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Research paper

Lesional skin in atopic dogs shows a mixed Type-1 and Type-2 immune responsiveness

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ABSTRACT

Canine atopic dermatitis (AD) is a chronic inflammatory and pruritic skin disease which shares several characteristics with its human counterpart. In chronic patch test lesions of human with AD mainly a Th1-type cellular response is found. Besides, non-lesional AD skin is already skewed for inflammation and therefore different from healthy skin.

The goal of this study was to characterize local immune responsiveness in chronic canine AD lesions as compared to that in non-lesional AD skin by defining T cell subset relevant cytokine- and transcription factor expression profiles. The gene expression of the Th1 cytokines IL-12p35, IL-12p40 and IFN- γ and their related transcription factors STAT4, SOCS5 and T-bet, the Th2 cytokines IL-4 and IL-13 and transcription factors STAT6, SOCS3 and GATA-3 and the regulatory cytokines IL-10 and TGF- β and the transcription factor FOXP3 was evaluated in healthy control and atopic dogs. In non-lesional (NLS) and chronic lesional skin (LS) of atopic dogs and control skin (CS) from healthy dogs mRNA expression of cytokines and transcription factors were measured by quantitative real-time PCR. Significantly different values were found for the following factors: IL-12p40 mRNA was lower in LS when compared to NLS. Expression of STAT4 was higher in LS compared to CS and NLS. More IL-13 and SOCS3 were found in LS and NLS when compared to CS and also in LS compared to NLS. GATA-3 was lower in LS compared to NLS. IL-10 expression was higher in both LS and NLS compared to CS and more IL-10 was present in LS compared to NLS. These findings indicate that both Th1- and Th2-type as well as T regulatory cells are present in NLS and LS in canine atopic skin.

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1. Introduction

Canine atopic dermatitis (AD) is a chronic inflammatory and pruritic skin disease with a prevalence of about 10–15% of the canine population. It shares several characteristics with its human counterpart, e.g. the genetic

predisposition to develop the disease, the early age of onset, the predilection sites of the affected skin and similarities in immunopathogenic mechanisms (Griffin and DeBoer, 2001; Sinke et al., 2002). In canine and human AD it is currently accepted that imbalances in lymphocyte populations and the related cytokine production play an important role in the pathogenesis of the disease. In both human and dogs non-lesional skin, although clinically unaffected, is different from healthy skin and contains increased numbers of CD4⁺ T cells (Sinke et al., 1997; Leung et al., 2004). In

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human it has been shown, by use of the atopy patch test as a model for the induction of eczematous lesions, that a biphasic T cell response occurs during epicutaneous allergen challenge (Grewe et al., 1995; Langeveld-Wildschut et al., 1996). In the initial phase the infiltrate is characterized by Th2 cells, mainly producing IL-4, IL-5 and IL-13, and eosinophils whereas after 24–48 h it is characterized by infiltration with Th1 cells and macrophages and presence of Th1 cytokines like IFN- γ and IL-12 (Thepen et al., 1996). Besides the Th1 and Th2 helper T cells, regulatory T cells (Treg) are thought to play a role in AD by production of cytokines with a regulatory function, such as IL-10 and TGF- β (Herrick and Bottomly, 2003; Larche, 2007; Wu et al., 2007). The action of these different cytokines in allergic inflammation is tightly regulated by transcription factors such as suppressors of cytokine signalling (SOCSs) and signal transducer and activator of transcription (STATs) (Elliott and Johnston, 2004; Chen and Khurana Hershey, 2007; Knisz and Rothman, 2007; Prefontaine et al., 2007). These groups of transcription factors are associated with specific subsets of cytokine-producing Th cells and cross-talk between different signalling pathways is mediated by these factors (Robinson and Lloyd, 2002). Genes encoding for STAT4, SOCS5 and T-bet are associated with Th1 cells, STAT6, GATA-binding protein 3 (GATA-3) and SOCS-3 with Th2 cells and FOXP3 with regulatory T cells (Arakawa et al., 2004; Prefontaine et al., 2007). SOCS5 protein is selectively expressed by Th1 cells. It can bind the IL-4R and suppress STAT6 phosphorylation (Knisz and Rothman, 2007). STAT6 inhibits expression of IFN- γ , IL-12 and TNF- α and collaborates with GATA-3, which promotes expression of Th2 cytokines and suppresses IL-12 and STAT4 expression thereby inhibiting Th1 development (Mowen and Glimcher, 2004; Ho et al., 2009). GATA-3, however, is inhibited by T-bet (Prefontaine et al., 2007). SOCS 3 is expressed in both Th1 and Th2 cells, but much higher in Th2 cells. SOCS3 expression correlates with disease severity in atopic patients and SOCS3 inhibits IL-12-mediated activation of the STAT4 pathway, thereby inducing Th2 differentiation (Seki et al., 2003; Yoshimura et al., 2007). FOXP3 is a critical transcription factor for regulatory T cells and interest in this factor stems from the observation that Treg cells inhibit effector T cells, both Th1 and Th2 in disease (O'Garra and Vieira, 2003; Huehn et al., 2009).

