Interleukin-31: its role in canine pruritus and naturally occurring canine atopic dermatitis


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Background – Interleukin-31 (IL-31) is a member of the gp130/interleukin-6 cytokine family that is produced by cell types such as T helper 2 lymphocytes and cutaneous lymphocyte antigen positive skin homing T cells. When overexpressed in transgenic mice, IL-31 induces severe pruritus, alopecia and skin lesions. In humans, IL-31 serum levels correlate with the severity of atopic dermatitis in adults and children.

Hypothesis/Objective – To determine the role of IL-31 in canine pruritus and naturally occurring canine atopic dermatitis (AD).

Animals – Purpose-bred beagle dogs were used for laboratory studies. Serum samples were obtained from laboratory animals, nondiseased client-owned dogs and client-owned dogs diagnosed with naturally occurring AD.

Methods – Purpose-bred beagle dogs were administered canine interleukin-31 (cIL-31) via several routes (intravenous, subcutaneous or intradermal), and pruritic behaviour was observed/quantified via video monitoring. Quantitative immunoassay techniques were employed to measure serum levels of cIL-31 in dogs.

Results – Injection of cIL-31 into laboratory beagle dogs caused transient episodes of pruritic behaviour regardless of the route of administration. When evaluated over a 2 h period, dogs receiving cIL-31 exhibited a significant increase in pruritic behaviour compared with dogs that received placebo. In addition, cIL-31 levels were detectable in 57% of dogs with naturally occurring AD (>13 pg/mL) but were below limits of quantification (<13 pg/mL) in normal, nondiseased laboratory or client-owned animals.

Conclusions – Canine IL-31 induced pruritic behaviours in dogs. Canine IL-31 was detected in the majority of dogs with naturally occurring AD, suggesting that this cytokine may play an important role in pruritic allergic skin conditions, such as atopic dermatitis, in this species.

Introduction
Canine atopic dermatitis (AD) is a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features.1 One clinical feature that dogs with AD commonly display is pruritus, which can have a significant impact on the quality of life for the pet as well as for the owner. However, the underlying pathways and mechanisms involved in triggering pruritic behaviours are not clear, hampering the development of effective anti-pruritic therapies.

Interleukin-31 (IL-31) is a recently identified cytokine implicated in pruritic skin conditions such as human AD. When initially characterized in transgenic mice, overexpression of IL-31 led to the development of several hallmark signs of AD, which included increased inflammatory cell infiltration into the skin, severe pruritus, alopecia and skin lesions.2 Interleukin-31 has been shown to be produced by activated T helper type 2 lymphocytes and by cutaneous lymphocyte antigen positive (CLA+) skin homing T cells from human AD patients, suggesting that these cells may represent a major source of this cytokine. Interleukin-31 has been found to be elevated preferentially in pruritic versus nonpruritic human skin conditions, and serum levels of IL-31 correlate with disease severity in human adults as well as children with AD.2–8

Interleukin-31 binds to a heterodimeric receptor consisting of IL-31 receptor A and oncostatin M receptor β. Upon ligand binding to this receptor complex, signal transduction cascades such as the Janus kinase–signal transducer and activator of transcription (JAK–STAT), mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways are activated.9 Receptors for IL-31 are found on a variety of cells, such as keratinocytes, macrophages and eosinophils, and participate in regulating immune responses in these cell types.9–11 Of great interest is the finding that these receptors are present on a subset of small-sized nociceptive neurons of mouse and human dorsal root ganglia,
suggesting that this cytokine may directly activate prurito-
genic signals in peripheral nerves.\textsuperscript{1,2,12}

The cloning of canine interleukin-31 (cIL-31) has been pre-
viously reported.\textsuperscript{13} These investigators were able to
detect cIL-31 mRNA in freshly isolated canine peripheral
blood mononuclear cells after concanavalin A treatment,
suggesting that IL-31 may be produced by canine T cells;
however, they were not able to detect cIL-31 mRNA in
skin biopsy specimens from dogs diagnosed with AD,
which calls into question the role of IL-31 in canine AD.
To extend investigations of canine IL-31 to assessments
of biological activity and protein levels in disease, the
present study was conducted to evaluate the role of IL-31
in canine pruritus using purpose-bred beagle dogs and to
evaluate whether IL-31 is present in the serum of animals
with naturally occurring AD.

