

Cytokine expression in feline allergic dermatitis and feline asthma

Caitlin E. Older*¹ , Alison B. Diesel†, Johanna C. Heseltine†, Amanda Friedeck†, Carolyn Hedke‡, Stephanie Pardike‡, Katrina Breitreiter§, Michael A. Rossi¶, James Messamore‡, Gary Bammert‡, Andrea J. Gonzales‡  and Aline Rodrigues Hoffmann*² 

Departments of *Veterinary Pathobiology, and †Small Animal Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, USA

‡Veterinary Medicine Research and Development, Global Therapeutics Research, Zoetis Inc, Kalamazoo, MI 49007, USA

§South Austin Cat Hospital, Austin, TX 78747, USA

¶Coastal Veterinary Dermatology & Ear Clinic, Houston, TX 77059, USA

¹Present address: Warmwater Aquaculture Research Unit, Agricultural Research Service, USDA, 127 Experiment Station Road, Stoneville, MS 38776, USA

²Present address: Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, 2015 16th Ave, Gainesville, FL 32608, USA

Correspondence: Caitlin E. Older, USDA, Agricultural Research Service, Warmwater Aquaculture Research Unit, 127 Experiment Station Road, Stoneville, MS 38776, USA. E-mail: c.older@hotmail.com

Background – The pathogenesis of feline allergic dermatitis (FAD) is unclear, with several differences from allergic dermatitis in dogs and humans.

Hypothesis/objectives – To survey cytokine expression levels in healthy cats and cats affected with allergic dermatitis or asthma.

Animals – Formalin-fixed, paraffin-embedded skin biopsies from 22 cats with allergic dermatitis and 21 cats without allergic dermatitis were used for cutaneous assays. Serum was obtained from 17 healthy cats, 18 cats with allergic dermatitis, and 18 cats with a presumptive diagnosis of asthma.

Methods and materials – Cutaneous mRNA expression was evaluated with quantitative PCR [interleukin (IL)-31 and IL-31 Receptor A] and RNA *in situ* hybridisation (ISH) [IL-5, IL-31, IL-31RA, IL-33 and Oncostatin M receptor (OSMR)- β]. IL-31 protein concentrations were evaluated in serum with an enzyme-linked immunosorbent assay. Serum levels of 19 additional cytokines were evaluated using a Luminex panel.

Results – IL-31, IL-31RA, IL-5 and IL-33 mRNA expression were either expressed in low quantities or undetectable in most samples. By contrast, OSMR- β expression was significantly higher in the skin of allergic versus healthy cats ($P < 0.0001$). Although serum IL-31 was detected in a larger number of cats with allergic dermatitis than healthy cats, and concentrations appeared to be higher in cats with allergies, this difference was not statistically significant. Cats affected by asthma also exhibited insignificantly higher concentrations of IL-31 in the serum.

Conclusions and clinical relevance – Our results suggest that feline allergic diseases may exhibit different pathomechanisms from allergic diseases affecting other species. These findings are useful in guiding further therapeutic development toward targets that may have a role in the pathogenesis of feline allergic skin disease.

Introduction

Allergic dermatitis in cats is unique, with regard to both clinical signs and pathomechanisms. Atopic dermatitis is well-documented in humans and dogs, with similarities between the two species in lesion types, immunoglobulin (Ig)E involvement, and immune response. In cats, the term “atopic dermatitis (AD)” is not well-accepted for the cutaneous hypersensitivity reaction to environmental allergens. This is due to the distinct clinical patterns,

potential lack of IgE involvement, and differing immunological profile compared to people and dogs. The disease affecting cats is more appropriately referred to as “atopic-like dermatitis”, “nonflea, nonfood hypersensitivity dermatitis” (NFNFD) or “feline atopic skin syndrome” (FASS).^{1–3} Unlike for human and canine AD, little research has been done regarding pathomechanisms of FASS. Feline asthma, which affects an estimated 1–5% of cats,⁴ is another incompletely understood feline disease that is thought to have an allergic aetiology. Unlike FASS, feline asthma appears to share similarities with human asthma,^{4,5} indicating that cats may be a potential animal model for human asthma. Feline asthma can be challenging to diagnose and, like feline allergic dermatitis, can be difficult to manage.