However, in dogs with spontaneously AD no reliable model for the induction of eczematous lesions exists. For this reason it is not possible to collect biopsies from acute lesions (within 48 h after initiation of inflammation). In contrast, chronic lesional skin (LS) is clinically well-defined. Some groups have described the inflammatory cell infiltrate in non-lesional (NLS) and lesional skin phenotypically (Olivry et al., 1997; Sinke et al., 1997) but relatively few studies have been done to obtain a better insight in the functional aspects of T helper cell subsets. These were executed in either a low number of animals or with semi-quantitative methods (Olivry et al., 1999; Nuttall et al., 2002a). A better understanding of the tightly regulated pathways in which both cytokines and transcription factors play important roles, may finally lead to strategies to suppress the inflammatory reaction in both canine and human AD.

We hypothesised that LS of dogs with AD displays a Th1-polarized cytokine and transcription factor profile as is observed in chronic cutaneous patch test lesions in human with AD. Furthermore it was expected that as NLS of AD dogs contains more inflammatory cells, this will be reflected in higher mRNA expression of specific transcription factors and cytokines, as compared to the skin of healthy control dogs (CS).

Hence, it was the aim of this study to characterize the immune response in chronic AD lesions and non-lesional AD skin by differentiating T cell subsets on the basis of their cytokines and transcription factors profiles.

Real-time quantitative PCR was used to determine Th1, Th2 and regulatory cytokines and transcription factors in non-lesional and chronic lesional skin versus healthy control skin.

2. Materials and methods

2.1. Design and animals

Two groups of dogs were used in this study. Group I consisted of privately-owned AD dogs ($n=28$) presented to the Department of Clinical Sciences of Companion Animals, Utrecht University. All dogs fulfilled the diagnostic criteria for atopic dermatitis (Willemse, 1986; Favrot et al., 2010). This group consisted of 19 Labradors Retrievers and one of each of the following breeds: Flatcoated Retriever, Gordon Setter, Boxer, Vizsla, French Bulldog, Jack Russell Terrier, Podenco Canario, Dachshund and German Shepherd; male and female dogs were equally represented. Ages ranged between 1 and 8 years (median 3.6 years). Group II included seven healthy control dogs owned by the Department and comprised of five male Beagle dogs and two female mongrel dogs with ages ranging between 4 and 11 years (median 8 years). All dogs were withdrawn from treatment with glucocorticoids for at least 6 weeks before entering the trial. Treatment for secondary bacterial or yeast infections were allowed before and throughout the study. From all dogs 6 mm punch biopsies of the skin were obtained under general anesthesia. All AD dogs suffered from chronic AD. From these dogs two LS biopsies and two NLS biopsies were collected for qPCR. CS and NLS specimens were all taken from the lateral thorax, whereas the LS biopsies were obtained from affected predilection sites. After collection, the skin biopsies were immediately snap-frozen in liquid nitrogen and stored at -70°C until used for RNA isolation. The procedures were approved by the Utrecht University Animal Ethics Committee, as required under Dutch legislation.