Materials and methods

Cloning and expression of cIL-31

Using total RNA isolated from canine testicular tissue and oligo-(dT)\textsubscript{20}
primers, complementary DNA was synthesized with the Super-
Script\textsuperscript{\textregistered} III First-Strand Synthesis System for RT-PCR (Invitrogen,
Carlsbad, CA, USA) according to the manufacturer’s protocol.
Poly-
merase chain reactions were performed to amplify the cIL-31 gene
from complementary DNA using primers TEF-1237 (5\textsuperscript{'-} AGAT-
CTGCACTAGCTCCACAGCCACACAGGACCATCCAG-3\textsuperscript{'} and TEF-1240
(5\textsuperscript{'-} GTGACTCTGTAGGTCCAGGTCCAGTGTATTAGTGAC-3\textsuperscript{'}). The PCR prod-
uct was cloned into pCRII-Blunt II-TOPO\textsuperscript{\textregistered} according to the manufac-
turer’s protocols (Life Technologies, Grand Island, NY, USA) and
further subcloned into the expression construct pSOO524. The cIL-
31 expression construct was either transiently transfected into Free-
Style\textsuperscript{\textregistered} 293 suspension culture cells following the manufacturers’
protocol (Life Technologies) or stably transfected into CHO cells
using a site-specific integration system.\textsuperscript{14}

Protein purification and analysis of recombinant
cIL-31

Canine interleukin-31 was produced by cultured FreeStyle\textsuperscript{\textregistered} 293
cells or CHO cells. Conditioned media from these cells was collected,
dialysed with buffer (20 mmol/L Tris, pH 8.0, and 40 mmol/L NaCl)
and purified by anion exchange chromatography (Q Sepharose). Pro-
tein identity was confirmed by N-terminal sequencing and by liquid
chromatography–mass spectrometry (LC-MS) analysis of a tryptic
digest of the protein.

Cell culture

The DH82 canine monocytic cell line (American Type Culture Collec-
tion, Manassas, VA, USA) was used to evaluate cIL-31 cytokine
function. DH82 cells were plated into CoStar 96-well flat-bottomed
cell culture plates (Corning, Tewksbury, MA, USA) at a density of
1 × 10\textsuperscript{5} cells per well in MEM growth media (Life Technologies)
containing 15% heat-inactivated fetal bovine serum, 2 mmol/L
Glutamax, 1 mmol/L sodium pyruvate, 50 \mu g/L gentamicin and
10 ng/mL canine interferon-\gamma (R&D Systems, Minneapolis, MN,
USA) for 24 h at 37\textdegree C in humidified air supplemented with 5% CO\textsubscript{2}.
The following day, cells were exposed to MEM growth media
without serum or interferon-\gamma for 2 h. Following serum deprivation,
cells were treated with cIL-31 for 5 min. Cytokine treatment was termi-
nated by removing medium and then adding AlphaScreen Sure-
Fire\textsuperscript{\textregistered} lysis buffer (Perkin Elmer, Waltham, MA, USA) and freezing
samples at −20\textdegree C.

Signal transduction pathway activation

Cell lysates were used to evaluate phosphorylation of signal transdu-
cer and activator of transcription 3 (STAT3) and extracellular signal-
regulated kinase 1/2 (ERK1/2). Activation of STAT3 was detected using the Perkin Elmer AlphaScreen SureFire\textsuperscript{\textregistered} STAT3 p-Y705 kit,
and activation of ERK1/2 was detected using the Perkin Elmer Alpha-
Screen SureFire\textsuperscript{\textregistered} MAPK p-T202/Y204 kit, following the manufac-
turer’s protocol. Specifically, 4 \mu L of cIL-31-treated cell lysates was
sequentially incubated with streptavidin-coated donor beads bound
with biotinylated capture antibody, then with protein A-coated accep-
tor beads bound with antibody that recognized the phosphoryla-
tion site on the target protein. Assay plates were placed on a Perkin
Elmer Envision plate reader to cause excitation of the donor beads at
680 nm. Upon excitation of a donor bead, a singlet oxygen transfer
occurs from the donor to an acceptor bead. Any acceptor bead in
close proximity to a donor bead (due to the binding of capture
and detection antibodies to the desired target protein) emits light at
520–620 nm as a result of a cascade of energy transfer triggered by
the singlet oxygen. Light emission was detected by the Envision
plate reader. Data were expressed as mean relative signal units, and
the EC\textsubscript{50} for induction of phosphorylated STAT3 (pSTAT3) and
phosphorylated MAPK (pMAPK) was determined by a nonlinear fit
model in GraphPad Prism 5.0 (GraphPad Software, San Diego, CA,
USA).