Recent advances in drug development have allowed for human and, to a lesser degree, canine patients to be

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treated with host-specific biologicals. Few feline-specific biologicals have been produced and tested. For feline asthma, treatment usually involves glucocorticoids and bronchodilators, which can lessen the response to allergens, but does not restore immune tolerance or normal airway physiology.⁶ Glucocorticoids also can be used for medical management of feline FASS, in addition to antihistamines and/or ciclosporin.¹ Current therapies are not always effective and can lead to adverse effects when administered long-term,^{7,8} so alternative therapies are desirable. Targeting soluble mediators of allergic disease is one potential area for therapeutic development.

For some human patients with allergic asthma^{9,10} and AD,¹¹ monoclonal antibody therapies and Janus kinase (JAK) inhibitors offer beneficial management of disease. In dog and cats, there are currently no biological therapies approved for the treatment of asthma; however, a JAK1 inhibitor (ocloclitinib)¹² that is approved for the treatment of canine AD, was found to reduce eosinophilic airway inflammation in cats with experimentally induced asthma.¹³ Canine AD patients also may benefit from lokivetmab (Cytopoint),^{14,15} a caninised monoclonal antibody against interleukin (IL)-31, a major pruritogenic cytokine involved in the disease.^{12,16–19}

A few studies have begun to describe the role of IL-31 in feline allergic skin disease. In one study, injection of recombinant feline IL-31 in healthy cats resulted in pruritus, and when oclacitinib was given 1 h before injection, pruritus was reduced.²⁰ Oclacitinib also has been shown to reduce pruritus and lesion scores in cats with naturally-occurring FASS.²¹ In one abstract, cats with a presumptive diagnosis of allergic dermatitis demonstrated higher circulating serum concentrations of IL-31 compared to healthy controls.²² Additionally, feline IL-31 has exhibited similar activity to canine IL-31 in cell-based assays, where it has been shown to utilise the JAK/STAT pathway for signalling.²³ As mentioned previously, administering oclacitinib to cats with allergic asthma reduced eosinophilic airway inflammation.¹³ Collectively, these results indicate that the JAK/STAT pathway may be a useful target in the treatment of feline allergic disease. In feline patients affected by allergic dermatitis, perhaps targeting IL-31 would be useful.

Development of therapeutics that target soluble mediators of allergic disease requires a more comprehensive understanding of the pathomechanisms associated with these diseases. Allergic diseases often are thought to involve a strong T-helper (Th)2 response and thus have become important targets for development of therapeutics.^{10,11} However, the role of these inflammatory mediators in feline allergic diseases is still unknown.²⁴ In the present study we aim to elucidate the role of select cytokines in feline allergic dermatitis and asthma by evaluating the concentrations of IL-31 and other Th2 cytokines in the skin and serum of cats affected by allergic dermatitis and in the serum of cats affected by asthma.

Methods and materials

This study was approved by the Texas A&M University Institutional Animal Care and Use Committee (2018-0201 CA).

Evaluation of cutaneous cytokine expression

Formalin-fixed, paraffin-embedded tissue sample collection

Formalin-fixed, paraffin-embedded tissue (FFPE) samples from 22 cats with allergic skin disease were retrieved from the Dermatopathology Specialty Service (DSS) archive at Texas A&M University. All cats were >1-year-old at the time of sample collection. Biopsies showed histopathological changes compatible with allergic skin disease, as determined by examination of haematoxylin & eosin-stained slides by a board-certified anatomical pathologist. FFPE samples from 21 cats without allergic skin disease were obtained from the Texas A&M University DSS and surgical pathology service archives (see Supporting information Table S1). Control samples included marginal normal skin from noninflammatory disease processes. The selected controls primarily were from patients presented with follicular cysts, noninvasive epithelial neoplasms or well-demarcated spindle cell sarcomas, which were resected with very wide margins. All slide sections utilised as controls for this study did not present any neoplastic cells; instead, they were all normal haired skin without any signs of inflammation or neoplasia.

