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Altered cutaneous expression of β -defensins in dogs with atopic dermatitis

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ABSTRACT

Canine atopic dermatitis (AD) is a chronic allergic skin disorder with an immunopathogenesis comparable to that in humans with AD. The high frequency of recurrent infections with *Staphylococcus pseudo intermedius* and *Malassezia pachydermatis* may indicate a defective innate immune response in the skin of atopic dogs. Production of β -defensins constitutes an important role in skin defense but information on canine β -defensin localization and regulation is scarce. We conducted a gene-expression study of 16 canine β -defensins (cBDs) in 11 tissues of healthy dogs, which revealed a variable expression of cBDs in different organ systems of the dog. In skin, three β -defensins, cBD1, cBD103 and cBD107, were extensively expressed, while inconsistent expression of five other β -defensins was detected. Using immunohistochemistry abundant expression of cBD103 peptide was detected in the epidermis, hair follicles and sebaceous glands, comparable to hBD3 expression in human skin. To examine the gene-expression of β -defensins in atopic dogs, full thickness skin biopsy specimens (non-lesional and lesional) of 10 atopic dogs and 7 healthy dogs were examined with real-time PCR. A significant 12-fold increased expression of cBD1 was detected in lesional atopic skin compared to healthy skin, while non-lesional skin showed a 5-fold increase. Contrary to cBD1, expression of cBD103 was slightly (2-fold) downregulated in skin of atopic dogs. Gene-expression levels of S100A8, a marker for atopic dermatitis, were also highly upregulated in skin of atopic dogs, confirming the diagnostics of the skin biopsies. Taken together these results provide new evidence for a possible defect in the innate immune response of dogs with atopic dermatitis, and indicate the potential of the dog as a model for human AD.

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1. Introduction

The mammalian innate immune system plays a pivotal role in the first-line defense strategy of epithelial and mucosal surfaces. Small cationic peptides, such as β -defensins and cathelicidins, are important effector molecules of this evolutionary ancient immune system. These antimicrobial peptides are able to directly kill microbial pathogens by their interaction with anionic components in the microbial membrane (Toke, 2005), but also several immunomodulatory roles have been described that can help in bacterial killing (Dhople et al., 2006; Hirsch et al., 2008).

Atopic dermatitis (AD) is a genetically predisposed inflammatory and pruritic allergic skin disease that affects approximately 10% of all dogs. The characteristic clinical features of AD are associated with IgE antibodies most commonly directed against environmen-

tal allergens (Halliwell, 2006; Reedy et al., 1997; Scott et al., 2001). Canine AD shares many immunological similarities with human AD and displays comparable clinical features (Leung and Bieber, 2003; Marsella et al., 2006; Nuttall et al., 2002a; Pucheu-Haston et al., 2008; Sinke et al., 2002; Willemse, 1986). In both species, recurrent skin infections with microbial agents often exacerbate the clinical manifestations of the disease. The high frequency of infections with *Staphylococcus aureus* and *Malassezia* spp. in human atopic skin (Leung et al., 2004) and *Staphylococcus pseudo intermedius* and *Malassezia pachydermatis* infections in canine atopic skin (Scott et al., 2001), indicates that the skin of atopic patients has a defective innate immune response. Indeed, the gene-expression of three β -defensins in atopic human skin, hBD1, hBD2 and hBD3, is altered compared to healthy skin (Gambichler et al., 2006; Gambichler et al., 2008; Howell et al., 2006; Howell et al., 2005; Nomura et al., 2003; Ong et al., 2002). Moreover, the secretion of hBD3 from the keratinocytes towards the skin surface is decreased in human atopic skin due to a suppressive effect of T_H2 cytokines IL-4 and IL-13 (Howell et al., 2006; Kisich et al., 2008; Nomura et al., 2003).