Assessment of pruritus in animals

All animal procedures were approved by the Institutional Animal Care
and Use Committee (Pfizer Animal Health, Kalamazoo, MI, USA) and
were performed in compliance with the Animal Welfare Act, Regula-
tions, 9 CFR Parts 1, 2 and 3, and with the Guide for the Care and
Use of Laboratory Animals, issued by the US Institute for Laboratory
Animal Research Commission of Life Sciences (National Academy

Purpose-bred beagle dogs (Marshall BioResources, North Rose,
NY, USA) were used in these experiments. Dogs were acclimated
for at least 1 h to single-housed runs equipped with ceiling cameras.
To evaluate the effects of cIL-31 administration via various routes
on pruritic behaviour, cIL-31 (10 \mu g) or vehicle control [phosphate-buf-
fered saline (PBS) containing equivalent amounts of mammalian host
cells proteins to those present in the cIL-31 preparation] was adminis-
tered intradermally (i.d.), subcutaneously (l.s.c.) or intravenously (l.v.).
Pruritic behaviours (e.g. scratching, licking, chewing, scooting, head
shaking and body rubbing) were monitored using video surveillance.
Pruritic behaviours were measured as the time (in seconds) over a
4 h baseline period or 4 h after cIL-31 administration by one or more
observers who were blinded to the treatment.

To evaluate the pruritic effects of cIL-31 in a statistically pow-
ered study, vehicle control-treated animals were compared with
cIL-31-treated animals. Pruritic behaviour was evaluated for 2 h
starting approximately 30 min after vehicle control or cIL-31 injec-
tion (1.76 \mu g/kg, i.v.) by one or more observers who were
blinded to the treatment. Observed pruritic behaviour was mea-
sured using a categorical scoring system. ‘Yes/no’ determinations of
displayed pruritic behaviour were made during consecutive, dis-
crete 1 min intervals. The number of minutes categorized as ‘yes’
for displayed pruritic behaviours for an animal was then summed.
The maximal achievable score for a 2 h (120 min) observation
period was 120.

Canine serum samples

Blood was collected in 5 mL plastic BD Vacutainer\textsuperscript{\textregistered} SST\textsuperscript{\textregistered} tubes
(Beckton Dickinson & Co., Franklin Lakes, NJ, USA) with owners’
signed informed consent when required, allowed to clot then sepa-
rated according to the manufacturer’s protocol. Serum was collected
from the following populations of dogs and frozen prior to measure-
ments of serum cIL-31.

1 Experimentally sensitized dogs. Twenty-four purpose-bred bea-
gle dogs (Marshall BioResources) prior to and 1 week after the
last exposure to house dust mite (HDM) allergen. Animals were
sensitized to Dermatophagoides farinae by receiving a series of
three 0.5 mL injections containing 10 \mu g of allergen (Greer
Laboratories, Inc., Lenoir, NC, USA), 2.0 mg Rehydralgel

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(Reheis, Inc., Berkeley Heights, NJ, USA) and 0.4 mL sterile PBS. The injections were administered 2 weeks apart. All animals were approximately 9 months of age, and 12 neutered males and 12 spayed females were evaluated.

2 Flea-allergic dogs. Thirty research dogs with established flea allergy (Youngs Veterinary Research Services, Turlock, CA, USA) prior to flea infestation or approximately 1 week after infestation with adult cat fleas (Ctenocephalides felis) began. The majority of the dogs in this colony were of mixed breed. The mean age was 10.5 years. This colony consisted of 14 intact females, two spayed females, 11 intact males and three neutered males.

3 Pet dogs without allergic disease. Eighty-seven client-owned dogs with subclinical periodontal disease but otherwise determined to be in good health. Samples were collected across 18 veterinary clinics in the USA to perform serum chemistries and titre assessments as part of a screening protocol for entry into a study. All owners had provided written consent for remaining serum to be used in research. No additional samples were collected for this portion of the study. Approximately 86% of the dogs were purebred and approximately 18% of the total population were retrievers (13%) and golden (5%). The mean age was 3.2 years. The population consisted of 9% intact females, 47% spayed females, 13% intact males and 31% neutered males.

4 Pet dogs with nonseasonal atopic dermatitis. Two hundred and twenty-three client-owned dogs diagnosed with chronic, non-seasonal AD of at least 1 year duration diagnosed by a board-certified dermatologist (based on modified criteria of Willemse and Prélaud) prior to flea infestation or approximately 1 week after infestation with adult cat fleas. The mean age was 5.8 years. This population of dogs consisted of 3% intact females, 51% spayed females, 3% intact males and 43% neutered males.

Anti-IL-31 monoclonal antibody production

Anti-canine IL-31 monoclonal antibodies were produced at Maine Biotechnology Services (Portland, ME, USA). CF-1 mice were immunized on a biweekly schedule with cIL-31. Postimmunization, mouse sera and primary fusion products were screened for reactivity to cIL-31 by ELISA. Hybridomas were generated and subcloned by limiting dilution to ensure monoclonal cultures.

