PAR-2 and TSLP, related to activation of allergic inflammation by protease from allergen, are needed in this dog model of AD.

Acknowledgements

We thank Domenico Santoro (Department of Dermatology, College of Veterinary Medicine, University of Florida), who helped with clinical evaluation in this study.

References

- Hillier A, Griffin CE. The ACVD task force on canine atopic dermatitis (I): incidence and prevalence. *Vet Immunol Immunopathol* 2001; 81: 147–151.
- Bieber T. Atopic dermatitis. *Ann Dermatol* 2010; 22: 125–137.
- Jacquet A. Interactions of airway epithelium with protease allergens in the allergic response. *Clin Exp Allergy* 2011; 41: 305–311.
- Coughlin SR. Thrombin signaling and protease-activated receptors. *Nature* 2000; 407: 258–264.
- Rattenholl A, Steinhoff M. Role of proteinase-activated receptors in cutaneous biology and disease. *Drug Dev Res* 2003; 59: 408–416.
- Rattenholl A, Steinhoff M. Proteinase-activated receptor-2 in the skin: receptor expression, activation and function during health and disease. *Drug News Perspect* 2008; 21: 369–381.
- Lee SE, Jeong SK, Lee SH. Protease and protease-activated receptor-2 signaling in the pathogenesis of atopic dermatitis. *Yonsei Med J* 2010; 51: 808–822.
- Ziegler SF. The role of thymic stromal lymphopoietin (TSLP) in allergic disorders. *Curr Opin Immunol* 2010; 22: 795–799.
- Kim HJ, Kim YJ, Kang MJ *et al.* A novel mouse model of atopic dermatitis with epicutaneous allergen sensitization and the effect of *Lactobacillus rhamnosus*. *Exp Dermatol* 2012; 21: 672–675.
- Post S, Nawijn MC, Hackett TL *et al.* The composition of house dust mite is critical for mucosal barrier dysfunction and allergic sensitization. *Thorax* 2012; 67: 488–495.
- Kouzaki H, O'Grady SM, Lawrence CB *et al.* Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2. *J Immunol* 2009; 183: 1427–1434.
- Komatsu N, Saijoh K, Kuk C *et al.* Human tissue kallikrein expression in the stratum corneum and serum of atopic dermatitis patients. *Exp Dermatol* 2007; 16: 513–519.
- Maeda S, Maeda S, Shibata S *et al.* Molecular cloning of canine protease-activated receptor-2 and its expression in normal dog tissues and atopic skin lesions. *J Vet Med Sci* 2009; 71: 577–582.
- Maeda S, Maeda S, Ohno K *et al.* Protease-activated receptor-2 induces proinflammatory cytokine and chemokine gene expression in canine keratinocytes. *Vet Immunol Immunopathol* 2013; 153: 17–25.
- Klukowska-Rötzler J, Chervet L, Müller EJ *et al.* Expression of thymic stromal lymphopoietin in canine atopic dermatitis. *Vet Dermatol* 2013; 24: 54–59.
- Nakajima S, Igyártó BZ, Honda T *et al.* Langerhans cells are critical in epicutaneous sensitization with protein antigen via thymic stromal lymphopoietin receptor signaling. *J Allergy Clin Immunol* 2012; 129: 1048–1055.
- Demehri S, Morimoto M, Holtzman MJ *et al.* Skin-derived TSLP triggers progression from epidermal-barrier defects to asthma. *PLoS Biol* 2009; 19: e1000067.
- Marsella R, Girolomoni G. Canine models of atopic dermatitis: a useful tool with untapped potential. *J Invest Dermatol* 2009; 129: 2351–2357.
- Marsella R, Olivry T, Nicklin C *et al.* Pilot investigation of a model for canine atopic dermatitis: environmental house dust mite challenge of high-IgE-producing beagles, mite hypersensitive dogs with atopic dermatitis and normal dogs. *Vet Dermatol* 2006; 17: 24–35.
- Hightower K, Marsella R, Flynn-Lurie A. Effects of age and allergen exposure on transepidermal water loss in a house dust mite-sensitized beagle model of atopic dermatitis. *Vet Dermatol* 2010; 21: 88–95.
- Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001; 29: 2002–2007.
- Comoy EE, Pestel J, Duez C *et al.* The house dust mite allergen, *Dermatophagoides pteronyssinus*, promotes type 2 responses by modulating the balance between IL-4 and IFN- γ . *J Immunol* 1998; 160: 2456–2462.
- Kheradmand F, Kiss A, Xu J *et al.* A protease-activated pathway underlying Th cell type 2 activation and allergic lung disease. *J Immunol* 2002; 169: 5904–5911.
- Soumelis V, Reche PA, Kanzler H *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; 3: 673–680.
- Ramelli G, Fuertes S, Narayan S *et al.* Protease-activated receptor 2 signalling promotes dendritic cell antigen transport and T-cell activation *in vivo*. *Immunology* 2010; 129: 20–27.
- Santoro D, Marsella R, Ahrens K *et al.* Altered mRNA and protein expression of filaggrin in the skin of a canine animal model for atopic dermatitis. *Vet Dermatol* 2013; 24: 329–336.
- Arshad SH. Indoor allergen exposure in the development of allergy and asthma. *Curr Allergy Asthma Rep* 2003; 3: 115–120.
- Litonjua AA, Carey VJ, Burge HA *et al.* Exposure to cockroach allergen in the home is associated with incident doctor-diagnosed asthma and recurrent wheezing. *J Allergy Clin Immunol* 2001; 107: 41–47.
- Wang J, Visness CM, Calatroni A *et al.* Effect of environmental allergen sensitization on asthma morbidity in inner-city asthmatic children. *Clin Exp Allergy* 2009; 39: 1381–1389.
- Rosenstreich DL, Eggleston P, Kattan M *et al.* The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med* 1997; 336: 1356–1363.
- Arizmendi NG, Abel M, Mihara K *et al.* Mucosal allergic sensitization to cockroach allergens is dependent on proteinase activity and proteinase-activated receptor-2 activation. *J Immunol* 2011; 186: 3164–3172.
- Ebner S, Nguyen VA, Forstner M *et al.* Thymic stromal lymphopoietin converts human epidermal Langerhans cells into antigen-presenting cells that induce proallergic T cells. *J Allergy Clin Immunol* 2007; 119: 982–990.
- Liu YJ, Soumelis V, Watanabe N *et al.* TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annu Rev Immunol* 2007; 25: 193–219.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Indirect immunofluorescence evaluation of PAR-2 over time in the AD dog model versus normal dogs ($\times 200$).