2.2. RNA isolation and cDNA synthesis

Total RNA was isolated using a combination of the TRizol reagent (Invitrogen, Breda, the Netherlands) and the RNeasy Mini Kit (Qiagen, Leusden, the Netherlands) according to the manufacturers instructions. In short, the skin tissue was disrupted and homogenized in Trizol reagent using a Biopulverizer (Biospec #59013, Biospec Inc., Bartlesville, OK) and Ultra-turrax (T8, IKA® Labortechnik GmbH, Staufen, Germany). RNeasy columns were

Table 1

Nucleotide sequences of canine specific primers used for quantitative PCR analysis of cDNA from canine control skin, non-lesional skin and lesional skin.

Gene	Forward primer 5' → 3'	Reverse primer 5' → 3'	Ta (°C)
β-Glucuronidase (BGLR) ^a	AGACGCTTCCAA/GTACCCC	AGGTGTGGTGTAGAGGAGCAC	62.0
Ribosomal protein S5 (RPS5) ^a	TCACCTGGTGAG/AACCCCT	CCTGATTACACGGCGTAG	62.5
Ribosomal protein S19 (RPS19) ^a	CCTTCCTCAAAA/GTCTGGG	GTCTCATCGTAGGGAGCAAG	61.0
IL-12p35 ^c	TAATGGATCCCAAGAGGCAG	TCAAGGGAGGATTCTGTGG	62.5
IL-12p40 ^c	GGACGTTTCACATGCTGGT	CCACTCTGACCTCTCTGCT	59.0
IFN-γ ^c	AGCGCAAGCGGATAAATG	GCGGCCTGGAACAGATT	55.8
IL-4 ^c	CCAAAGAACAACAGCGATAAGGAA	GTTTGCCATGCTGCTGAGGTT	61.0
IL-13 ^c	GAGGAGCTGGTCAACATCA	TGCAGTCGGAGACATTGA	59.0
IL-10 ^c	CCCCGGCTGAGAACACGAC	AAATGCGCTCTTCACTGCTCCAC	63.0
TGF-β ^b	CAAGGATCTGGCTGGAAGTGA	CCAGGACCTTGCTGTACTGCTGT	65.0
GATA-3 ^c	TACGTCCCGAATACAGCTC	ACTCCTGCCTTCTGTGCT	64.0
STAT-4 ^c	ACTGGAAGAGGCGACAACAG	GCCTTCTGAGTTGGAACAGG	59.0
STAT-6 ^c	AACTGCAGCGGCTCTATGTC	CATGTGCAGCAGAAGGTGT	64.0
SOCS-3 ^c	ACACCAGCTGCGCTCAAGACCT	CGCCTCGCCGCCCTGCA	63.0
SOCS-5 ^c	TCTGCCGTGCAGTAATCTGT	GCCTTGACTGGTTCTCGTTC	65.0
Foxp3 ^c	CAAATGGTGTCTGCAAGTGG	GTGCTCTGCCCTTCTCATCT	59.0
T-bet ^c	AATCAGCACCAGACGGAGAT	GTCCACGAACATCCGGTAAT	61.2

^a Brinkhof et al. (2006).^b Spee et al. (2005).^c Veenhof et al. (2010).

used for clean-up of the RNA including DNase digestion (Qiagen Rnase-free DNase kit). RNA was quantified spectrophotometrically using Nanodrop ND-1000 (Isogen Life Sciences, IJsselstein, the Netherlands). cDNA synthesis was performed as previously described (Brinkhof et al., 2006; Schlotter et al., 2009). Samples were screened for contamination with genomic DNA by qPCR of non-reverse-transcribed RNA templates.