Anti-canine IL-31 hybridomas were grown in RPMI 1640 base medium supplemented with 10% ultra-low IgG fetal bovine serum, 2 mM/L GlutaMAX, 100 U/mL penicillin, 100 µg/mL streptomycin and 55 µmol/L 2-mercaptoethanol. Antibodies were purified from the culture supernatants by protein A or protein G affinity chromatography.

Canine interleukin-31 immunoassays

A Gyrolab sandwich immunoassay was used to quantify cIL-31 levels in canine serum. Serum samples were diluted 1:2 in Rexxip buffer (Gyrolab, Warren, NJ, USA) and run on Bioaffy 1000 mL CDs (Gyrolab) using the Gyrolab xP workstation. Canine interleukin-31 was captured with a biotin-labelled anti-IL-31 monoclonal antibody and detected with an Alexaflour 647-labelled anti-IL-31 monoclonal antibody. Sample concentrations of cIL-31 were extrapolated from an eight-point standard curve with a dynamic range of 0.013–250 ng/mL using a five-parameter fit equation with Gyrolab Evaluation software. The lower limit of quantification was determined to be 13 pg/mL based on the performance of quality control standards at this concentration. Specifically, the 13 pg/mL standards gave values at least two standard deviations above background, accuracy measurements consistently within 20% of intended concentrations, and precision or the percentage coefficient of variance (%CV) within 20%.

Statistical analysis

Data generated from the evaluation of pruritic effects of cIL-31 administered i.v. in beagle dogs were analysed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). PROC MIXED for mixed linear models was used to analyse pruritic score. The model included a fixed effect of treatment and random effects for room, block within room and error. Least squares means were used as estimates of treatment means. Standard errors for treatment means were calculated at 90% confidence intervals for treatment means constructed. All tests (significance of effects and treatment comparisons) were conducted at the two-sided 10% level of significance.

Results

Identification and functional assessment of canine IL-31

Canine interleukin-31 was cloned by RT-PCR from total RNA isolated from canine testicular tissue, a tissue shown to express IL-31 mRNA by other investigators. The nucleotide sequence generated for cIL-31 was identical to the one independently determined and reported (GenBank AB455159). Protein produced from the generated cIL-31 mammalian expression systems was confirmed to be cIL-31 by N-terminal sequencing and tryptic mapping (see Supporting information Figure S1). To confirm biological activity of the expressed and purified protein, cIL-31 was evaluated for its ability to activate the JAK-STAT, MAPK and PI3K pathways, because these signal transduction pathways have been reported to be involved in the signalling of human and mouse IL-31. Specifically, cIL-31 treatment led to STAT3 and ERK1/2 phosphorylation in DH82 cells with EC50 values of 53.2 and 84.5 ng/mL, respectively (Figure 1a,b). Phosphorylation of these proteins is indicative of JAK-STAT and MAPK pathway activation, respectively. The phosphorylation of Akt, a marker of PI3K activity, was constitutively turned on in this cell line, so induction of this pathway (PI3K/Akt) by cIL-31 could not be evaluated adequately (see Supporting information Figure S2).

Administration of cIL-31 in vivo to purpose-bred beagle dogs caused transient episodes of pruritic behaviour ranging from two- to 10-fold increases above baseline measurements, regardless of the route of administration (Table 1). Behaviours varied among animals. For example, some animals primarily exhibited behaviours such as scratching or head shaking (e.g. dog no. 4807448), whereas others spent most of their time licking (e.g. dog no. 4746538). One animal did not appear to respond to IL-31 injections at all (dog no. 4802098). When pruritic behaviours were displayed, they were readily seen within 4 h after cIL-31 administration and tended to return to baseline levels within 24 h (data not shown). No other obvious clinical signs were observed in the animals. Phosphate-buffered saline vehicle containing residual host cell
proteins at equivalent concentrations to those present in the cIL-31 preparations (mock protein) did not induce pruritic behaviours above baseline levels (e.g. dog no. 3770044). When evaluated over a 2 h period using a categorical scoring system, dogs receiving cIL-31 (i.v.) exhibited a statistically significant increase in mean pruritic score when compared with the vehicle control treatment groups (Figure 2).

Detection of cIL-31 cytokine in dogs with naturally occurring atopic dermatitis

A variety of canine populations were evaluated for the presence of cIL-31 in serum. Levels were not detectable (<13 pg/mL) in the serum from purpose-bred beagle dogs prior to and after sensitization to HDM (n = 24 dogs), mixed breed dogs prior to and after flea infestation (n = 30 dogs) or client-owned dogs with periodontal disease but otherwise considered to be in good health, regardless of breed (n = 87 dogs). In the dogs with naturally occurring AD, cIL-31 was detectable (≥13 pg/mL) in 57% (127 of 223) of the animals, with 52% (117 of 223) of the samples showing serum cIL-31 levels between 13 and 1000 pg/mL, and 4% (10 of 223) showing levels above 1000 pg/mL (Table 2 and Supporting information Table S1).