Figure S2. Indirect immunofluorescence evaluation of TSLP over time in the AD dog model versus normal dogs ($\times 200$).

Figure S3. Subjective evaluation of immunofluorescence intensity of PAR-2 (a) and TSLP (b) in the AD dog model versus normal dogs.

Résumé

Contexte – Les récepteurs PAR-2 (protease-activated receptor) jouent un rôle crucial dans l'inflammation et la barrière cutanée. PAR-2 est activé par des enzymes protéolytiques d'allergènes, stimule les TSLP (thymic stromal lymphopoietin) et les cytokines T-helper 2. Dans la dermatite atopique de l'homme (AD) une augmentation de l'expression de PAR-2 et de TSLP a été décrite.

Hypothèses/Objectifs – Comparer les patrons de coloration de PAR-2 et TSLP entre les chiens sains et des beagles atopiques. L'hypothèse testée était qu'une augmentation de l'expression est présente dans la peau de chien atopique comparée à la peau de chiens sains.

Sujets – Huit chiens atopiques et cinq chiens sains ont été testés pendant 3 jours avec des acariens de la poussière de maison.

Méthodes – Des biopsies cutanées ont été prélevées pour évaluer l'intensité, la distribution et l'intégrité des patrons de coloration cellulaire à jour 0, 3 et 10 à la fois objectivement et subjectivement. Les signes cliniques ont été notés et comparés entre les groupes.

Résultats – Les chiens atopiques ont montré des scores cliniques augmentés aux jours 3 (pic du test) et 10 (résolution) et un patron de coloration condensé significatif de TSLP dans la couche basale à tous les temps en comparaison aux chiens sains. Ils montraient un patron en patch significatif pour PAR-2 aux jours 0 et 3 et tous les temps pour TSLP en comparaison avec les chiens sains. L'intensité elle-même n'était pas significativement augmentée chez les chiens atopiques en comparaison avec les chiens sains à la fois pour PAR-2 et pour TSLP.

Conclusions et importance clinique – Ces données préliminaires ne confirment pas une différence dans la quantité d'expression mais plutôt dans sa distribution. Des études utilisant des inhibiteurs de PAR-2 et TSLP pourraient jeter la lumière sur leur importance clinique.

Resumen

Introducción – el receptor de la proteasa activada (PAR-2) juega un papel fundamental en la inflamación y en la función de barrera de la piel. El receptor de proteasa activada tipo 2 se activa mediante enzimas proteolíticas de alérgenos y estimula la linfopoietina del estroma del timo (TSLP), promoviendo el desarrollo de citoquinas ayudantes de tipo T2. En humanos con dermatitis atópica (AD), hay una expresión incrementada de PAR-2 y de TSLP.

Hipótesis/Objetivos – comparar los patrones de expresión de PAR-2 y TSLP entre perros normales y perros atópicos de raza Beagle. La hipótesis a probar era que existiría una expresión incrementada en perros atópicos comparados con los perros normales.

Animales – ocho perros atópicos y cinco perros sanos fueron expuestos durante tres días ácaros del hogar.

Métodos – se tomaron muestras de biopsias de la piel para medir la intensidad, distribución, integridad y patrón de tinción en los días 0, 3 y 10 tanto objetivamente como subjetivamente. Los signos clínicos se valoraron y se compararon entre los grupos.

Resultados – los perros atópicos mostraban un marcado incremento de los signos clínicos al día tres (pico de la exposición) y 10 (resolución) y un patrón de tinción significativamente condensado para TSLP en el estrato basal en todos los tiempos en comparación con los perros normales. Mostraron un patrón multifocal significativo para PAR-2 en los días 0 y 3, y para que TSLP en todos los tiempos comparados con los perros normales. La intensidad en sí misma no estaba significativamente incrementada en los perros atópicos comparados con los perros normales tanto para PAR-2 como para TSLP.

Conclusiones e importancia clínica – estos resultados preliminares confirman que no hay una diferencia en la expresión sino más bien en el patrón de distribución de estas moléculas. Nuevos estudios utilizando inhibidores de PAR-2 o de TSLP pueden aportar más información de su relevancia clínica.

Zusammenfassung

Hintergrund – Der Protease-aktivierte Rezeptor (PAR)-2 spielt eine wesentliche Rolle bei der Entzündung und der Hautbarriere. Der Protease-aktivierte Rezeptor-2 wird durch proteolytische Enzyme von Allergenen aktiviert und stimuliert das thymische stromale Lymphopoietin (TSLP), wodurch Zytokine der T2-Helferzellen gefördert werden. Bei Menschen mit atopischer Dermatitis (AD) wurde eine vermehrte Expression von PAR-2 und TSLP publiziert.

Hypothese/Ziele – Ein Vergleich der Färbekarakteristika von PAR-2 und TSLP bei normalen und atopischen Beagles. Die untersuchte Hypothese besagte, dass in atopischer Hundehaut im Vergleich zu gesunder Kontrollhaut eine vermehrte Expression auftritt.

Tiere – Acht atopische und fünf normale Hunde wurden drei Tage lang mit Hausstaubmilben provoziert.

Methoden – Es wurden Hautbiopsien entnommen, um die Intensität, die Verteilung, die Integrität und die Zellfärbemuster an den Tagen 1, 3 und 10 objektiv sowie subjektiv zu beurteilen. Die klinischen Symptome wurden bewertet und zwischen den Gruppen verglichen.

