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Molecular Cloning of Canine Protease-Activated Receptor-2 and its Expression in Normal Dog Tissues and Atopic Skin Lesions

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ABSTRACT. Protease-activated receptor-2 (PAR-2) belongs to a new G protein-coupled receptor subfamily and is activated by serine proteases. PAR-2 has been demonstrated to play an important role in inflammation and immune response in allergic diseases. In this study, we cloned canine PAR-2 cDNA from the canine kidney by RT-PCR. The canine PAR-2 clone contained a full-length open reading frame encoding 397 amino acids that had 84% and 80% homology with human and mouse homologues, respectively. Canine PAR-2 mRNA was detected in the heart, lung, liver, pancreas, stomach, small intestine, colon, kidney, adrenal gland, spleen, thyroid gland, thymus, skeletal muscle, lymph node, fat and skin of three healthy dogs. The expression pattern of PAR-2 mRNA in canine tissues was similar to that in humans. The expression level of PAR-2 mRNA in skin was not different between the atopic dermatitis (AD) and healthy dogs, suggesting that the level of PAR-2 mRNA transcription may not be associated with development of canine AD. The canine PAR-2 cDNA clone obtained in this study will be useful for further investigation of the immunopathogenesis of canine allergic diseases.
KEY WORDS: atopic dermatitis, canine, molecular cloning, protease-activated receptor-2.

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Protease-activated receptor-2 (PAR-2) belongs to the family of G protein-coupled, seven transmembrane region receptors, that is activated by specific proteolytic cleavage of its extracellular amino terminus [7]. Cells with PAR-2 expression are activated when the cleavage forms a new amino terminus, a tethered ligand, and interacts with another region of the receptor, which is demonstrated by increased levels of Ca influx with peptides based on tethered ligand sequences [17, 24]. In PAR-2-deficient mice after surgical trauma, the mean leukocyte rolling flux fraction is significantly lower and mean rolling velocity is significantly higher than in wild-type (WT) mice [11], and this implies that the onset of inflammation is delayed in the absence of PAR-2. Later research has demonstrated that ICAM-1, an adhesion molecule, is enhanced by PAR-2 agonist in primary human keratinocytes [3]. These reports suggest that PAR-2 plays an important role in skin inflammation.

House dust mite (HDM), *Dermatophagoides farinae and D. pteronyssinus*, allergens are the most important aeroallergens in asthma [18]. Several studies have reported that HDM allergens have protease activities [6, 10, 14, 22, 26, 27]. Serine proteases, such as trypsin or mast cell tryptase, cleave the N-terminus of PAR-2 at the specific amino acid sequence SKGR↓SLIG, forming a tethered ligand that activates the receptor. Previous studies have also shown that Der p 1, the major mite allergen, can activate PAR-2 and induce IL-6 and IL-8 release in respiratory epithelial cells [1]. These results suggest that protease derived from HDM allergens may play an important role in the deterioration of allergic diseases by selective activation of epithelial cells.

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In 42 dogs with atopic dermatitis (AD), HDM was the most common positive allergen in both an intradermal skin test and antigen-specific IgE assay [13]. Recently, immunofluorescent staining for Der f 1 has demonstrated that mite allergens could infiltrate subepidermal sites after epicutaneous application of Der f slurry in a model of canine AD [19], which suggests that the defective epidermal barrier in atopic skin may enhance penetration of HDM. Furthermore, epicutaneous sensitization with HDM allergens has been shown to induce elevation of mite-specific IgE and pruritic dermatitis, resembling spontaneous AD in dogs [19]. Additionally, atopy patch testing with HDM enhanced mRNA transcription of cytokines (IL-6, IL-12p35, IL-13, IL-18 and IFN-γ) and a chemokine, TARC/CCL17, in lesional skin from high-IgE Beagles [12]. Lymphocytes and keratinocytes, the principle epidermal cells, have been recognized as a major source of cytokine production [8]. It is interesting to note that trypsin induces GM-CSF and IL-6 gene expressions in normal human keratinocytes via PAR-2 activation [25]. Thus, it is conceivable that HDM allergens may directly activate PAR-2 expressed on keratinocytes to release cytokines leading to skin inflammation in canine AD.