RNA extraction and quantitative PCR

From each tissue sample, RNA was extracted from 50 µm FFPE tissue. RNA extraction was performed with the MO BIO FFPE Tissue RNA Isolation Kit (formerly MO BIO, now Qiagen; Hilden, Germany) according to the manufacturer's protocol. Following extraction, RNA quantification was performed using the Qubit High Sensitivity RNA kit (Life Technologies Corporation; Carlsbad, CA, USA). cDNA was synthesised using the iScript Reverse Transcription Supermix for RT-qPCR (real-time quantitative) PCR (Bio-Rad Laboratories; Hercules, CA, USA), following the manufacturer's protocol. The qPCRs were performed using the iTaq Universal SYBR Green Supermix or iTaq Universal Probes Supermix (both Bio-Rad Laboratories). qPCRs targeting the IL-31 and IL-31RA genes, in addition to the ribosomal protein S7 (RPS7) and TATA-box-binding protein 1 (TBP1) genes were performed in duplicate using the oligos shown in Table 1.

Primers and probes were designed using PRIMER3 (v0.4.0)^{25,26} and checked for specificity with primer-BLAST.²⁷ Initially, four different primer sets were tested for various normalising genes, and three assays each for IL-31 and IL-31RA were designed and tested. Assays were tested preliminarily via a temperature-gradient PCR, followed by gel electrophoresis to visualise a single band of the appropriate size and lack of primer dimers, and then tested for an appropriate efficiency (90–110%) using a 10-fold dilution curve in duplicate. The best assays for IL-31 and IL-31RA and the best two normalising assays then were utilised on experimental samples. Based on these preliminary tests, optimal qPCR parameters included: 95°C for 30 s; 40 cycles of 95°C for 5 s, 57°C (RPS7, IL-31 and IL-31RA) or 60°C (TBP1) for 30 s, and a plate read; as well as a melt curve for the RPS7 and TBP1 assays. Resulting data were acquired from CFX Manager (Bio-Rad Laboratories). Mean Cqs were obtained for each sample from all four assays. Data from the IL-31 and IL-31RA qPCR assays were normalised to the expression of both the RPS7 and TBP1 genes using the $\Delta\Delta C_q$ method and compared between-sample groups using the $\Delta\Delta C_q$ method.

RNA in situ hybridisation

Slides were baked at 60°C for 15 min and then subjected to RNA-in situ hybridisation (RNA-ISH) using RNAScope probes (Advanced Cell Diagnostics, Inc., Newark, CA, USA)²⁸ targeting the genes for IL-31, IL-31RA, OSMR, IL-33 and IL-5 in cats on a BOND RX Fully Automated Research Stainer (Leica Biosystems; Wetzlar, Germany). Hybridisation reactions with probes targeting PPIB (catalog no. 455018) were performed to ensure RNA integrity in each sample and dapB (312038) to ensure no background signals. Probes for other targets were designed by Advanced Cell Diagnostics according to the following sequences from Genbank for each gene: XM_011287838.2 (IL-31), XM_019834296.1 (IL-31RA), XM_006928041.2 (OSMR), XM_006939157.2 (IL-33) and NM_001009845.2 (IL-5). Signals for all

Table 1. Oligos used in quantitative PCRs

Oligo	Sequence (5'–3')	Final concentration (nM)
RPS7		
RPS7_460F	GTC CCA GAA GCC GCA CTTT	400
RPS7_549R	CAC GGA TTC TCT TGC CCA CA	400
TBP1		
TBP1_746F	GAA TAA GAG AGC CCC GAA CC	400
TBP1_824R	TGC TCT TCA CTC TTG GCT CC	400
IL-31		
IL31_498F	CAA CCA AGT GAC GTC CGA AA	900
IL31_561R	GAC TAC CTG AAG AAG GAG ATT G	900
IL31_533Probe	ACG GCC CAT GTC TAA GGG ACT TTT GCA	400
IL-31RA		
IL31RA_619F	TTG GGT GTC AAA CGA ATG GT	900
IL31RA_737R	ACT TCC ATC CAG TAG GCA CT	900
IL31RA_654P	ACG GCC TGT GTT GGC TCC TGT T	400

probes were developed using the RNAscope 2.5 HD red assay kit (Advanced Cell Diagnostics, Inc.), except for the PPIB assay, which was observed with the RNAscope 2.5 HD brown assay kit (Advanced Cell Diagnostics, Inc.). Hybridisation signals were scored in the epidermis using the semiquantitative scoring criteria recommended by the manufacturer (Advanced Cell Diagnostics, Inc.) where scoring is based on the number of dots/cell (scoring criteria and example at <https://acdbio.com/technical-support/solutions>).