2.3. Primer design

Primer sets for cytokines and transcription factors (Table 1) were developed using known dog sequences available from Ensembl (www.ensembl.org) or NCBI (www.ncbi.nih.gov/genbank/index.html) and the design was performed with Oligo Explorer 1.1.0 software (www.genelink.com/tools/gi-downloads.asp) as previously described (Spee et al., 2005; Brinkhof et al., 2006; Veenhof et al., 2010). To reduce chances of amplifying traces of genomic DNA, the primers were positioned

in different exons. All PCR products had sizes between 100 and 150 bp. For the canine endogenous reference genes, β-glucuronidase (BGLR/GUSB), ribosomal protein S5 (RPS5) and ribosomal protein 19 (RPS 19) primers were used (Table 1) (Brinkhof et al., 2006).

2.4. Quantitative PCR

Real-time qPCR employing the high affinity, double-stranded DNA-binding dye SYBR Green read in a Bio-Rad My-iQ detection system (IQ SYBR Green Supermix and My-iQ, Bio-Rad, Veenendaal, The Netherlands), was performed in duplicate according to the manufacturer's instructions. Primers (Eurogentec, Maastricht, the Netherlands) had a final concentration of 400 nM each and 1.0 μl of cDNA template was used in a reaction volume of 25 μl on 96-well iCycler iQ plates (Bio-Rad). Q-PCR reactions were performed as described before (Brinkhof et al., 2006). Standard curves constructed by plotting the relative starting amount versus threshold cycles were generated using

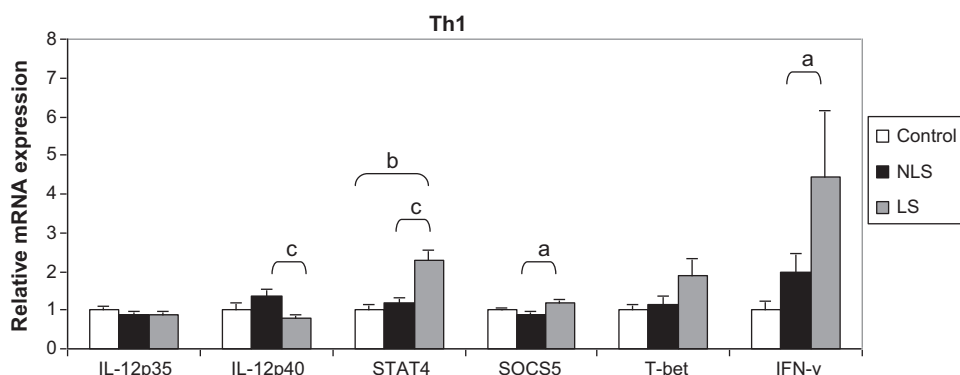


Fig. 1. Relative mRNA levels of Th1 cytokines and transcription factors in control skin, non-lesional AD skin (NLS) and lesional AD skin (LS). Mean ± SEM of grouped Th1-type mRNA. Values represent ratios of mRNA expression in each group, compared with control dogs: (a) $p \leq 0.05$; (b) $p \leq 0.01$; (c) $p \leq 0.001$; Group means of NLS and LS versus CS were compared by using the multivariate ANOVA (MANOVA) of the general linear model (GLM). Differences between the group means of NLS versus LS were compared using repeated measures analysis of the GLM. White bars: control skin (CS); Black bars: non-lesional atopic skin (NLS); Grey bars: lesional atopic skin (LS).