Ergebnisse – Die atopischen Hunde zeigten im Vergleich zu den normalen Hunden einen signifikanten Anstieg der klinischen Werte an den Tagen 3 (Höhepunkt der Provokation) und 10 (Ende) und ein signifikant verstärktes Färbeverhalten für TSLP im Stratum basale zu allen verglichenen Zeitpunkten. Sie zeigten im Vergleich zu normalen Hunden an den Tagen 0 und 3 ein signifikantes fleckiges Muster für PAR-2 sowie zu allen Zeitpunkten für TSLP. Die Intensität selbst war nicht signifikant erhöht bei den atopischen Hunden im Vergleich zu den normalen Tieren für sowohl PAR-2 wie auch TSLP.

Schlussfolgerungen und klinische Bedeutung – Diese vorläufigen Ergebnisse können einen Unterschied in der Quantität der Expression weniger bestätigen als vielmehr ihren Verlauf. Studien, welche PAR-2 und TSLP-Inhibitoren verwenden, könnten ein Licht auf ihre klinische Relevanz werfen!

摘要

背景 — 蛋白酶激活受体(PAR)-2在炎症反应和皮肤屏障中起重要作用。蛋白酶激活受体-2被过敏原的水解蛋白酶激活,并刺激胸腺基质淋巴细胞生成素(TSLP)生成,促进T-辅助细胞2细胞因子释放。患有异位性皮炎(AD)的人,PAR-2和TSLP的表达会增加。

假设/目的 — 比较正常和患有异位性皮炎的比格犬PAR-2和TSLP着色模式。假设异位性皮炎患者比正常犬表达增加。

动物 — 八只异位性皮炎患者犬和五只正常犬,接受3天的屋尘螨刺激。

方法 — 于第0、3、10天,取皮肤活检样本,分别客观和主观测量样本的强度、分布、整体性和细胞染色模式。给临床症状评分并对比两组。

结果 — 与正常犬相比,异位性皮炎患者犬的第3天(刺激峰期)和第10天(缓解期),临床分数明显增加,所有时间点的基底层TSLP明显深染。与正常犬相比,PAR-2在第0和3天明显不规则分布,TSLP在所有时间点明显不规则分布。与正常犬相比,PAR-2及TSLP自体完整性没有显著增加。

总结与临床意义 — 这些初步发现不能确定表达数量的不同,而是模式不同。试验应用PAR-2或TSLP抑制剂来阐明他们之间的临床关联。

要約

背景 — プロテアーゼ活性化受容体(PAR)-2は炎症および皮膚バリアにおいて重要な役割を果たす。プロテアーゼ活性化受容体-2はアレルゲンのタンパク分解酵素により活性化され、胸腺間質性リンパ球新生因子(TSLP)、Tヘルパー2サイトカイン促進を刺激する。アトピー性皮膚炎(AD)に罹患したヒトでは、PAR-2とTSLPの発現の増加が報告されている。

仮説/目的 — 正常犬とアトピービーグル犬の間で、PAR-2およびTSLPの染色パターンを比較すること。健康なコントロール皮膚と比較し、アトピー犬の皮膚で発現の増加が認められるという仮説を調査した。