So far, human, mouse and rat PAR-2 genes have been cloned [2, 15, 16, 20]; however, canine PAR-2 has not been identified. Thus, we report molecular cloning of the canine PAR-2 gene and its transcription levels in various dog tissues and atopic skin lesions in order to improve understanding of the involvement of PAR-2 in canine allergic diseases.

MATERIALS AND METHODS

Cloning of canine PAR-2: Total RNA was extracted from the kidney of a healthy dog with a commercially available

Table 1. Sequences and nucleotide positions of the oligonucleotide primers used for molecular cloning of canine PAR-2 cDNA

Primers		Primer Sequence (5'–3')	Nucleotide positions
P1	Forward	ACCCCCAGTAACCTTCTGCT	901–920
P2	Reverse	TTGCATCTGCCTGACAGAAC	1094–1113
P3	Reverse	TCAGTAAGAGGTTTTGACAC	1174–1194
P4	Forward	TCGTGAGGCAGACTATGCAC	632–651
P5	Reverse	GTTAAGGGTGGACAGGCAGC	989–1008
P6	Forward	CTTGGCAGACCTCCTTTCTG	354–373
P7	Reverse	AAGGGCTGGAATGTGCATAG	644–663
P8	Forward	ATGCGAAGCGTGCGCGCCGC	1–20
P9	Reverse	CCCCATAAACCCAGTTGTTG	414–433
P10	Forward	TCACTGAAACTTCTGGTCTCG	–118– –98
P11	Reverse	CAGGACACACCCATCACAG	1243–1262

kit (RNeasy Lipid Tissue Mini Kit, Qiagen Inc., Valencia, CA, U.S.A.). The use of dogs in this study was approved by the Institutional Animal Care and Use Committee of Gifu University. A cDNA sample was synthesized from 0.5 μg of total RNA with reverse transcriptase (ReverTra Dash, Toyobo, Osaka, Japan). Oligonucleotide primers to amplify canine PAR-2 cDNA were designed based on the predicted sequences of canine PAR-2 (GenBank/EMBL/DDBJ accession number XM_546057; Table 1). Using these primer pairs, canine PAR-2 cDNA was amplified from the canine kidney with a Taq polymerase (TAKARA Ex Taq, Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. The PCR amplifications consisted of pre-denaturing (94°C for 2 min), 35 cycles of denaturation (94°C for 1 min), annealing (60°C for 1 min) and extension (72°C for 1 min); and then a final extension (8 min). PCR generated a single DNA fragment for each primer pair. The PCR products were purified from agarose gel with a commercially available kit (Wizard SV Gel and PCR Clean-Up System, Promega Corp., Madison, WI, U.S.A.) and were sequenced by an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). In the present study, more than 7 independent clones of PCR products were sequenced to avoid errors in sequence analyses.

Transcription analysis of PAR-2 mRNA in normal dog tissues: Transcription analysis of PAR-2 mRNA in normal dog tissues was performed using quantitative real-time PCR (Thermal Cycler Dice Real Time System, Takara Bio Inc.). Three healthy beagles were used to investigate the transcription of canine PAR-2 in normal dog tissues. Various tissues were collected from the dogs and snap-frozen in liquid nitrogen. The tissue samples were stored at –80°C until use. Total RNA samples were extracted from the heart, lung, liver, pancreas, stomach, small intestine, colon, kidney, adrenal gland, spleen, thyroid gland, thymus, skeletal muscle, lymph node, fat and skin using the method described above. Genomic DNA was removed from the samples with a commercially available kit (TURBO DNA-free Kit, Applied Biosystems). Transcription of PAR-2 mRNA was quantified by one-step real-time PCR (One Step SYBR PrimeScript RT-PCR Kit II, Takara Bio Inc.) according to the manufacturer's instructions. Transcription of ribosomal protein L13a (RPL13A) and ribosomal protein L32 (RPL32) mRNA was used as an internal control. The sequences of the primer pairs used for quantitative real-time PCR are shown in Table 2. A standard curves method was employed for quantification of PAR-2 mRNA transcription. One-step real-time PCR amplifications consisted of reverse transcription (42°C for 5 min and 95°C for 10 sec), 40 cycles of PCR reaction (95°C for 5 sec and 65°C for 30 sec) and then dissociation (95°C for 15 sec, 60°C for 30 sec and 95°C for 15 sec). All samples were examined in duplicate, and the mean amount of PAR-2 mRNA was calculated from the standard curve.