Discussion

This report describes the generation of canine interleukin-31 protein and the biological function of this cytokine in canine systems. Canine IL-31 was found to activate the JAK-STAT pathway as well as the MAPK pathway in canine cells. Upon administration of cIL-31 to dogs, a significant increase in pruritic behaviours was observed. This study is the first to describe the biological function of IL-31 in canine models. These study results also corroborate the findings from others who have shown the same signalling cascades activated by mouse and human IL-31 and have observed pruritic phenotypes in mice infused with or engineered to overexpress IL-31. Interleukin-31 may therefore play a role in inducing pruritus across a variety of species.

The types of pruritic behaviours observed in dogs after IL-31 injection included scratching, licking, chewing, scooting, head shaking and body rubbing; however, not all behaviours were seen in each animal. Instead, the types of behaviours displayed by each animal varied. One animal (dog no. 4802098) displayed as much pruritic behaviour during baseline monitoring as most dogs did after IL-31 injection, and IL-31 injection in this dog did not appear to increase the amount of pruritus displayed by this animal as determined by video monitoring and quantification of time spent scratching over a 4 h observation period. This dog could have been nonresponsive to IL-31 or may have already had endogenously circulating levels of IL-31.

Table 1. Effects of canine interleukin-31 (cIL-31) administration via different routes on pruritic behaviour in dogs

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Total cIL-31 dose (µg)</th>
<th>Delivery route*</th>
<th>Observed pruritus (s) over 4 h intervals (mean ± SD)</th>
<th>Fold increase in pruritus after cIL-31 (versus baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4340761</td>
<td>10</td>
<td>i.d.</td>
<td>28 ± 26</td>
<td>162</td>
</tr>
<tr>
<td>4807448</td>
<td>10</td>
<td>i.d.</td>
<td>263 ± 59</td>
<td>862</td>
</tr>
<tr>
<td>4746538</td>
<td>10</td>
<td>i.d.</td>
<td>124 ± 67</td>
<td>1096</td>
</tr>
<tr>
<td>4701488</td>
<td>10</td>
<td>i.d.</td>
<td>417 ± 80</td>
<td>916</td>
</tr>
<tr>
<td>3770044</td>
<td>0 (mock protein)</td>
<td>i.d.</td>
<td>348 ± 111</td>
<td>254</td>
</tr>
<tr>
<td>4802098</td>
<td>10</td>
<td>s.c.</td>
<td>988 ± 223</td>
<td>782</td>
</tr>
<tr>
<td>4814975</td>
<td>10</td>
<td>s.c.</td>
<td>312 ± 37</td>
<td>885</td>
</tr>
<tr>
<td>4477138</td>
<td>10</td>
<td>s.c.</td>
<td>31 ± 15</td>
<td>201</td>
</tr>
<tr>
<td>4711921</td>
<td>10</td>
<td>s.c.</td>
<td>232 ± 84</td>
<td>1547</td>
</tr>
<tr>
<td>4340761</td>
<td>10</td>
<td>i.v.</td>
<td>103 ± 125</td>
<td>996</td>
</tr>
<tr>
<td>4701488</td>
<td>10</td>
<td>i.v.</td>
<td>480 ± 235</td>
<td>989</td>
</tr>
<tr>
<td>4477138</td>
<td>10</td>
<td>i.v.</td>
<td>163 ± 123</td>
<td>1147</td>
</tr>
<tr>
<td>3770044</td>
<td>0 (mock protein)</td>
<td>i.v.</td>
<td>359 ± 78</td>
<td>137</td>
</tr>
</tbody>
</table>

Observed pruritus (in seconds) over 4 h intervals is listed for baseline observations (means ± SD) and observations taken after cIL-31 treatment. Fold increase in pruritus from baseline is also calculated.

* i.d., intradermal injection (0.2 mL volume); s.c., subcutaneous injection (0.2 mL volume); or i.v., intravenous injection (1 mL volume).
† Replicate baseline observations were made on separate days but at the same time of day. Data represent means ± SD.
‡ Administration of cIL-31 was performed on a different day from baseline observations, but at the same time of day.

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Purpose-bred beagle dogs 24 0
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nificantly induces pruritic behaviour in dogs compared with the vehi-
( back transformed least square means) are shown. Canine IL-31 sig-
were compared. Pruritic behaviour was evaluated for 2 h starting
and 1.75
l

population

Canine
canine populations

Number of animals with detectable serum IL-31 in various
occurring AD. Interleukin-31 was not detected in normal
populations were analysed for IL-31 protein levels, IL-31
mediators involved in naturally occurring, chronic disease
models do not always accurately reflect pathways or
present study, because observations from laboratory
pruritic skin diseases, such as AD, was of interest in the
was not evaluated in this study but will be an important
factor to consider in the future.