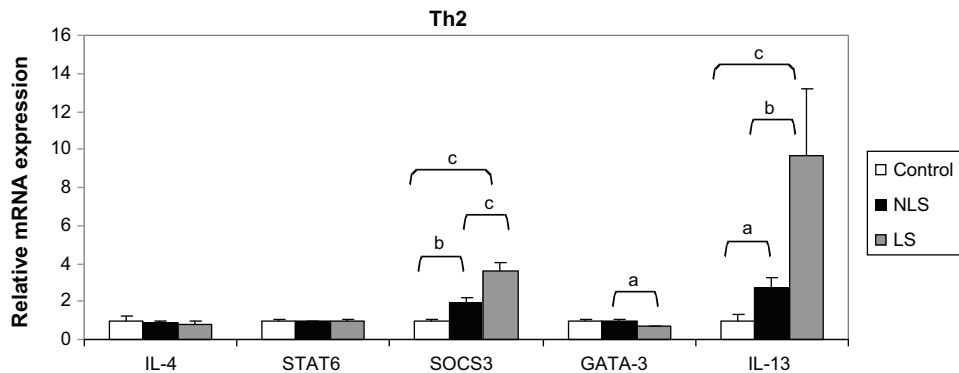


Fig. 2. Relative mRNA levels of Th2 cytokines and transcription factors in control skin, non-lesional AD skin (NLS) and lesional AD skin (LS). Mean $\pm$ SEM of grouped Th1-type mRNA. Values represent ratios of mRNA expression in each group, compared with control dogs: (a) $p \leq 0.05$; (b) $p \leq 0.01$; (c) $p \leq 0.001$; see Fig. 1 for statistical methods used. White bars: control skin (CS); Black bars: non-lesional atopic skin (NLS); Grey bars: lesional atopic skin (LS).

serial 4-fold dilutions of pooled cDNA fractions from control skin, non-lesional and lesional AD skin, Con-A stimulated peripheral blood mononuclear cells (PBMC) and liver. cDNA from PBMC and liver contains mRNA of all included cytokines and transcription factors, which is needed to construct proper standard lines. The amplification efficiency, $E = (10^{(1/\text{slope})} - 1) \times 100\%$, of all standard lines were $>95\%$ and $<105\%$ and all melting curves described a single distinctive peak. For each experimental sample the relative expression of the gene of interest and of the endogenous reference genes GUSB, RPS19 and RPS5 were determined from the appropriate standard curve (Schlotter et al., 2009). The expression of the gene of interest was normalized to the mean expression of the endogenous references for each animal. The normalized values were divided by the normalized values of the calibrator (control group) to generate relative expression levels. The relative expression levels of the genes of interest are reported as mean $\pm$ SEM for NLS and LS of all dogs. Data analysis was performed with My-IQ software (Bio-RAD, Veenendaal, The Netherlands).

2.5. Statistical analysis

All statistical analyses were performed with SPSS 12.0.1 for Windows (SPSS Benelux BV, Gorinchem, the Netherlands). The two-sided level of significance was set at $p \leq 0.05$. Group means of NLS and LS versus CS were compared by using the multivariate ANOVA (MANOVA) of the general linear model (GLM). Differences between the group means of NLS versus LS were compared using repeated measures analysis of the GLM. Relationships between variables were evaluated by calculation of Pearson's correlation coefficient.

3. Results

3.1. mRNA expression of cytokine and transcription factors in LS compared to CS and NLS

STAT4 in LS had a higher expression when compared to CS ($p < 0.01$) as well as to NLS ($p < 0.001$). More SOCS5 ($p < 0.05$) and IFN- γ ($p < 0.05$) was present in LS when com-

pared to NLS (Fig. 1). In contrast IL-12p40 expression in LS was lower ($p = 0.001$) when compared to NLS.

LS had a higher expression of SOCS3 and IL-13 when compared to CS ($p < 0.001$ and $p = 0.001$, respectively) and also when compared to NLS ($p < 0.001$ and $p < 0.01$, respectively; Fig. 2). The expression of GATA-3 in LS is lower compared to NLS ($p < 0.05$), whereas lower expression observed for GATA-3 in LS compared to CS ($p = 0.07$) may only be classified as a trend ($0.05 < p < 0.1$).

IL-10 was higher expressed in LS when compared to both NLS ($p < 0.001$) and CS ($p \leq 0.01$; Fig. 3). Foxp3 showed a trend to higher expression in LS compared to NLS ($p = 0.08$).

3.2. mRNA expression of cytokine and transcription factors in NLS compared to CS and LS

NLS had a higher expression of SOCS3 ($p < 0.001$) and IL-13 ($p < 0.05$) when compared to CS (Fig. 2). IL-10 was higher expressed in NLS ($p < 0.05$) when compared to CS (Fig. 3).