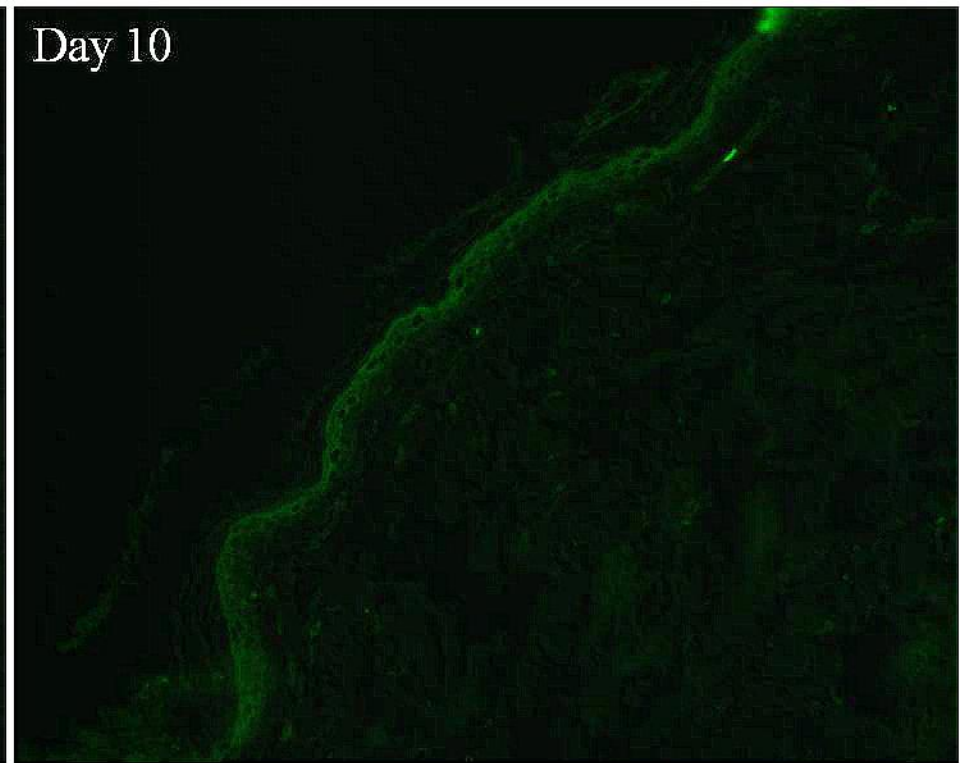
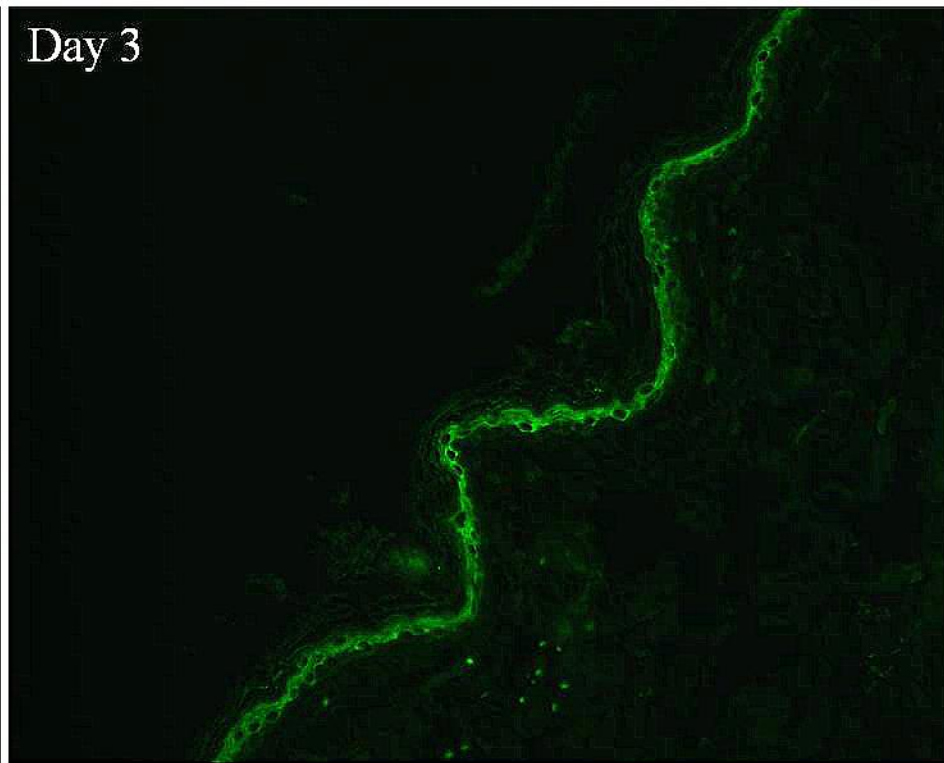
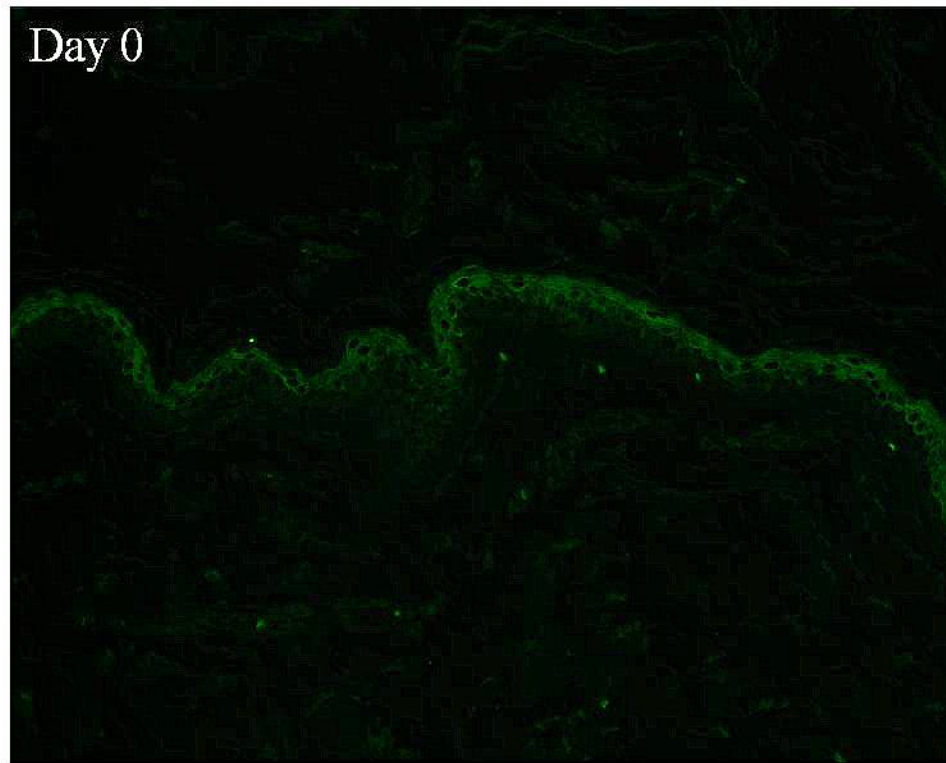
供与動物 — 8頭のアトピー犬と5頭の正常犬を3日間ハウスダストマイトに暴露した。

方法 — 0日目、3日目ならびに10日目に濃度、分布、整合性、および細胞染色パターンを測定するために皮膚生検を行った。臨床症状をスコア化し、グループ間で比較した。