Evaluation of system cytokine expression

Serum sample collection

Blood samples were processed for serum collection immediately after collection, aliquoted and stored at -80°C until all samples were collected. Samples collected at collaborating clinics were processed for serum collection and shipped overnight on ice packs, then aliquoted and stored at -80°C until used for the assays described below.

Cats classified as having allergic skin disease included those with a history of pruritus/overgrooming and/or that showed typical cutaneous reaction patterns associated with feline cutaneous hypersensitivity disease (eosinophilic skin lesions including plaques, granulomas and indolent ulcers; miliary dermatitis; self-induced alopecia; and/or head and neck pruritus), for which infectious and parasitic causes of pruritus were ruled out. Of the 18 cats with allergic skin disease, 12 had a current "flare" of clinical signs and the remaining six had a historic diagnosis (absence of clinical signs at the time of sampling) (Table S2). Cats were included in the asthma group if they had owner-reported clinical signs or physical examination findings of respiratory disease (specifically coughing or wheezing episodes), a negative Baermann test, negative faecal flotation and negative heartworm antibody test. Thoracic radiographs were obtained for cats with asthma (read by a radiologist), but not all exhibited compatible radiographic changes. Healthy cats were >1 year of age, with no history of chronic disease and had not received medication (other than flea, tick or heartworm preventative) within the last two months. Cats affected by either allergic dermatitis or asthma were >1 year of age and had not received oral steroids or cyclosporine within the previous 14 days, injectable steroids within the past month, Depo-medrol within the past two months, or topical steroids within the previous seven days.

Serum testing

Serum samples were subjected to measurement of IL-31 with a Simoa HD-1 Analyzer (Quanterix Corporation; Billerica, MA, USA) and used for the MILLIPLEX MAP feline cytokine/chemokine magnetic bead panel (MilliporeSigma; Burlington, MA, USA), which targets 19 cytokines (sFas, Flt-3 ligand, GM-CSF, IFN- γ , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-12 p40, IL-13, IL-18, KC, MCP-1, PDGF-BB, RANTES,

SCF, SDF-1, TNF- α), on a FLEXMAP 3D (Luminex Corp.; Austin, TX, USA). The IL-31 assay was performed in duplicate using 80 μL serum. The MILLIPLEX MAP panel was performed according to the manufacturer's protocol, with 10 μL serum used from each sample in duplicate. Detailed methods can be found in Appendix S1.

Statistical methods

Data were tested for normality using the Shapiro–Wilks test in JMP Pro 14 (SAS Institute; Cary, NC, USA), followed by Kruskal–Wallis or Wilcoxon rank-sum tests where appropriate. Enzyme-linked immunosorbent assay data were compared based on the three groups (healthy, allergic dermatitis, asthma), as well as between healthy and allergic animals and between pairs (e.g. asthma vs. allergic dermatitis). For serum data from the MILLIPLEX MAP panel, samples with quantities below the limit of detection (LoD, as determined by manufacturer) were set to LoD/2 before being subjected to Kruskal–Wallis or Wilcoxon rank-sum tests. For serum IL-31 data, samples with quantities below the LoD were set to the lowest quantity detected, 0.1 pg/mL.

Results

qPCR

The normalising genes RPS7 and TBP1 were quantified in the skin of both allergic and control cats with an average cycle quantification value (Cq) of 29.36 for RPS7 and 33.99 for TBP1 assays. Although samples from cats with allergies showed a trend of higher IL-31 and IL-31RA expression, these mRNA could be detected only in a small number of samples ($n = 16$ for IL-31; $n = 8$ for IL-31RA; Figure 1), and only in very low quantities, with average Cqs of 38.05 for IL-31 and 38.64 for IL-31RA.