The information on function, localization and regulation of expression of canine β -defensins (cBDs) is scarce. Genome anal-

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ysis has revealed that the canine β -defensin gene family consists of 43 members, based on sequence similarity with other mammalian β -defensins (Patil et al., 2005). The gene-expression of cBD1 and cBD103 has recently been described in healthy canine skin (Candille et al., 2007; Wingate et al., 2009), while gene-expression of cBD122 and cBD127 was detected in only some of the skin samples tested. Interestingly, a mutation in cBD103 was the cause of black coat color in Labrador retriever dogs, showing a surprising new function of β -defensins (Candille et al., 2007). Furthermore cBD122 (previously called cBD1-like protein) was cloned from canine testis and proved to have antimicrobial activity against a broad spectrum of bacteria (Sang et al., 2005). Information on any of the other canine β -defensins is currently lacking. In order to extend our knowledge on canine β -defensins and to provide further understanding of the skin barrier formed by antimicrobial peptides, we investigated the tissue expression of various β -defensins and determined the altered gene-expression of cBD1 and cBD103 in the skin of dogs with AD.

2. Materials and methods

2.1. Animals

Seventeen dogs were included in this study. Group I consisted of 10 privately-owned atopic dogs presented to the Utrecht University Department of Clinical Sciences of Companion Animals. These animals fulfilled the diagnostic criteria for atopic dermatitis (Prélaud et al., 1998; Willemse, 1986) and were treated for bacterial and yeast infections prior to inclusion. Male and female dogs were equally represented and the group consisted of different purebred dogs. Their age ranged between 1 and 6 years (3.5 ± 2 years). Group II included seven healthy control dogs (four female dogs) owned by the Utrecht University Animals Laboratory and aged 2 ± 0.5 years. Five of these dogs were Beagles and two were mongrels.

2.2. Biopsies and tissue samples

Biopsies were taken using a 6 mm disposable punch (Kai Industries Co., Ltd., Solingen, Germany). In the dogs with AD two skin biopsies (one lesional and one non-lesional) were taken under sedation. From healthy dogs several peripheral blood and tissue samples were collected after the dogs had been euthanized with an overdose of sodiumpentobarbital IV for reasons not related to the study: from skin, liver, duodenum, colon, lymph nodes, spleen, lung, kidney, testis, and bone marrow. All samples were immediately snapfrozen in liquid nitrogen after collection and stored at -80°C until further use. The procedures were approved by the Utrecht University Ethical Committee, as required under Dutch legislation.

2.3. RNA extraction from tissues

Total cellular RNA was isolated from tissues by disruption of approximately 100 μg tissue using Magnalyzer Beads (Roche Diagnostics GmbH, Germany). RNA was isolated according to the manufacturer's recommendation using the High Pure RNA Tissue Kit (Roche Diagnostics GmbH, Mannheim, Germany). RNA from skin and bone marrow was isolated using Trizol reagent® (Invitrogen Life Technologies), which gave higher yield and purity for these tissues than the commercial kit. Total RNA from PBMCs was isolated from EDTA anti-coagulated blood collected by venepuncture. Equal volumes of blood and Polymorphprep™ solution (Lucron Bioproducts, De Pinte, Belgium) were centrifuged for 30 min at $500 \times g$ at 20°C . PBMCs were harvested and resuspended in HEPES buffered saline (pH 7.4). Subsequently, RNA was extracted as above with the High Pure RNA Tissue Kit®. After isolation, RNA quality and concentration were analyzed spectrophotometrically using a

Nanodrop-1000 (Isogen Life Sciences, IJsselstein, The Netherlands) and on 1.5% agarose gels. Genomic DNA traces were removed from total RNA with RNase-free DNaseI (Invitrogen Life Technologies).

2.4. Reverse transcriptase PCR

RNA samples were converted to cDNA using iScript (Bio-rad Laboratories, Richmond, CA) according to the manufacturer's recommendation. PCR reactions were performed using FastStart DNA Taq-polymerase (Roche Diagnostics GmbH, Mannheim, Germany). Primers were developed using software from Primer 3 version 0.4.0 (<http://frodo.wi.mit.edu/>). Primer pairs were investigated for specificity and uniqueness with the Basic Local Alignment and Search Tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The formation of secondary structures was determined on the DNA mfold server (<http://mfold.bioinfo.rpi.edu/cgi-bin/dna-form1.cgi>). PCR reactions were performed on the MyCycler Thermal Cycler (Bio-rad Laboratories, Richmond, CA) in 20 μl at the following conditions: denaturing at 95°C for 6 min, followed by 40 cycles of 30 s at 95°C , 30 s at the specific annealing temperature and 45 s at 72°C ; and a final extension step at 72°C for 7 min. Primer concentrations were 200 nM for all primer pairs. Primer sequence and annealing temperature are specified in Table 1. The PCR products were sequenced by Baseclear (Leiden, the Netherlands) to confirm specificity of the PCR reaction.