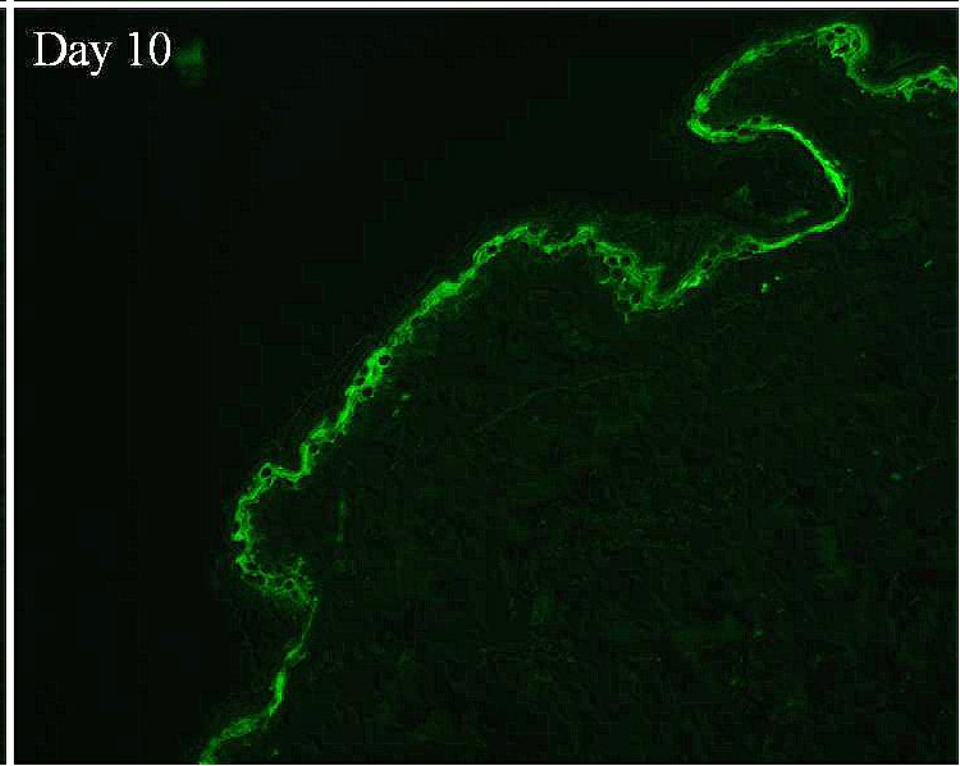
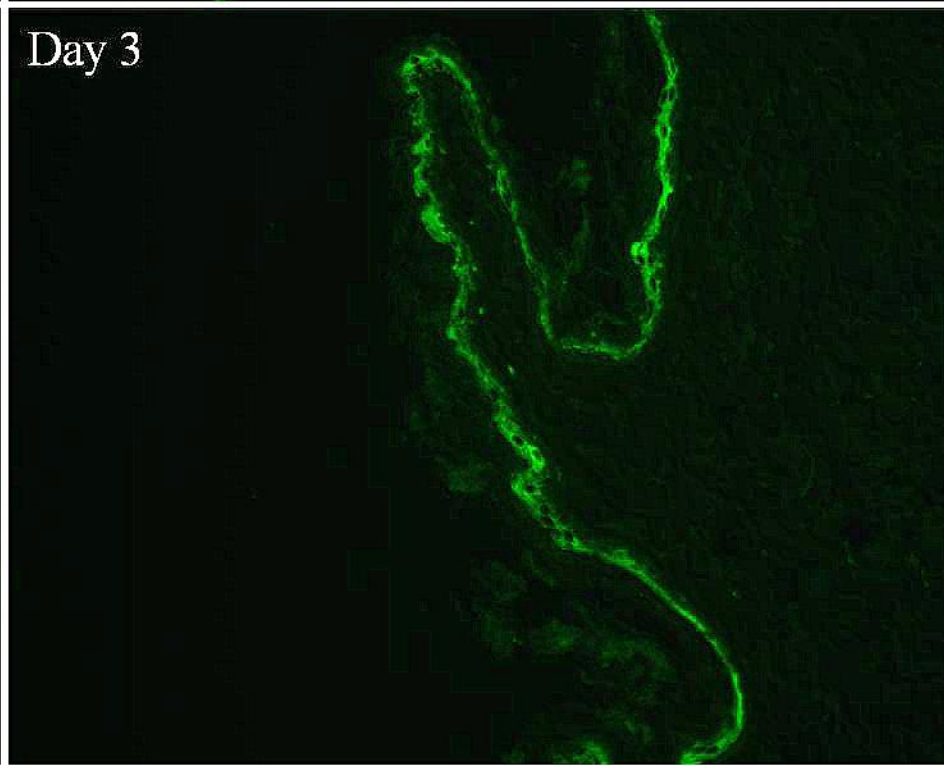
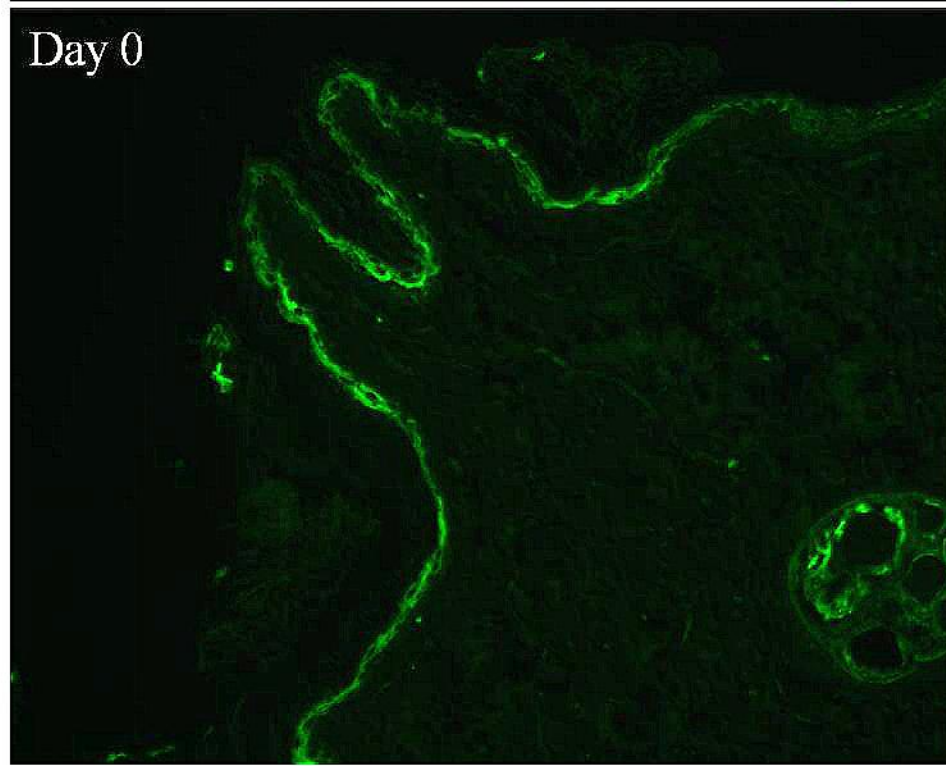
結果 — アトピー犬は3日目(暴露のピーク)と10日目(消失時)において臨床スコアが優位に増加し、正常犬と比較し、すべての時間で基底層におけるTSLPの染色パターンが優位に濃縮していた。それらは正常犬と比較し、PAR-2に対して0日目と3日目において、また、TSPLに対してすべての時間で優位な斑状パターンを示した。濃度そのものはPAR-2とTSLPの両方において、正常動物と比較し、アトピー犬で優位な増加は見られなかった。