Evaluating the relevance of IL-31 in naturally occurring
pruritic skin diseases, such as AD, was of interest in the
present study, because observations from laboratory
models do not always accurately reflect pathways or
mediators involved in naturally occurring, chronic disease
conditions. When serum samples from a variety of canine
populations were analysed for IL-31 protein levels, IL-31
was detected in 57% of dogs diagnosed with naturally
occurring AD. Interleukin-31 was not detected in normal
of IL-31. This animal was a neutered male 4 years of age
and the heaviest dog in the study, weighing 26.8 kg. The
correlation between body weight and IL-31 serum levels
was not evaluated in this study but will be an important
factor to consider in the future.

Table 2. Number of animals with detectable serum IL-31 in various
canine populations

| Canine population                      | Number of animals evaluated | Number of animals with detectable cIL-31 in serum*
|---------------------------------------|-----------------------------|-----------------------------------------------
| Purpose-bred beagle dogs              | 24                          | 0                                             |
| Purpose-bred beagle dogs sensitized to house dust mite | 24                          | 0                                             |
| Mixed breed dogs with no fleas        | 30                          | 0                                             |
| Mixed breed dogs infested with fleas  | 30                          | 0                                             |
| Healthy client-owned dogs of multiple breeds | 87                          | 0                                             |
| Naturally occurring atopic dermatitis in client-owned dogs of multiple breeds | 223                         | 127                                           |

*Protein levels of cIL-31 were measured in canine populations using immunoassay techniques. The number of animals evaluated and the number of animals in which cIL-31 was detected (≥13 pg/mL) are listed for each population. A value <13 pg/mL is below the limit of quantification.

Although IL-31 was detected in 57% of dogs with AD, a large percentage of animals with AD (43%) did not display detectable levels of IL-31 (<13 pg/mL). These animals may have had circulating levels of IL-31 that were below our assay limits of detection, or possibly IL-31 levels were acting locally within target tissues and not released into the circulation. Alternatively, IL-31 dysregulation may not play a significant role in the aetiology of AD in these animals with undetectable IL-31 levels. The latter interpretation is consistent with the concept that canine AD is a multifactorial disease that involves complex interactions between susceptibility genes, skin barrier dysfunction, immune dysregulation and neuroimmune interactions that collectively produce a hypersensitivity to environmental allergens and a pruritic allergic skin condition in dogs. Owing to the complexity of the disease, it is not surprising that not all dogs exhibit the same molecular or cellular changes.

Continued investigations of IL-31 in the canine AD pop-
ulation to determine whether serum levels correlate with a
variety of parameters, such as age, breed, weight, sex
and disease severity, will be important to improve our
understanding of the role of IL-31 in this disease. Several
investigators interested in the role of IL-31 in human AD
have already extended their evaluations to the protein
level and have generated data correlating serum levels of
IL-31 to disease severity.3,7 Specifically, studies have
shown IL-31 to be elevated in the serum of human AD
patients compared with healthy control subjects and that
serum IL-31 levels correlate with disease severity in
adults as well as children with AD.

Interleukin-31 in canine AD has been a topic of interest
to other groups. Mizuno et al13 used RT-PCR techniques
to evaluate IL-31 mRNA levels in a variety of canine
tissues and in the skin of dogs with AD. This group was able
to detect canine IL-31 mRNA in a variety of tissues; how-
ever, they were not able to detect IL-31 mRNA in the skin
of dogs with naturally occurring AD (n = 9). They
hypothesized that the biological function of IL-31 may be
different in dogs versus other species or that evaluations
of IL-31 protein could be more informative than looking at
mRNA levels, given that mRNA levels do not always
mimic changes that can be seen at the protein level. In our
study, we were able to extend assessments of IL-31 to
the protein level using quantitative immunoassay tech-
niques and were able to detect elevated levels in the
dogs, in pruritic, flea-allergic dogs or in dogs sensitized to
the HDM allergen D. farinae. These findings suggest that
IL-31 may be dysregulated in dogs with AD and may con-
tribute to the pathobiology of this chronic allergic skin dis-
ease. It is not surprising that IL-31 was not detected in
HDM-sensitized dogs, because these dogs were not pru-
ritic after the sensitization protocol. However, IL-31 levels
were also not detected in the serum from flea-allergic
dogs, even though these animals displayed pruritic
behaviours. One explanation could be that this cytokine is
not a key mediator of pruritus in flea allergy dermatitis.
Alternatively, IL-31 could be acting locally within the skin
and not readily detected in the serum. The length of flea
infestation may also play a role. These animals were
exposed to fleas for only 1 week prior to sampling. Inter-
leukin-31 levels were not assessed in animals with pro-
longed infestation with fleas.