Table 1

Primers used for reverse transcriptase PCR and quantitative PCR. Indicated are sequence, annealing temperature in PCR reactions and expected product size.

Gene	Primer pairs	<i>T_m</i>	Product size
cBD1	F 5'-AGCCTCTCTCCCTTCTCTGG-3' R 5'-GCCATCGATCTGTAAAGA-3'	55	217
cBD102	F 5'-TTGCCTGCTGAAGCTAGTCC-3' R 5'-GAGGGCATCTGAATGAAAA-3'	54	168
cBD103	F 5'-GCCGCTGCTTACTGTACCT-3' R 5'-CCTCATGACCAACAGGCTTC-3'	55	112
cBD104	F 5'-CGTTCCTCTTCTTTTGCTG-3' R 5'-TGCATAGACATTGGGGCATA-3'	55	192
cBD105	F 5'-TTTTTATCAGGGTGCCAAAGC-3' R 5'-CGTCGGCAACAGAAAAAGTT-3'	57	167
cBD107	F 5'-CAGTGTGAGAAGATGGATGG-3' R 5'-CAAAGTGGTGTGAGTTCAGC-3'	56	92
cBD108	F 5'-CTCGCCCTGCTCTTCTTCT-3' R 5'-CGTGGCCATTAAACACCTC-3'	57	139
cBD122	F 5'-CCTGAAGACATGAAGCTTT-3' R 5'-TGAGATCAGACTTGGGACAGG-3'	56	268
cBD123	F 5'-GAAGCTCCTTTGGCTGACTG-3' R 5'-TTTCTCTCGGCTGGAACCTG-3'	55	185
cBD124	F 5'-TCTGGCTTCTTCGGTGTCT-3' R 5'-TTCCATAGGGGAGACAGCAC-3'	57	217
cBD134	F 5'-GCCTCTCCTCGTTGTGCTTA-3' R 5'-CGGGTTCTCTTGATACAGC-3'	55	187
cBD135	F 5'-GGTCTTGTGGCTCTTGTGT-3' R 5'-TGTTAATGCAGACAAACGTG-3'	55	228
cBD136	F 5'-TCAGGGAATGGATTGTTGG-3' R 5'-GGGTGGCAAATTTTCTTCA-3'	57	159
cBD138	F 5'-TCCTCCTTGTGTCTTGTGCC-3' R 5'-GAGCATCCATGGGCTTTTG-3'	55	112
cBD139	F 5'-CCCTTCTCATTTTCGTGTCC-3' R 5'-TGGCATGCGAGTCATACAAT-3'	57	134
cBD140	F 5'-GACTCCTCCATGTGGTCTC-3' R 5'-GTCTCTGGCTGCTCTCTT-3'	59	217
S100A8	F 5'-AAGGATGCAGACACCTGGT-3' R 5'-TATTATTCGGCAGGACAGC-3'	57	165

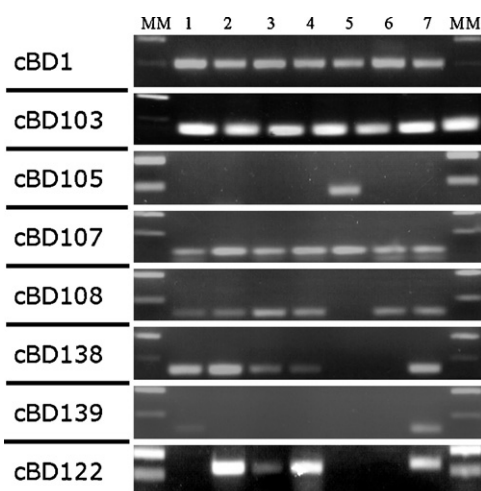


Fig. 1. Gene-expression of canine β -defensins in skin of seven healthy dogs. Numbers represent individual dogs, MM is 200 bp ladder.