結論および臨床的な重要性 — これらの予備的な所見は発現パターンではなく、発現量における違いを裏付けるものではなかった。PAR-3やTSLP阻害剤を利用した研究はそれらの臨床的な関連性に関して光を灯すことができるかもしれない。

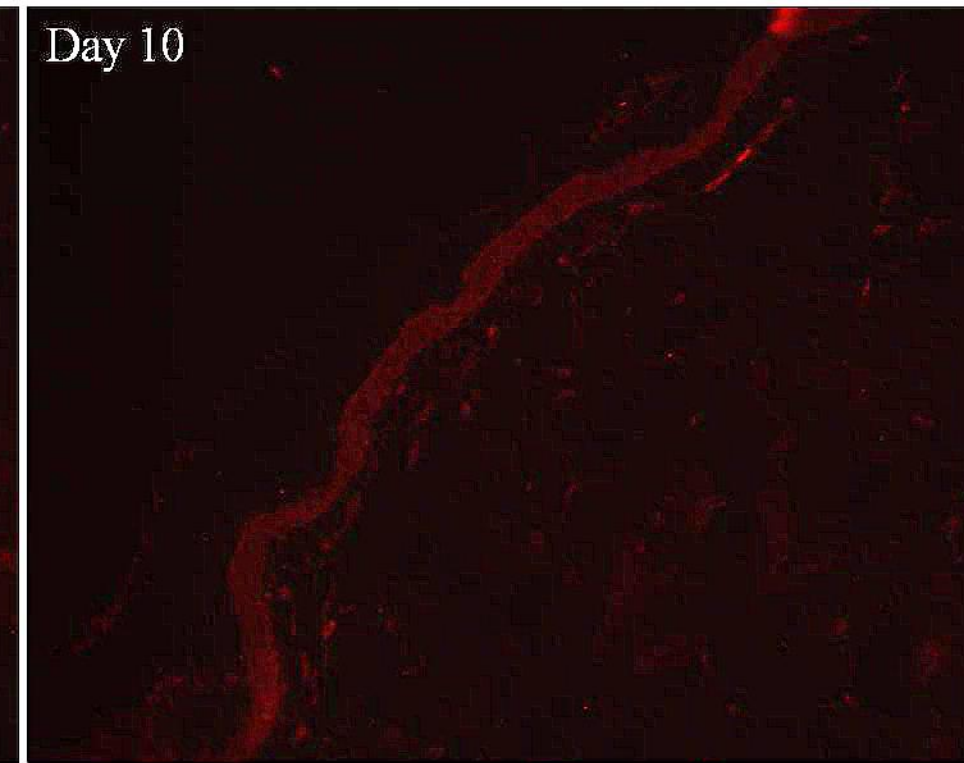
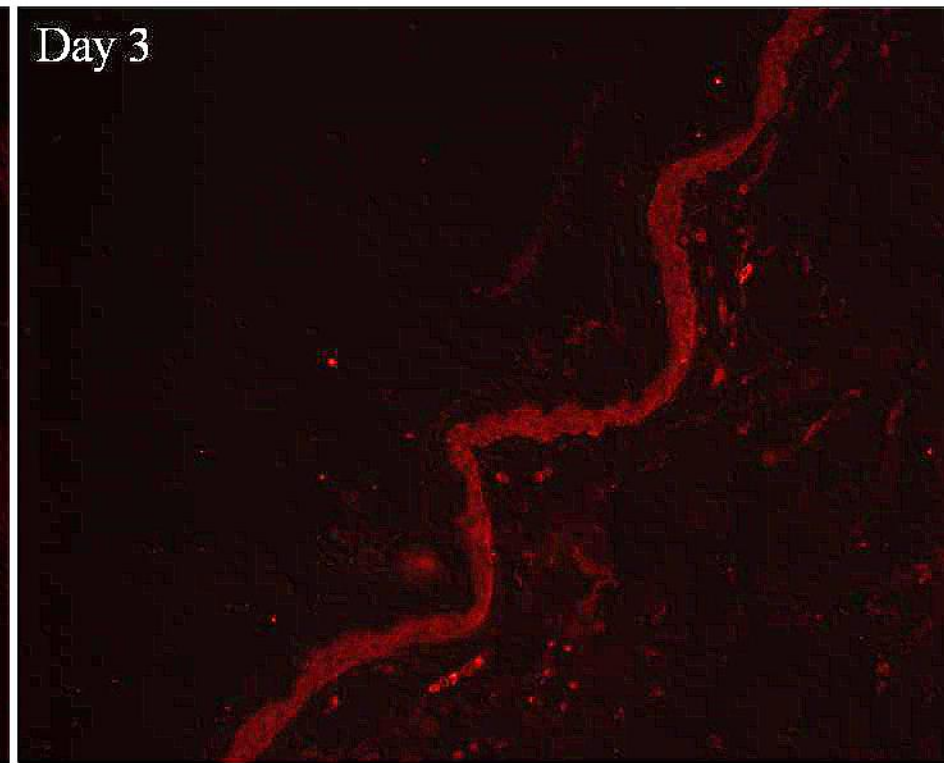
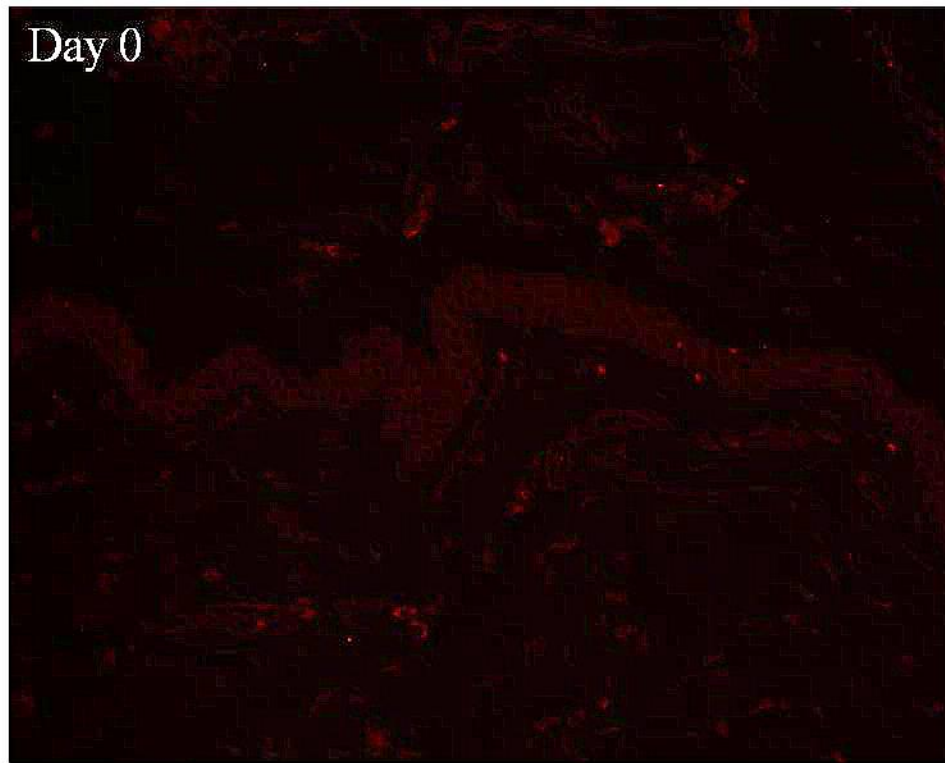
Normal