Table 2. Sequences of the oligonucleotide primers used for quantitative real-time PCR

Primer Set		Primer sequences (5'–3')
RPL32	Forward	TGGTTACAGGAGCAACAAGAAA
	Reverse	GCACATCAGCAGCACTTCA
RPL13A	Forward	GCCGGAAGGTTGTAGTCGT
	Reverse	GGAGGAAGGCCAGGTAATTC
PAR-2	Forward	TGAAGATCGCCTACCACATCCA
	Reverse	CCAATACCGTTGCACACTGA

Transcription analysis of PAR-2 mRNA in skin lesions: Control skin samples were collected from five healthy beagles that did not show any clinical signs of skin diseases. Lesional and non-lesional skin samples were collected from ten dogs with AD that were referred to the Animal Medical Center of Gifu University for problems with pruritic skin diseases. All ten dogs had compatible historical and clinical findings of AD, including seasonal or recurrent and chronic pruritus. Dogs with other skin diseases causing pruritus, such as infestation of parasites, or bacterial or fungal infections, were excluded based on routine dermatologic examinations and a therapeutic trial of antibiotics. Food hypersensitivity was excluded by a food elimination test with a commercial prescription diet (Hill's z/d ultra, Hill's Pet Nutrition, Inc., Topeka, KS, U.S.A.) for 8 weeks. Skin biopsy was carried out in both AD and healthy dogs using a 4 mm disposable punch (DERMAPUNCH, Nipro Medical Industries Ltd., Tokyo, Japan) under local anesthesia with 2% lidocaine hydrochloride (Xylocaine, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan). A pair of skin biopsy sam-

ples was obtained from sites of both lesional and non-lesional skin in each dog with AD. The gross findings for lesional skin were generally consistent among the dogs and included lichenification and hair loss showing the characteristics of a chronic lesion rather than an acute lesion. All skin samples were immediately submerged in RNAlater (Qiagen Inc.) and stored at –20°C until extraction of total RNA. Following homogenization of the skin samples, total RNA was extracted, and genomic DNA was removed from the samples as described above. Expression of PAR-2 mRNA was examined by quantitative real-time PCR. Transcription of PAR-2 mRNA was evaluated as described above.

Statistical analysis: The transcription levels of PAR-2 mRNA were statistically analyzed among the lesions, non-lesions and normal skin using Dunnett's test. All analyses were performed using a statistical software package (JMP version 5.1.2, SAS Institute, Cary, NC, U.S.A.). Statistical significance was defined as $P<0.05$.

RESULTS

Molecular cloning of canine PAR-2: The full-length sequence of canine PAR-2 cDNA was determined by combining the sequences of the overlapping 5', central and 3' DNA fragments obtained in this study. Canine PAR-2 cDNA was 1,251 bp long and contained the entire open reading frame of 1,194 bp encoding 397 amino acid residues with seven hydrophobic, presumably membrane-spanning, domains (GenBank/EMBL/DDBJ accession number AB458680; Fig. 1). The seven transmembrane domains of canine PAR-2 were presumed by comparison with those of humans and mice (Fig. 1). The deduced amino acid sequence of the canine PAR-2 cDNA cloned in this study was shown to have 84% and 80% similarity with those of its human [2, 15] and mouse [16] counterparts, respectively (Fig. 2).