2.5. Quantitative PCR

A quantitative PCR reaction was set up for cBD1, cBD103, cBD107 and S100A8 using the specific primers sequences and annealing temperatures depicted in Table 1. PCR reactions were performed in 25 μ l on a MyiQ single-color real-time PCR detection system (Bio-rad Laboratories, Richmond, CA) using SYBR green. The PCR reactions were initiated by a 12 min denaturation period at 95 °C, followed by 40 cycles consisting of 30 s at 95 °C, 30 s of annealing at the specific temperature for each primer set, and 30 s of elongation at 72 °C. All reactions were performed in triplicate and a melting curve analysis was performed to determine purity of the amplified products. Primer sequences and cycling details for quantitative determination of the housekeeping genes RPS5, RPS19, BM2 and HPRT have been described before (Brinkhof et al., 2006). Real-time PCR data were further analyzed on the MyiQ software (Bio-Rad).

2.6. Immunohistochemistry of cBD103

For the detection of cBD103 in canine skin and human skin a polyclonal rabbit antibody against hBD3 (Orbigen, San Diego, CA) was used at a dilution of 1:800, as described earlier for human skin (Sørensen et al., 2006). Skin biopsies were fixed in neutral 10% phosphate-buffered formalin (pH 7.2) for 24 h, dehydrated and embedded in paraffin. Slices (5 μ m) were cut with a microtome and mounted on glass slides. These slides were deparaffinized in xylene and rehydrated in graded alcohol. For antigen retrieval, the slides were incubated for 10 min in a boiling citrate solution (pH 6) in a microwave oven. Thereafter, slides were cooled down in the same solution at room temperature for

20 min. After dehydration in graded alcohol, slides were incubated in methanol containing 3% hydrogen peroxide to block the endogenous peroxidase. Next, sections were thoroughly rinsed in phosphate-buffered saline containing 0.05% Tween (TPBS). Slides were incubated in a blocking solution containing 10% normal goat serum (Jackson ImmunoResearch, West Grove, PA) and 2.5% bovine serum albumin (BSA) (Serva, Heidelberg, Germany) in PBS for 30 min. Subsequently, slides were incubated with the primary antibody diluted in PBS with 2.5% BSA. Thereafter, slides were rinsed (three times 5 min) with TPBS and incubated with a secondary biotinylated goat anti-rabbit antibody (Vector Laboratories Inc., Burlingame, CA) diluted 1:250 for 30 min. Three washing steps of 5 min in TPBS were followed by 30 min incubation with the ABC reagent (Vector Laboratories Inc.). Color was developed with Nova RED (Vector Laboratories Inc.). Slides were counterstained with Mayer's hematoxylin for 60 s, dehydrated and mounted in Pertex (Klinipath). Digital images were obtained with a coolpix camera (Nikon). As negative control the commercially available isotype antibody (Rabbit IgG-purified immunoglobulin reagent grade; Sigma-Aldrich, Inc., Missouri, USA) was used in a dilution of 1:800.

2.7. Statistical analysis

Non-parametric analysis of the real-time PCR results was performed because the data were not normally distributed. The Mann–Whitney test was applied to test for significance between healthy skin and non-lesional or lesional atopic skin. A Wilcoxon signed rank test was applied to test for significance between the paired data of lesional and non-lesional atopic skin of the same dog. Data were considered significant if $p < 0.05$.

3. Results

3.1. Gene-expression of canine β -defensins

The gene-expression of 16 β -defensins was determined in 11 different tissues of 7 healthy dogs. The tissues other than skin were chosen to represent the lymphatic, urogenital, respiratory and digestive system of the body. As is shown in Fig. 1, three β -defensins, cBD1, cBD103 and cBD107 were expressed in all skin samples, while five other β -defensins were inconsistently expressed and were only detected in some of the skin samples (cBD105, cBD108, cBD122, cBD138, cBD139). Levels of defensin mRNA in the other tissues showed that the gene-expression pattern of the tested cBDs was relatively broad with every tissue containing several of the β -defensins tested (Table 2). The testis contained most β -defensins, while four β -defensins (cBD104, cBD134, cBD135, cBD136) were not detected in any of the tissues tested. The primers of these 4 β -defensins were successfully used on genomic DNA indicating a true lack of expression in these tissues (data not shown). Of interest is