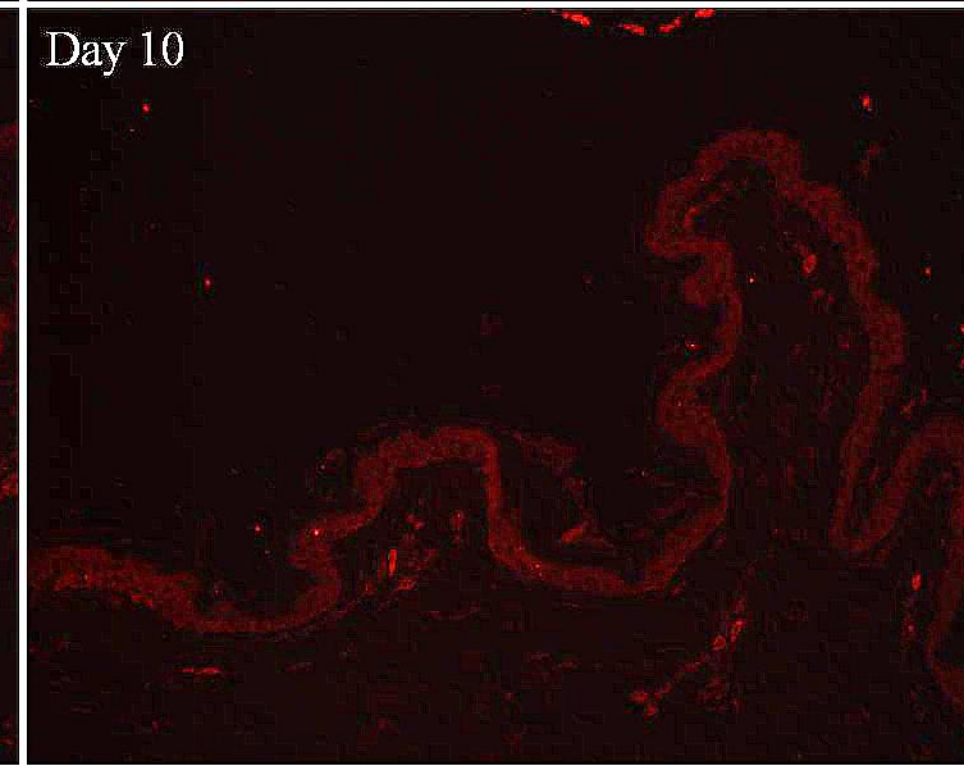
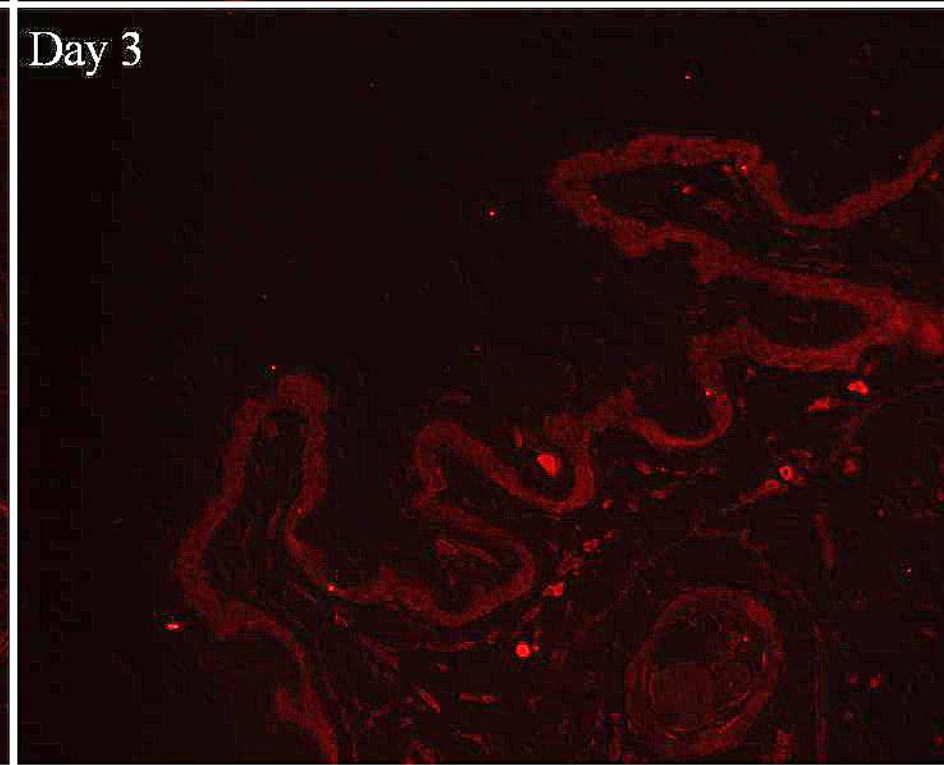
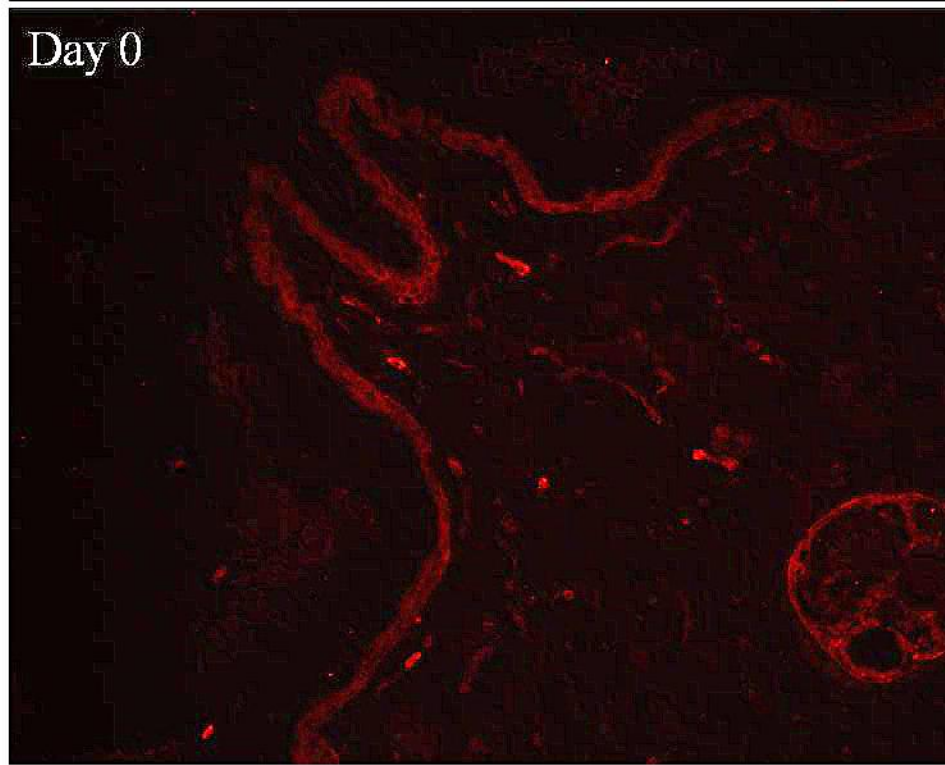
Atopic