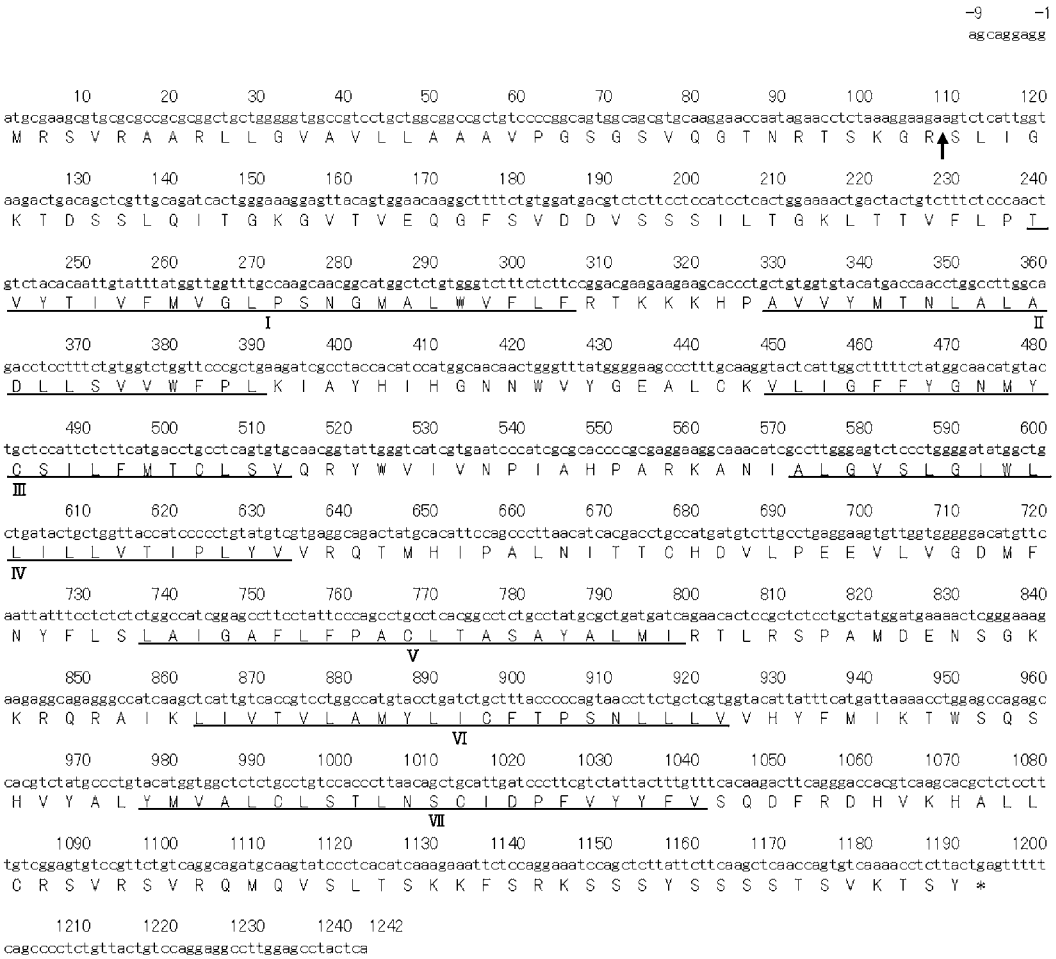

Fig. 1. Nucleotide and deduced amino acid sequences of canine PAR-2 cDNA (GenBank/EMBL/DDBJ accession number AB458680). The canine PAR-2 cDNA was 1,251 bp long and contained the entire open reading frame, which was composed of 1,194 bp encoding 397 amino acid residues. The solid lines indicate the seven predicted transmembrane domains in the amino acid sequence. The arrow points to the proposed protease cleavage site. The asterisk after the amino acid sequence shows the position of the termination codon.