Figure 2. Canine interleukin-31 (cIL-31) administered intravenously
induced pruritus in dogs. Pruritic scores from phosphate-buffered sal-
in (PBS) vehicle-treated (T01), PBS/protein vehicle-treated (T02)
and 1.75 µg/kg cIL-31 in PBS/protein vehicle-treated (T03) animals
were compared. Pruritic behaviour was evaluated for 2 h starting
approximately 30 min after cIL-31 injection. Mean pruritic scores
(back transformed least square means) are shown. Canine IL-31 sig-
nificantly induces pruritic behaviour in dogs compared with the vehi-
cle control-treated groups (*T01 versus T03, P = 0.0004; †T02
versus T03, P = 0.0003. No significant difference was observed
between T01 versus T02).
serum of dogs with AD. We were also able to evaluate a large population of dogs with naturally occurring AD (n = 224), which may have improved our chances of detecting IL-31 alterations in the canine AD population. Future studies should extend assessments of IL-31 protein levels to the skin of dogs with AD. Investigators have begun to perform these types of assessments in human AD patients and have found that IL-31 protein is elevated in the inflammatory infiltrates of skin biopsy specimens taken from AD subjects compared with skin biopsy specimens from patients with other types of skin diseases, suggesting that IL-31 dysregulation could be unique to AD.8

In summary, we demonstrated that canine IL-31 injected systemically or locally can induce pruritic behaviours in dogs and that this cytokine is elevated in a significant number of dogs with AD. We believe this pathway may play a role in the pathobiology of pruritic allergic skin conditions, such as canine AD, and may represent a novel pathway for therapeutic intervention.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article: Figure S1. Amino acid sequence of the canine IL-31 construct. The identity of purified canine IL-31 protein was confirmed using mass spectrometry to analyse a tryptic digest of the protein and also by N-terminal sequencing. Figure S2. Detection of phospho-Akt in cIL-31-treated DH82 cells. Using western blotting techniques, phospho-Akt levels were evaluated in DH82 cells treated with varying concentrations of IL-31 (0–10 μg/mL). Table S1. Individual cIL-31 serum levels in dogs with atopic dermatitis. Protein levels of cIL-31 were measured in serum using immunoassay techniques in client-owned animals diagnosed with atopic dermatitis. Quantitative levels of cIL-31 in serum are listed for each dog evaluated.

Résumé

Contexte – L’interleukine-31 (IL-31) est un membre de la famille des cytokines interleukine-6/gp130 qui est produit par des types cellulaires comme les lymphocytes T helper 2 et les cellules T cutanée lymphocytaire CLA positives (cutaneous lymphocyte antigen). Lorsque surexprimés chez des souris transgéniques, IL-31 provoque un prurit sévère, de l’alopécie et des lésions cutanées. Chez l’homme, les taux d’IL-31 sériques sont en corrélation avec la sévérité de la dermatite atopique chez les adultes et les enfants.

Hypothèse/Objective – Déterminer le rôle de l’IL-31 dans le prurit canin et la dermatite atopique canine naturelle (AD).

Animaux – Des chiens beagles ont été utilisés pour des études en laboratoire. Des échantillons de sérum ont été prélevés sur des animaux de laboratoire, sur des chiens sains et naturellement atopiques de cli-
Resumen

Introducción – la interleuquina 31 (IL-31) es un miembro de la familia de citoquinas gp130/IL-6 que se producen en células tales como los linfocitos T-2 ayudantes y linfocitos T cutáneos estimulados antígenicamente con tropismo cutáneo. Cuando esta interleuquina se expresa en exceso en ratones transgénicos induce prurito severo, alopecia y lesiones de la piel. En humanos, los niveles en suero de la IL-31 están correlacionados con la severidad de la dermatitis atópica en adultos y niños.

Hipothesis/Objetivos – determinar el papel de la IL-31 en el desarrollo del prurito canino y en dermatitis atópica espontánea (AD).

Animales – Se utilizaron perros de laboratorio de raza Beagle para los estudios laboratoriales. Se obtuvieron muestras de suero de los animales de laboratorio, de animales de propietarios particulares sin enfermedad, y de animales de propietarios particulares con AD espontánea.

Métodos – se administró IL-31 a perros de laboratorio de raza Beagle por varias rutas (intravenosa, subcutánea, intradermática) y se observó el comportamiento indicativo de prurito, cuantificándolo mediante observación en grabaciones de video. Se utilizaron técnicas cuantitativas de inmunoensayo para medir los niveles de IL-31 en el suero de los perros.