Table 2

Tissue gene-expression profile of canine β -defensins. (++) gene-expression in all samples, (+) gene-expression in >50% of the samples, (±) gene-expression in <50% of the samples, (–) no gene-expression.

	cBD1	cBD102	cBD103	cBD104	cBD105	cBD107	cBD108	cBD122	cBD123	cBD124	cBD134	cBD135	cBD136	cBD138	cBD 139	cBD 140
Skin	++	–	++	–	±	++	+	+	–	–	–	–	–	+	±	–
Duodenum	+	–	+	–	–	++	±	–	–	–	–	–	–	–	–	–
Colon	±	–	+	–	–	+	±	±	–	–	–	–	–	+	+	–
Liver	++	–	±	–	–	+	++	–	+	–	–	–	–	±	–	±
Kidney	++	–	++	–	–	+	++	–	+	–	–	–	–	±	++	++
Testis	±	++	++	–	+	++	++	++	++	++	–	–	–	++	–	++
Spleen	±	–	+	–	–	++	+	–	–	–	–	–	–	–	++	–
PBMCs	–	–	++	–	–	++	+	–	–	–	–	–	–	–	–	–
Bone marrow	–	–	++	–	–	++	++	–	–	–	–	–	–	–	–	–
Lymph node	–	±	–	–	–	++	+	±	+	–	–	–	–	±	+	–
Lung	++	–	++	–	–	+	++	++	++	–	–	–	–	+	–	±