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Dog     MRSVRAARLL GVAVLLAAAV PGS-GSVQGT NR-TSKGRSL IGKTDSSLQI TGKGVTVEQG  58
Human   ***PS**W** *A*I****SL SC*-*TI*** **-S****** ***V*GTSHV ********TV  58
Mouse   ***LSL*W** *GIT****S* SC*RTENLAP G*NN****** **RLETQPP* *****P**P*  60
                                                  I
Dog     FSVDDVSSSI LTGKLTTVFL PTVYTIVFMV GLPSNGMALW VFLFRTKKKH PAVVYMTNLA 118
Human   ****EF*A*V ********** *I******V* ********** ********** ***I**A*** 118
Mouse   **I*EF*A** ********** *V**I***VI ********** I********* ***I**A*** 120
        II                                        III
Dog     LADLLSVVWF PLKIAYHIHG NNWVYGEALC KVLIGFFYGN MYCSILFMTC LSVQRYWVIV 178
Human   ********I** ********** ***I****** N********* ********** ********** 178
Mouse   ********I** **A****L** ********** ********** ********** ********** 180
                          IV
Dog     NPIAHPARKA NIALGVSLGI WLLILLVTIP LYVVRQTMHI PALNITTCHD VLPEEVLVGD 238
Human   **MG*SRK** ***I*I**A* ********** ****K**IF* ********** ****QL**** 238
Mouse   **MG**RK** ***V****A* ****F***** ***MK**IY* ********** ********** 240
                     V                                                 VI
Dog     MFNYFLSLAI GAFLFPACLT ASAYALMIRT LRSPAMDENS GKKRQRAIKL IVTVLAMYLT 298
Human   ********** *V*****F** ****V****M ***S****** E***K***** ********** 298
Mouse   ********** *V******I** ****V***K* ***S****H* E*******R* *I******F* 300
                                                  VII
Dog     CFTPSNLLLV VHYFMIKTWS QSHVYALYMV ALCLSTLNSC IDPFVYYFVS QDFRDHVKHA 358
Human   ********** ****L**SQG ********I* ********** ********** H*****A*N* 358
Mouse   **A******* ****L***QR ********L* ********** ********** K*****ARN* 360

Dog     LLCRSVRSVR QMQVSLTSKK FSRKSSSYSS SSTSVKTSY     397
Human   *******T*K ********** H********* ***T*****    397
Mouse   *******T*N R**I**S*N* *****G**** *********     399
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Fig. 2. Comparison of the deduced amino acid sequence of canine PAR-2 cDNA with those of human and mouse homologues. The deduced amino acid sequence of canine PAR-2 cDNA cloned in this study was shown to have 84% and 80% similarity with those of the human and mouse counterparts, respectively. Asterisks indicate identity with amino acids of the canine PAR-2 sequence. Overlining denotes the seven predicted transmembrane domains. The arrow points to the proposed protease cleavage site.

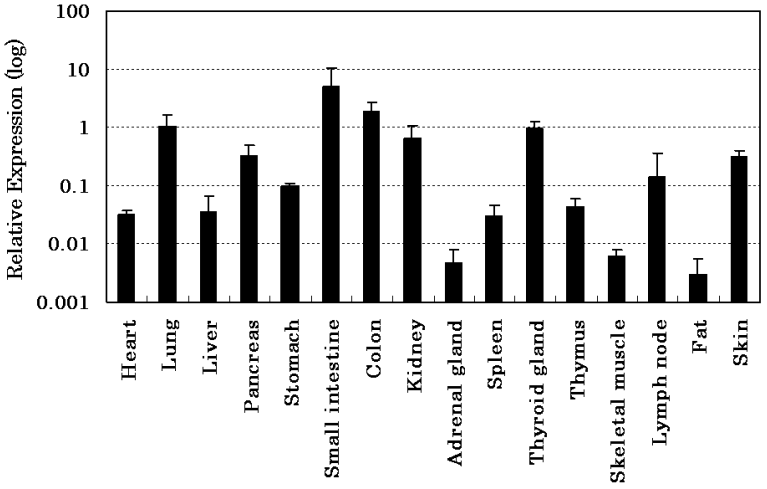

Fig. 3. Transcription levels of PAR-2 mRNA in various dog tissues. Transcription of ribosomal protein L13a (RPL13A) and ribosomal protein L32 (RPL32) mRNA was used as an internal control. Since both results were similar, data standardized based on RPL13A are shown. The mean amount of PAR-2 mRNA was calculated from a standard curve. The error bars represent the S.D.

Transcription of PAR-2 mRNA in normal dog tissues: Expression of PAR-2 mRNA was detected in all tissues investigated in this study. The highest level of transcription was observed in the small intestine and colon, whereas the lowest level was found in the adrenal gland, skeletal muscle and fat (Fig. 3). In skin, PAR-2 was constitutively expressed with transcription levels similar to those of the lung, pancreas, kidney, thyroid gland and lymph node (Fig. 3).