Resultados – la inyección de IL-31 a los perros Beagle de laboratorio produjo episodios transitorios de comportamiento indicativo de prurito, independientemente de la vía de administración. Cuando se evaluaron durante un periodo de 2 horas, los perros que recibieron IL-31 presentaron un incremento perceptible de comportamiento indicativo de prurito en comparación con los animales a los que se administró placebo. Además hubo niveles detectables de cIL-31 en un 57% de perros con AD espontánea (≥13 pg/mL), pero estuvieron por debajo de los niveles de detección (<13 pg/mL) en perros normales de laboratorio o sin enfermedad de propietarios particulares.

Conclusiones – la IL-31 canina induce comportamiento indicativo de prurito en perros. La IL-31 se detectó en el suero en la mayoría de los animales con AD espontánea, lo que sugiere que esta citoquina puede tener un papel importante en procesos alérgicos pruriginosos de la piel, tales como la dermatitis atópica, en esta especie.
Schlussfolgerungen – Canines IL-31 verursachte bei Hunden Juckreiz. Canines IL-31 wurde bei der Mehrheit der Hunde mit natürlich auftretender AD festgestellt, was darauf hinweist, dass diese Zytokine möglicherweise eine wichtige Rolle bei juckenden allergischen Hauterkrankungen, wie zum Beispiel bei der atopischen Dermatitis dieser Spezies, spielen könnten.

要約
背景 – イヌのIL-31は、CD103/CD31のサイトカインファミリーの1つで、ヘルパーT2リンパ球とリンパ球抗原非特異的皮膚に誘導されるT細胞によって産生される。トランスジェニックマウスで、過剰発現させたとき、IL31は皮膚の症状、脱毛および皮膚症状を誘発する。ヒトではIL-31の血清レベルは成人と児童のアトピー性皮膚炎の重症度と相関する。

仮説/目的 – イヌの発症帯と自然発症性イヌアトピー性皮膚炎（AD）でのIL-31の役割を調査する。

供与動物 – 目的に応じて繁殖したビーグル犬が実験に用いられた。血清サンプルは実験動物、病気のない飼い犬、自然発症性ADと診断された飼い犬から得られた。

方法 – 目的に応じて繁殖したビーグル犬に複数のエクササイズ（運動、皮下、あるいは皮膚内）で、イヌインターロイキン31（IL-31）を投与し、ビデオヨーでのモニターを介して飼い犬の行動を観察/測定した。イスのIL-31の血清レベルの測定には定量的免疫測定法を用いた。

結果 – cIL-31への実験ビーグル犬の注射は投与ルートに関わらず、飼い犬に関する行動を一過性に生じた。評価した2時間以上の間に、cIL-31を投与されていた犬はクレセントを投与されていた犬と比較して発症に関する行動が有意に増加した。さらに、cIL-31レベルは自然発症性ADの犬の57%で検出可能であった（0.13 pg / ml）が、正常な病気のない実験犬あるいは飼い犬では検出値以下であった（<13 pg / ml）。

結論 – イヌ IL-31 は犬に重要性に関する行動を誘発する。イス IL-31 は自然発症性 AD の犬の大多数で検出できるとのことこのサイトカインはこの動物種でアトピー性皮膚炎などの症状を誘発する可能性を示唆しているかもしれない。

摘要
背景 – 白介素31（IL31）は、酸性エラスミン、Ⅰ型細胞因子の家族、筋肉細胞が細胞の再構築性感生。IL31誘導産生の一過性、脱毛および皮膚症状、皮膚のIL-31血清レベルを成人と児童のアトピー性皮膚炎の重症度と相関する。

仮説/目的 – 健康IL-31がアトピー性皮膚炎（AD）における作用。

動物 – IL-31誘導による動物実験。血清検体に自発的動物、健康な健康的な健康的な健康的な病態下でIL-31の不規則性、脱毛および皮膚症状の発症特性。

方法 – IL-31誘導開始から実験動物を対象に、臨床検査及び薬剤投与を観察し、IL-31の濃度を測定し、検討した。

結果 – 不規則性の薬剤投与により、IL-31の集中化は観察され、刺激時のIL-31濃度は57%で検出可能であった（0.13 pg / ml）。IL-31濃度の検出値は以下の間では正常でないが、<13 pg / ml）。

結論 – IL-31はアトピー性皮膚炎の発症に、自発的陽性性皮膚炎の発症中に発現するIL-31が、 Intellectual動物や、薬剤投与による動物の病態下で検出され、IL-31濃度の検出値が低い（<13 pg / ml）。