3.3. Correlation

Significant interactions between the expression levels of the genes analyzed in each of the tissue groups are illustrated in Tables 2 and 3. However, significant relations should be interpreted in the context of the r -value and values below 0.7 should be considered cautiously. In CS no significant correlations are found. In NLS T-bet is highly positively correlated ($r > 0.7$) with STAT4 and with IFN- γ ($p < 0.01$). For LS the same is observed although the correlation factor is a bit lower ($0.6 < r < 0.7$). STAT4 is also positively correlated with IFN- γ ($0.6 < r < 0.7$; $p < 0.01$). Although other significant correlations are noticed, the r -value in these cases are around 0.5 or lower and should thus not be considered seriously.

4. Discussion

Canine AD, like human AD, is reported to be characterized by a dysregulation of the immune response comprising both Th2 and Th1 responses. In addition, the regulatory T cells are gaining interest in canine studies (Biller et al.,

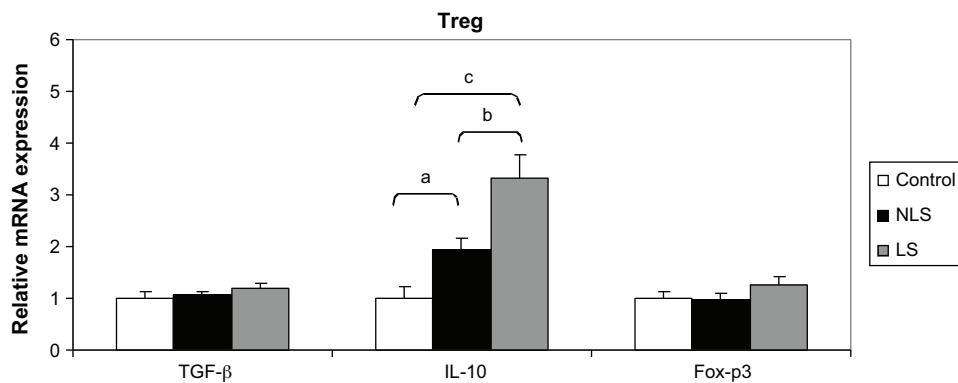


Fig. 3. Relative mRNA levels of T regulatory cytokines and transcription factors in control skin, non-lesional AD skin (NLS) and lesional AD skin (LS). Mean $\pm$ SEM of grouped Th1-type mRNA. Values represent ratios of mRNA expression in each group, compared with control dogs: (a) $p \leq 0.05$; (b) $p \leq 0.01$; (c) $p \leq 0.001$; see Fig. 1 for statistical methods used. White bars: control skin (CS); Black bars: non-lesional atopic skin (NLS); Grey bars: lesional atopic skin (LS).

2007; Keppel et al., 2008; Mizuno et al., 2009). To obtain a better insight into the types of Th cells possibly playing a role in the pathogenesis of spontaneously occurring canine AD, mRNA of several transcription factors and cytokines, specifically produced by Th1-type, Th2-type or Treg cells, was measured.

The investigated Th1-related transcription factors/cytokines were: IL-12p35, IL-12p40, IFN- γ , STAT4, SOCS5 and T-bet, these of the Th2 group: IL-4, IL-13, STAT6, SOCS3 and GATA-3 and of the T regulatory group: TGF- β , IL-10 and FoxP3.

Most significant differences are found when LS is compared to NLS and CS, which was expected, based on as well the clinical findings, in which LS is well differentiated from NLS and CS, as on former studies which described an inflammatory pattern to be present in lesional skin (Olivry et al., 1997; Sinke et al., 1997). It was hypothesized that LS would display a Th1-polarized cytokine and transcription factor profile. Higher expression was actually found for the Th1 transcription factors STAT4, SOCS5 and cytokine IFN- γ , but not for both chains of IL-12 and for T-bet.