Transcription of PAR-2 mRNA in skin lesions: Transcrip-

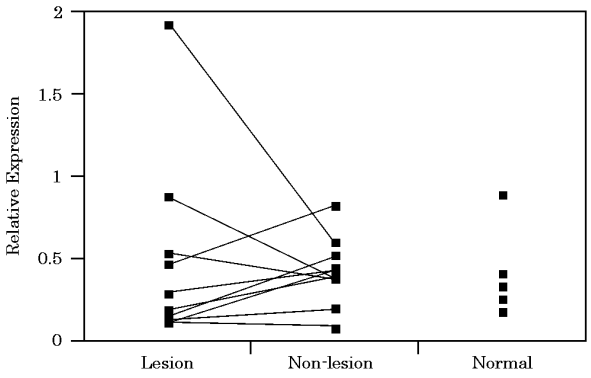

Fig. 4. Transcription levels of PAR-2 mRNA in lesional and non-lesional skin from AD dogs (n=10) and normal skin from healthy dogs (n=5). Transcription of ribosomal protein L13a (RPL13A) and ribosomal protein L32 (RPL32) mRNA was used as an internal control. Since both results were similar, data standardized based on RPL13A are shown. The mean amount of PAR-2 mRNA was calculated from a standard curve.

tion of PAR-2 mRNA was detected in all skin samples; however, there was no significant difference in transcription level among lesional, non-lesional and healthy skin (Fig. 4).

DISCUSSION

The nucleotide sequence reported in this study contained the entire open reading frame of canine PAR-2 cDNA. The amino acid sequence encoded by canine PAR-2 cDNA contains seven hydrophobic segments, probably corresponding to transmembrane regions. The amino acid sequence of canine PAR-2 was 84% and 80% identical to human and mouse PAR-2, respectively. This result suggests that PAR-2 is highly conserved and plays an important role in sustaining life. The extracellular N-terminus of canine PAR-2 was only 69% and 60% identical to human and mouse PAR-2, respectively; however, the eight residues surrounding the putative serine protease cleavage site (SKGR SLIG) were highly conserved, suggesting that this region is important for cleavage site recognition and receptor activation.

In this study, quantitative real-time PCR showed that PAR-2 mRNA was detected in the small intestine, colon, lung, pancreas, thyroid gland, lymph node, kidney and skin. The transcription pattern of PAR-2 mRNA in canine tissues was similar to that in humans [2, 15]. In addition, since the deduced amino acid sequence of canine PAR-2 had high similarity with those of the human and mouse, PAR-2 may have similar functions among the different species. PAR-2 mRNA in humans and dogs is expressed strongly in the small intestine and colon, which indicates that PAR-2 in the alimentary tract might be activated via pancreatic trypsin during digestion and might be involved in physiological and pathological functions. Indeed, it has been reported that intraperitoneal administration of PAR-2 agonist facilitates gastrointestinal transit in mice [9]. Moreover, colonic administration of PAR-2 agonist induces intestinal inflammation in WT mice, but not in PAR-2-deficient mice [5]. Considering that luminal trypsin and tryptase are elevated in the colons of inflammatory bowel disease (IBD) patients [4, 23], PAR-2 may be associated with the development of IBD.

PAR-2 was preferentially expressed in organs of epithelial origin, such the gastrointestinal organs, lung and skin; therefore, PAR-2 might function as a barrier in protection against external microbes and allergens. It has been suspected that PAR-2 in skin can induce inflammation; thus, we further explored whether the intensity of PAR-2 transcription in atopic skin lesions would be higher than in healthy skin; however, there was no significant difference in the transcription levels of PAR-2 mRNA among the skin samples from the healthy and AD dogs. In humans, it has been reported that PAR-2 immunoreactivity is enhanced in the lesional skin of AD patients [3]. Although canine PAR-2 expression has not been investigated on a protein level, the present results suggest that active transcription would not occur with the onset of allergic inflammation. Future studies will include immunohistochemical analysis of PAR-2 expression in canine atopic skin lesions. Thus, the deduced amino acid sequence in the present study should be useful in producing recombinant protein that can be used to screen antibodies.

In the lesional skin of patients with AD, PAR-2 has been shown to be markedly expressed on primary sensory nerve fibers, as well as keratinocytes, by immunohistochemistry [21]. Moreover, when applied intralesionally, PAR-2 agonist provokes enhanced and prolonged itch sensation in AD patients compared with healthy controls [21]. These data suggest that PAR-2 on the primary afferent nerve may play an important role in pruritus in AD patients. Considering that PAR-2 is highly conserved among animal species, PAR-2 may also participate in itching sensation in canine AD. Therefore, regulation of PAR-2 activation may be a novel therapeutic strategy not only for allergic inflammation but also for pruritus in canine AD.