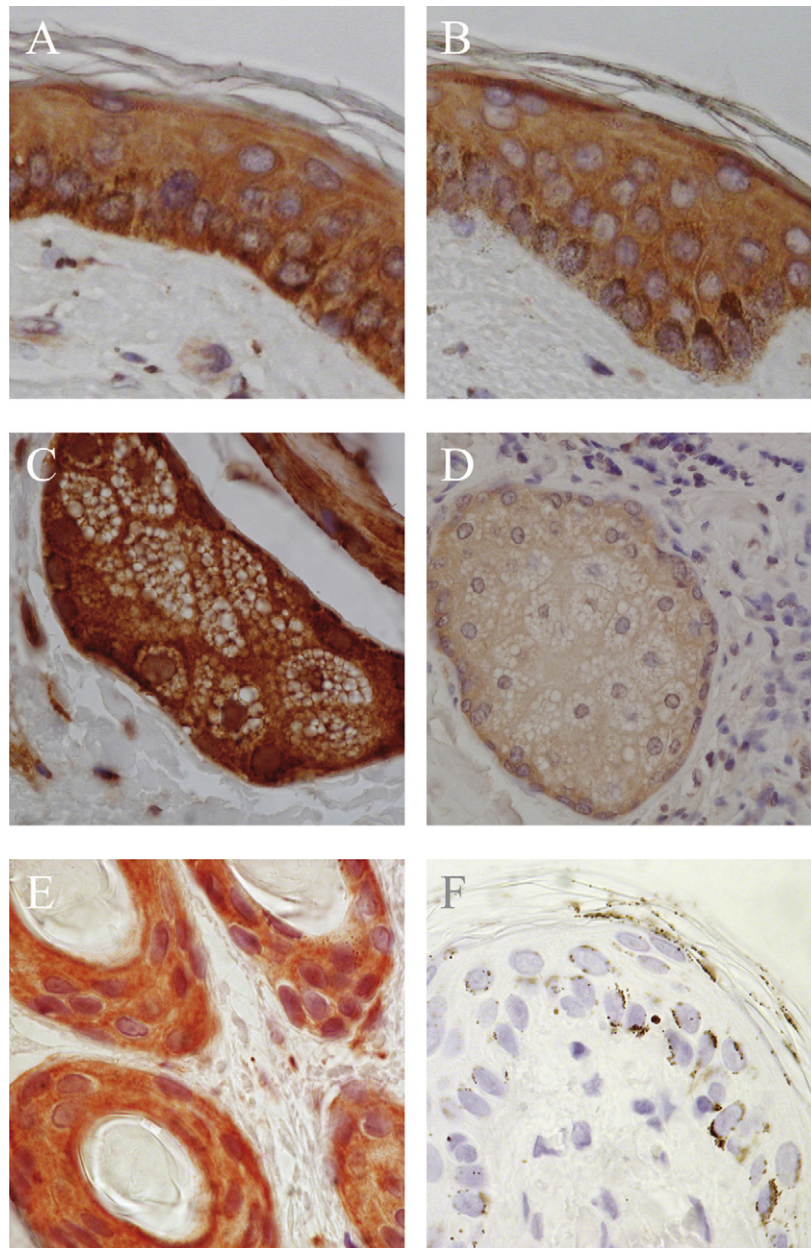


Fig. 2. Immunohistochemical staining of cBD103 and hBD3 in skin. Slides were stained with anti-hBD3 (1:800) and Nova-RED. Shown are: (A) canine and (B) human epidermis; (C) canine and (D) human sebaceous gland; (E) canine hair follicle; (F) negative control: canine skin using rabbit pre-immune serum as first antibody.

the presence of several cBDs in PBMCs, which could have resulted in positive signals in some of the other tissues if PMBC mRNA was co-isolated.

3.2. Localization of cBD103 in skin

The presence of cBD103 protein in skin was determined using immunohistochemistry. For this purpose an antibody raised against the human homologue hBD3 was used. The stained paraffin slides of canine and human skin are depicted in Fig. 2. In canine skin, intensive staining was observed in the entire epidermis (Fig. 2A), which is comparable to the findings in human skin (Fig. 2B). Furthermore, the sebaceous glands of canine and human skin showed strong staining indicating that these glands contain high levels of cBD103 (Fig. 2C and D, respectively). Finally, the hair follicles were also intensively stained in canine skin (Fig. 2E). Paraffin slices stained without the first antibody (data not shown) or with rab-

bit pre-immune serum (Fig. 2F) showed no staining indicating the specificity of the cBD103 staining. These results show that the expression of cBD103 is comparable to human skin and rule out that the observed cBD103 gene-expression in skin is due to PMBC mRNA.

3.3. Real-time PCR of *S100A8*, *cBD1* and *cBD103*

Four housekeeping genes were evaluated for stable expression in atopic and healthy skin samples using the freely available GeNorm program, which determines the number of housekeeping genes required for a reliable normalization of data (Vandesompele et al., 2002). Of these genes, HPRT and two ribosomal genes, RPS5 and RPS18, were stably expressed, while BM2 showed a more variable expression. The three stably expressed genes were subsequently used as reference genes to normalize qPCR data of the genes of interest.

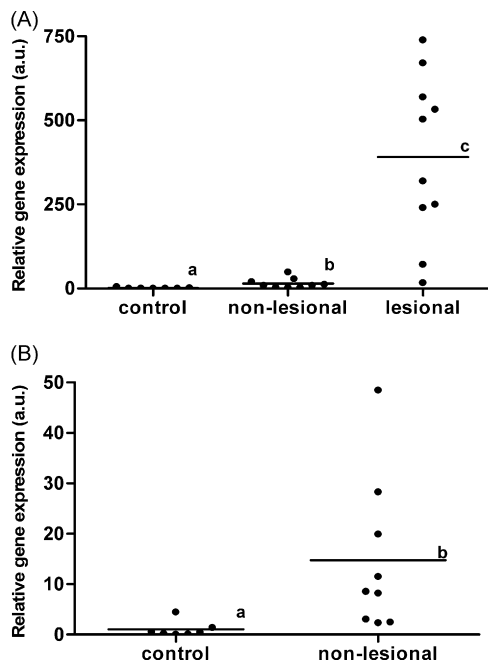


Fig. 3. Relative gene-expression of S100A8 in skin of healthy controls ($n = 7$) and atopic dogs ($n = 10$). (A) Expression of S100A8 expression in control, lesional and non-lesional skin samples, and (B) enlargement of A to more clearly show differences in S100A8 gene-expression of control and non-lesional skin samples. Shown are individual samples and the mean. Different labels (a–c) indicate significant differences ($p < 0.05$) between values.