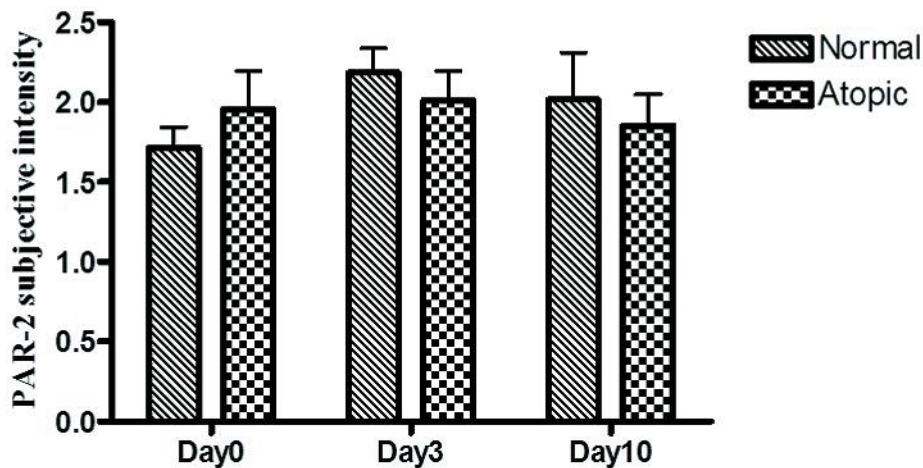
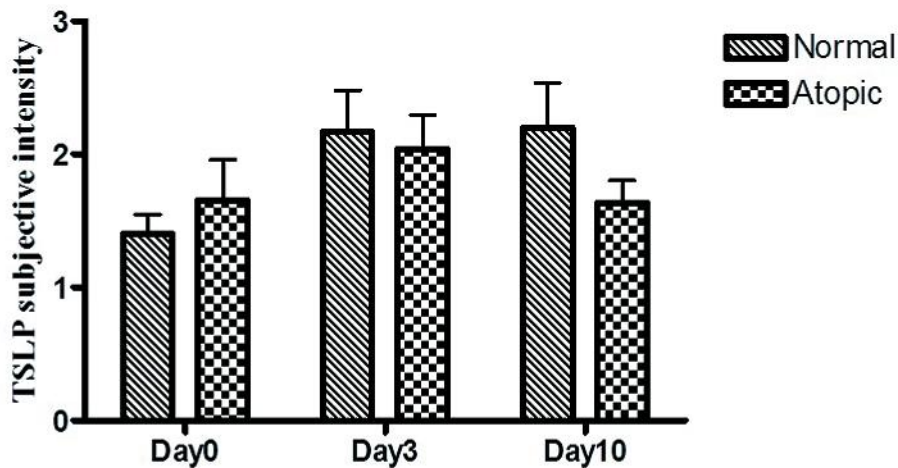


Normal



Atopic



a**b**

Supplementary figure legends

Supplementary Figure 1. Indirect immunofluorescence evaluation of PAR-2 over time in the AD dog model versus normal dogs.(× 200).

Supplementary Figure 2. Indirect immunofluorescence evaluation of TSLP over time in the AD dog model versus normal dogs.(× 200).

Supplementary Figure 3. Subjective evaluation of immunofluorescence intensity of PAR-2 **(a)** and TSLP **(b)** in the AD dog model versus normal dogs.

.