In conclusion, canine PAR-2 cDNA was cloned and sequenced, and its mRNA transcription was investigated in various normal tissues in healthy dogs. This study will provide fundamental information for clarification of the association of PAR-2 in the immunopathogenesis of canine AD.

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REFERENCES

1. Asokananthan, N., Graham, P. T., Stewart, D. J., Bakker, A. J., Eidne, K. A., Thompson, P. J. and Stewart, G. A. 2002. House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J. Immunol.* **169**: 4572–4578.
2. Bohm, S. K., Kong, W., Bromme, D., Smeekens, S. P., Anderson, D. C., Connolly, A., Kahn, M., Nelken, N. A., Coughlin,

S. R., Payan, D. G. and Bunnett, N. W. 1996. Molecular cloning, expression and potential functions of the human proteinase-activated receptor-2. *Biochem. J.* **314** (Pt 3): 1009–1016.

3. Buddenkotte, J., Stroh, C., Engels, I. H., Moormann, C., Shpacovitch, V. M., Seeliger, S., Vergnolle, N., Vestweber, D., Luger, T. A., Schulze-Osthoff, K. and Steinhoff, M. 2005. Agonists of proteinase-activated receptor-2 stimulate upregulation of intercellular cell adhesion molecule-1 in primary human keratinocytes via activation of NF-kappa B. *J. Invest. Dermatol.* **124**: 38–45.
4. Bustos, D., Negri, G., De Paula, J. A., Di Carlo, M., Yapur, V., Facente, A. and De Paula, A. 1998. Colonic proteinases: increased activity in patients with ulcerative colitis. *Medicina (B Aires)* **58**: 262–264.
5. Cenac, N., Coelho, A. M., Nguyen, C., Compton, S., Andrade-Gordon, P., MacNaughton, W. K., Wallace, J. L., Hollenberg, M. D., Bunnett, N. W., Garcia-Villar, R., Bueno, L. and Vergnolle, N. 2002. Induction of intestinal inflammation in mouse by activation of proteinase-activated receptor-2. *Am. J. Pathol.* **161**: 1903–1915.
6. Chua, K. Y., Stewart, G. A., Thomas, W. R., Simpson, R. J., Dilworth, R. J., Plozza, T. M. and Turner, K. J. 1988. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. Homology with cysteine proteases. *J. Exp. Med.* **167**: 175–182.
7. Coughlin, S. R. 1994. Protease-activated receptors start a family. *Proc. Natl. Acad. Sci. U.S.A.* **91**: 9200–9202.
8. Grone, A. 2002. Keratinocytes and cytokines. *Vet. Immunol. Immunopathol.* **88**: 1–12.
9. Kawabata, A., Kuroda, R., Nagata, N., Kawao, N., Masuko, T., Nishikawa, H. and Kawai, K. 2001. *In vivo* evidence that protease-activated receptors 1 and 2 modulate gastrointestinal transit in the mouse. *Br. J. Pharmacol.* **133**: 1213–1218.
10. King, C., Simpson, R. J., Moritz, R. L., Reed, G. E., Thompson, P. J. and Stewart, G. A. 1996. The isolation and characterization of a novel collagenolytic serine protease allergen (Der p 9) from the dust mite Dermatophagoides pteronyssinus. *J. Allergy Clin. Immunol.* **98**: 739–747.
11. Lindner, J. R., Kahn, M. L., Coughlin, S. R., Sambrano, G. R., Schauble, E., Bernstein, D., Foy, D., Hafezi-Moghadam, A. and Ley, K. 2000. Delayed onset of inflammation in protease-activated receptor-2-deficient mice. *J. Immunol.* **165**: 6504–6510.
12. Marsella, R., Olivry, T. and Maeda, S. 2006. Cellular and cytokine kinetics after epicutaneous allergen challenge (atopy patch testing) with house dust mites in high-IgE beagles. *Vet. Dermatol.* **17**: 111–120.