Real-time quantitative PCR was set up to detect the gene-expression levels of S100A8, cBD1 and cBD103. As depicted in Fig. 3A, S100A8 is highly upregulated in skin of atopic dogs. A 390-fold increase was observed in lesional skin compared to normal skin ($p < 0.01$), while non-lesional skin had a 15-fold increase ($p < 0.01$) (Fig. 3B). These results confirm the clinical status of our animals since increased levels of the pro-inflammatory molecule S100A8 can be considered a marker for AD in both dog and human (Merryman-Simpson et al., 2008; Sugiura et al., 2005).

Gene-expression levels of cBD1 showed a lower but similar pattern of increased gene-expression as S100A8 (Fig. 4A). A significant 12-fold induction was observed in lesional skin ($p < 0.01$), while cBD1 mRNA in non-lesional skin still showed a 5-fold increase compared to healthy control skin ($p < 0.01$). Levels of cBD3 were less affected by AD (Fig. 4B). A small decrease in gene-expression was observed in both lesional (1.8-fold) and non-lesional skin (2.5-fold) of atopic dogs compared to healthy skin, of which only the latter difference reached statistical significance ($p = 0.043$). The gene-expression levels of cBD102, cBD107 and cBD108 were determined in both atopic and control skin but mRNA levels were too low (or not present) to detect reliable quantitative differences between atopic and normal skin.

4. Discussion

In the present report we describe the tissue distribution of 16 canine β -defensins and the change in expression of cBD1 and cBD103 in the skin of dogs with atopic dermatitis compared to healthy skin. Moreover, the presence of cBD103 peptide in the skin was confirmed using immunohistochemistry.

In an analysis of the canine genome, Patil et al. (2005) have described the existence of the canine β -defensin family, which consists of 43 mainly putative canine β -defensins. This number of genes is comparable to the number of β -defensin genes found in rat,

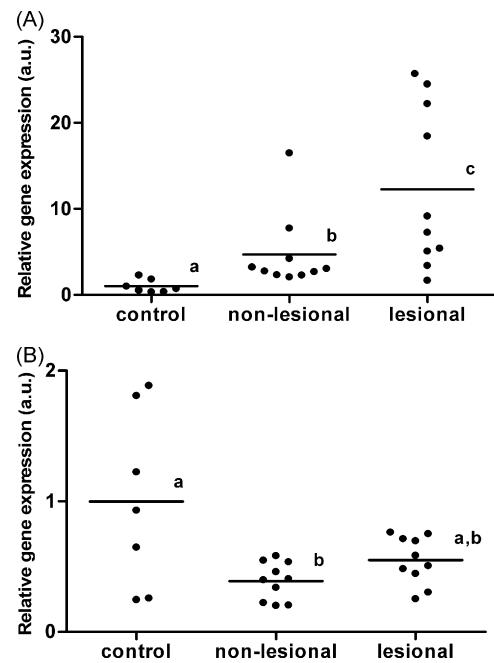


Fig. 4. Relative gene-expression of cBD1 and cBD103 in skin of healthy controls ($n = 7$) and atopic dogs ($n = 10$). (A) Expression of cBD1 and (B) expression of cBD103. Shown are individual samples and the mean. Different labels (a–c) indicate significant differences ($p < 0.05$) between values.

mouse and human. However, so far only a few of these canine genes have been supported by the existence of EST sequences. Furthermore, several members have the characteristic β -defensin cysteine motif but have an extended C-terminus, which could indicate that these proteins are functionally different from the 'classical' β -defensins. In this study we chose to study 16 members of this family based on homology with existing mammalian β -defensins, existing EST sequences and on the C-terminal sequence of the canine β -defensins.

The tissue distribution of canine β -defensins (Table 2) shows that most of the tested β -defensins are expressed in the testis. This is consistent with the expression profile of human, rat and porcine β -defensins, and indicates an important function for these peptides in innate immunity of the reproductive tract. In addition, a role of β -defensins in sperm maturation has been described (Tollner et al., 2004; Zhou et al., 2004). All other tested tissues expressed one or more β -defensins showing the broad expression of these peptides. In skin, the presence of cBD1 and cBD103 transcripts has been described before which was confirmed by our findings (Candille et al., 2007; Wingate et al., 2009). The presence of six other β -defensins in skin is new, although the expression was not consistent in all tested skin samples, with several samples testing negative for a specific β -defensin. This could indicate that expression of these β -defensins could be dependant on inflammation state, age or breed of the dog (Dorschner et al., 2003; Sørensen et al., 2005), but also other factors such as defensin copy numbers in the canine genome of the individual animals tested could have an effect. All these variables were not considered in these studies. A comparable non-constitutive expression was described in a recent study for cBD122, and also cBD127, the latter being a β -defensin that was not considered in our study due to the extended C-terminus (Wingate et al., 2009).