It was anticipated to find higher levels of IL-12 mRNA expression as this cytokine is reported to have a central role in Th1 responses in human (Trinchieri, 2003; Leung et al., 2004). However, lesional skin has a significantly lower amount of IL12-p40 when compared to non-lesional skin,

whereas IL-12p35 did not show differences between the skin groups. IL-12 is a heterodimeric cytokine composed of two subunits: p35 and p40. The genes encoding p35 and p40 are located on different chromosomes and therefore protein expression is independently regulated. When these subunits are co-expressed in the same cell they form the biologically active p70 heterodimer. The unit p40 can be secreted as a monomer or homodimer, whereas p35 can be secreted only when associated with p40 (Trinchieri, 2003; Watford et al., 2004). Messenger RNA encoding IL-12p35 is present in many cell types, which can explain the findings in this study. At the same time this ubiquitous expression makes it difficult to analyse its regulation. By contrast, p40 mRNA is restricted to cells that produce the biologically active heterodimer and might thus be a better reflection of IL-12 production. Hence, it was surprising to find even lower expression in LS. An explanation might be that IL-12 production by macrophages, eosinophils and inflammatory dendritic epidermal cells (IDECs), has already occurred and the resulting Th1-type cytokine milieu has been established in LS. Another explanation is that IL-12p40 also associates with p19 to form IL-23. Few other studies in canine AD measured IL-12 subunits. None of these studies found higher expression of one of the IL-12 subunits in lesional skin (Olivry et al., 1999; Nuttall et al., 2002b; Marsella et al., 2006). The finding in the present study of significantly higher IL-10 and IL-13 mRNA in LS, cytokines

Table 2

Significant correlation coefficients of Th1 related cytokines and transcription factors in non-lesional (NLS) and lesional (LS) skin of dogs with atopic dermatitis.

		STAT4	Tbet
SOCS5	NLS	0.420*	0.437*
	LS	0.456*	NS
STAT4	NLS	–	0.709**
	LS	–	0.621**
IL12p35	NLS	NS	0.440*
	LS	0.469*	0.391*
IFN- γ	NLS	0.642**	0.715**
	LS	0.552**	0.647**

NS: non-significant p -value.

* $p \leq 0.05$.

** $p \leq 0.01$.

Table 3

Significant correlation coefficients of Th2 related cytokines and transcription factors in non-lesional (NLS) and lesional (LS) skin of dogs with atopic dermatitis.

		GATA3	IL4	SOCS3
STAT6	NLS	0.523**	NS	NS
	LS	0.507**	0.403*	NS
IL-13	NLS	–0.503**	NS	NS
	LS	NS	NS	0.457*
GATA-3	NLS	–	NS	NS
	LS	–	0.455*	NS

NS: non-significant p -value.

* $p \leq 0.05$.

** $p \leq 0.01$.

known to inhibit IL-12 production (Watford et al., 2004) support the low IL-12 levels found.

Related to these lower levels of IL-12 in LS, also lower levels of IFN- γ and STAT4 would have been expected. However, significantly higher expressions of both factors in LS are found. Activation of STAT4, involved in controlling Th1 gene expression, induces production of IFN- γ (Watford et al., 2004), but normally depends on IL-12. Besides IL-12, the higher expressions of STAT4, IFN- γ and SOCS5 fit with the hypothesis that lesional skin is skewed towards a Th1-like environment.

The significant correlations between the Th1 factors point out their important relationship.

An important inhibitor of IL-12 mediated STAT4 activation is SOCS3 (Seki et al., 2003) which, in agreement with human AD and asthma patients, was indeed found to be significantly higher expressed (Seki et al., 2003). STAT6, also a potent inhibitor of IL-12 expression (Prefontaine et al., 2007), does not show any differences in expression between the various skin groups. GATA-3, highly expressed in Th2 cells (Ho et al., 2009) can be upregulated by IL-4 through STAT6 (Mowen and Glimcher, 2004). This study shows no increased expression of both these factors which may in part explain the findings for GATA-3. SOCS-5 association with the IL-4R α chain results in a decreased STAT-6 activation, a mechanism to downregulate IL-4 signalling in Th1 cells (Seki et al., 2002) which fits with the findings in this study.