13. Masuda, K., Sakaguchi, M., Fujiwara, S., Kurata, K., Yamashita, K., Odagiri, T., Nakao, Y., Matsuki, N., Ono, K., Watari, T., Hasegawa, A. and Tsujimoto, H. 2000. Positive reactions to common allergens in 42 atopic dogs in Japan. *Vet. Immunol. Immunopathol.* **73**: 193–204.
14. McCall, C., Hunter, S., Stedman, K., Weber, E., Hillier, A., Bozic, C., Rivoire, B. and Olivry, T. 2001. Characterization and cloning of a major high molecular weight house dust mite allergen (Der f 15) for dogs. *Vet. Immunol. Immunopathol.* **78**: 231–247.
15. Nystedt, S., Emilsson, K., Larsson, A. K., Strombeck, B. and Sundelin, J. 1995. Molecular cloning and functional expression of the gene encoding the human proteinase-activated receptor 2. *Eur. J. Biochem.* **232**: 84–89.
16. Nystedt, S., Emilsson, K., Wahlestedt, C. and Sundelin, J. 1994. Molecular cloning of a potential proteinase activated receptor. *Proc. Natl. Acad. Sci. U.S.A.* **91**: 9208–9212.
17. Nystedt, S., Larsson, A. K., Aberg, H. and Sundelin, J. 1995. The mouse proteinase-activated receptor-2 cDNA and gene. Molecular cloning and functional expression. *J. Biol. Chem.* **270**: 5950–5955.
18. Platts-Mills, T. A., Vervloet, D., Thomas, W. R., Aalberse, R. C. and Chapman, M. D. 1997. Indoor allergens and asthma: report of the Third International Workshop. *J. Allergy Clin. Immunol.* **100**: S2–24.
19. Pucheu-Haston, C. M., Jackson, H. A., Olivry, T., Dunston, S. M. and Hammerberg, B. 2008. Epicutaneous sensitization with Dermatophagoides farinae induces generalized allergic dermatitis and elevated mite-specific immunoglobulin E levels in a canine model of atopic dermatitis. *Clin. Exp. Allergy* **38**: 667–679.
20. Saifeddine, M., al-Ani, B., Cheng, C. H., Wang, L. and Hollenberg, M. D. 1996. Rat proteinase-activated receptor-2 (PAR-2): cDNA sequence and activity of receptor-derived peptides in gastric and vascular tissue. *Br. J. Pharmacol.* **118**: 521–530.
21. Steinhoff, M., Neisius, U., Ikoma, A., Fartasch, M., Heyer, G., Skov, P. S., Luger, T. A. and Schmelz, M. 2003. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. *J. Neurosci.* **23**: 6176–6180.
22. Stewart, G. A., Ward, L. D., Simpson, R. J. and Thompson, P. J. 1992. The group III allergen from the house dust mite Dermatophagoides pteronyssinus is a trypsin-like enzyme. *Immunology* **75**: 29–35.
23. Tarlton, J. F., Whiting, C. V., Tunmore, D., Bregenholt, S., Reimann, J., Claesson, M. H. and Bland, P. W. 2000. The role of up-regulated serine proteases and matrix metalloproteinases in the pathogenesis of a murine model of colitis. *Am. J. Pathol.* **157**: 1927–1935.
24. Vu, T. K., Hung, D. T., Wheaton, V. I. and Coughlin, S. R. 1991. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **64**: 1057–1068.
25. Wakita, H., Furukawa, F. and Takigawa, M. 1997. Thrombin and trypsin induce granulocyte-macrophage colony-stimulating factor and interleukin-6 gene expression in cultured normal human keratinocytes. *Proc. Assoc. Am. Physicians* **109**: 190–207.
26. Weber, E., Hunter, S., Stedman, K., Dreitz, S., Olivry, T., Hillier, A. and McCall, C. 2003. Identification, characterization, and cloning of a complementary DNA encoding a 60-kd house dust mite allergen (Der f 18) for human beings and dogs. *J. Allergy Clin. Immunol.* **112**: 79–86.
27. Yasueda, H., Mita, H., Yui, Y. and Shida, T. 1986. Isolation and characterization of two allergens from Dermatophagoides farinae. *Int. Arch. Allergy Appl. Immunol.* **81**: 214–223.