The presence of cBD103 gene transcripts in PMBCs prompted us to determine the presence and localization of cBD103 peptide in canine skin. Immunohistochemistry clearly showed that cBD103 peptide is expressed in whole epidermis, hair follicles and the

sebaceous glands (Fig. 2). This localization is comparable to hBD3 localization in human (de Jongh et al., 2005; Sawamura et al., 2005; Sørensen et al., 2006) and strengthens the dog as model system for atopic dermatitis. Unfortunately, the same strategy to use antibodies against human homologues could not be used for cBD1, because of the relatively low sequence homology between canine and human β -defensin 1.

In our studies, the gene-expression of cBD1 was significantly higher in canine atopic skin compared to healthy skin (Fig. 4A), an effect comparable to its human homologue hBD1, which is also altered in atopic skin (Gambichler et al., 2008). Furthermore, in analogy with a large human study regarding the expression of hBD1 in patients with chronic AD (Gambichler et al., 2008), an increased expression of cBD1 was found in both lesional and non-lesional canine atopic skin. The higher cBD1 levels could be a direct effect of skin infections since it is known that microbe-derived molecules can stimulate the expression of hBD1 (Harder et al., 2001). The expression of cBD1 might also be upregulated by TNF- α , since significantly higher levels of this proinflammatory cytokine were found in the lesional skin of dogs with AD (Maeda et al., 2002; Nuttall et al., 2002b).

The expression of cBD103 was approximately 2-fold lower in the skin of atopic dogs, although this only reached statistical significance for non-lesional skin (Fig. 4B). The tendency towards a decreased gene-expression for cBD103 in lesional and non-lesional skin of atopic dogs is not in direct agreement with human studies, which report an unchanged or increased levels of hBD3 in the skin of atopic patients (Gambichler et al., 2008; Howell et al., 2006; Kisich et al., 2008). However, it is known that T_H2 cytokines, IL-4 and IL-13, which are abundantly present in human atopic skin, can reduce the expression of hBD3 in cultured human keratinocytes (Howell et al., 2006; Kisich et al., 2008). In lesional canine skin increased levels of IL-4 mRNA have also been described. In addition, allergen challenges of high IgE beagles, a canine model for atopic dermatitis, resulted in an increased expression of IL-13, another T_H2 cytokine (Marsella et al., 2006). Therefore, an adaptive immune system skewed towards a T_H2 response in atopic dog could influence the expression of cBD103, resulting in a downregulation or defective upregulation of this canine β -defensin. Although there is no evidence regarding the antibacterial and antifungal effects of cBD103 against the canine skin pathogens *S. intermedius* and *M. pachydermatis*, it has been described that the human homologue, hBD3, is able to kill the human skin pathogen *S. aureus* in a salt-independent manner (Harder et al., 2001; Kisich et al., 2007). This indicates that a decreased level of hBD3 (or cBD103) could be linked to increased infection of the skin. Interestingly, a recent study has shown that keratinocytes of human atopic patients insufficiently mobilize hBD3 effectively onto the stratum corneum to kill *S. aureus* (Kisich et al., 2008). This would indicate that not the amount of β -defensins, but the actual localization of the peptide affects microbial status of the skin. It would be interesting to determine if canine keratinocytes show the same defective mobilization capacity of cBD103.

In conclusion, we determined for the first time the expression of 16 β -defensins in 11 different tissues of the dog. The expression of cBD1 and cBD103 was quantitatively determined in healthy canine skin and in lesional and non-lesional skin of dogs with AD. The increased expression of cBD1 and the tendency towards a decreased expression of cBD103 in the skin of atopic dogs indicate an altered reaction of the cutaneous innate immune system, which could partially explain the clinical observation of recurrent infections in dogs with AD. In addition, the results further confirm the similarity between the skin of human and dog and strengthen the usefulness of the dog as a model for human atopic dermatitis.

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