The significantly higher expression of IL-13 in LS coincides with results in a canine AD model (Marsella et al., 2006) and with many human studies in AD. These human studies have emphasized the importance of IL-13 in allergic asthma and atopic dermatitis in the recruitment of inflammatory cells to the sites of inflammation in AD (Purwar et al., 2006; Purwar et al., 2008). A recent study using a transgenic mouse model showed the possible direct tissue effects of IL-13 as these mice show a skin phenotype which reflects AD lesions observed in human in several aspects (Zheng et al., 2009). IL-13, like IL-4, is produced by Th2 cells, mast cells, eosinophils and basophils (Gessner et al., 2005). Although the differences in the contribution of IL-4 and IL-13 in allergic inflammation are difficult to elucidate as they signal through the shared IL4R α chain, it is evident that in vivo IL-13 occasionally dominates the response where also IL-4 could use the receptor-complex (Wills-Karp and Finkelman, 2008).

The present study shows a significantly greater expression of IL-10 in LS compared to NLS and CS. These results are in contrast to other studies in dogs with naturally occurring AD and in a dog model of AD, in which no significant differences were observed (Nuttall et al., 2002a; Marsella et al., 2006). A study in high-IgE-Beagles experimentally-sensitized to house dust mites, even showed a lower IL-10 mRNA expression in whole blood following environmental allergen challenge (Maeda et al., 2007). No differences were observed for the expression of TGF- β between the different skin types, whereas other studies showed a decrease of TGF- β in LS (Nuttall et al., 2002b) and both PBMC of IgE-sensitized dogs and PBMC from dogs experimentally-sensitized to Japanese cedar pollen (Fujiwara et al., 2003; Maeda et al., 2007). These studies considered a role for IL-

10 and TGF- β as important mediators preventing allergic inflammation and suggested that AD lesions might be associated with suppression of regulatory cytokines. Indeed, studies into human AD consider a role for T regulatory cells producing IL-10 and also increased production of TGF- β has been shown to contribute to regulatory T cell function (Jutel et al., 2003; Larche, 2007). In human, it is evident that IL-10 can be produced from many different cell types such as dendritic cells, macrophages and several regulatory T cell populations (Couper et al., 2008). These specific cell types are an important part of the inflammatory cell population also found in canine AD skin (Olivry et al., 1997; Olivry and Hill, 2001) and might all be responsible for making IL-10. Moreover IL-10 production by one of these cell populations influences other IL-10 producing cells thereby regulating each other (Couper et al., 2008). For Foxp3, expressed by CD4+CD25+T regulatory cells, in this study no differences were found between the skin groups which coincides with findings in human AD skin lesions that lack functional regulatory T cells (Ou et al., 2004; Verhagen et al., 2006).

The summarizing results of this study do not indicate a purely Th1-polarized profile in chronic lesional skin of AD dogs but rather a mixed expression of Th1-type and Th2-type transcription factors and cytokines.

Although these findings in dogs suffering from spontaneously occurring AD are different from human patch test results, studies in spontaneous lesional skin from people with atopic eczema are in agreement with ours (Akdis, 2011; Guttman-Yassky et al., 2011). Like in human AD the regulatory T cell compartment needs further elucidation. However, the high mRNA expression found for IL-10 might indicate that the inflammatory effects of the mixed Th1/Th2-type of infiltrate is counteracted by IL-10 irrespective of the source of this cytokine. Finally, both LS and NLS are different from CS as evidenced by higher expressions for SOCS3, IL-13 and IL-10. If this reflects an activation status in predisposed dogs which may develop into lesional skin under certain conditions, is unclear at the moment.

Future studies are needed to evaluate the biological significance of these findings, e.g. Western blots to confirm protein synthesis from mRNA and immunohistochemistry to relate the different cell types to transcription factors and cytokines produced.

Conflict of interest

The authors state no conflict of interest.

Acknowledgment

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