RNA-ISH

Strong mRNA expression of OSMR- β (Figure 2) was observed; however, IL-31RA and IL-33 mRNA expression was weak. IL-5 and IL-31 signals were rare and not visible in some sections. PPIB and DapB control probes confirmed RNA integrity and no background signal, respectively. Cytoplasmic and nuclear signals for all targets were primarily in the epidermis and follicular epithelial cells in the hair bulb and outer root sheath, and occasionally observed in sebaceous and apocrine glands, arrector pili muscle and infiltrating inflammatory cells. Significant differences in expression of IL-5 ($P = 0.0285$) and IL-33 ($P = 0.042$) were found, with samples from allergic cats

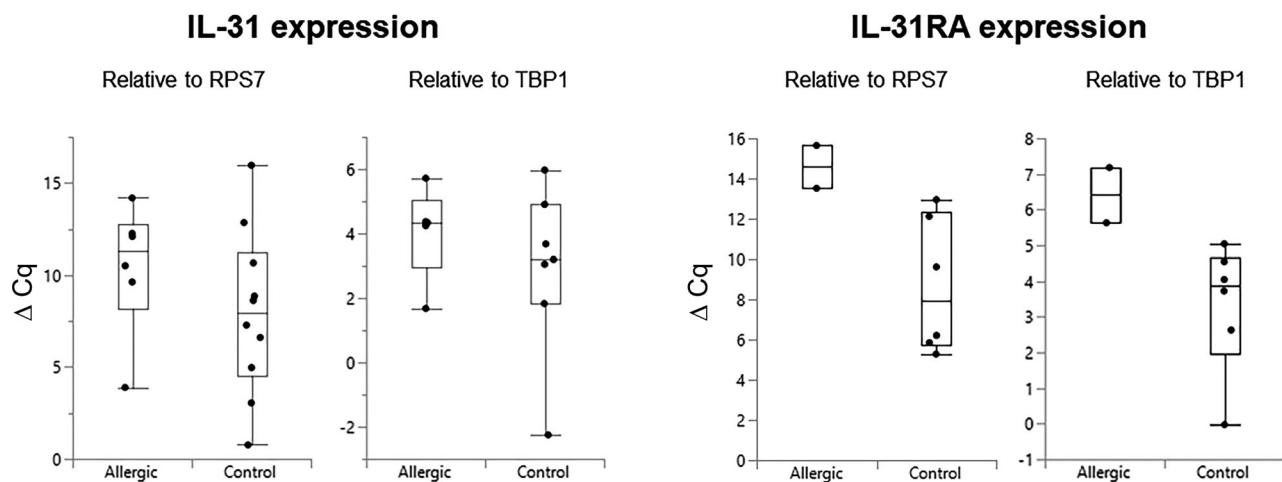


Figure 1. Results of interleukin (IL)-31 and IL-31RA quantitative PCR assays.

Results shown are from samples where at least one replicate was positive ($n = 16$ for IL-31, $n = 8$ for IL-31RA). Expression is shown relative to the ribosomal protein S7 (RPS7) and TATA-box-binding protein 1 (TBP1) genes.

having higher scores for both. However, average scores for both groups were low (<1) and the differences between them were low in magnitude. OSMR- β signals were significantly more abundant in allergic animals

($P < 0.0001$) and exhibited a substantial difference in scores between allergic and unaffected animals (Table 2).

Serum protein concentrations

Within the multiplex assay quantifying 19 different cytokines/chemokines, five cytokines (IL-12, IL-8, SDF1, RANTES, SCF) were measured successfully in all samples (Table 3). Although IL-31 was found in slightly higher concentrations in affected cats (mean 1.50 pg/mL) compared to healthy cats (0.73 pg/mL), it could be found at detectable concentrations only in 19 of 53 samples and was the cytokine with the lowest detected quantities. No cytokines had significantly different levels between the groups, but there were notable differences in the number of samples where IL-31 could be measured. While 10 of 18 allergic dermatitis samples had detectable quantities, only four of 17 healthy samples and four of 18 asthmatic samples had quantifiable levels. Additionally, SCF concentrations were below the limit of quantification for four of 18 allergic dermatitis and three of 18 asthma samples, but were quantifiable in all healthy samples. When comparing the samples from cats with allergic dermatitis with flares ($n = 12$) and those without current flares ($n = 6$), RANTES ($P = 0.0492$) and IL-12 p40 ($P = 0.0492$) were found to be present at higher concentrations in cats with flares. Statistical analyses yielded no significant difference between cats with allergic dermatitis with flares and the healthy cats.

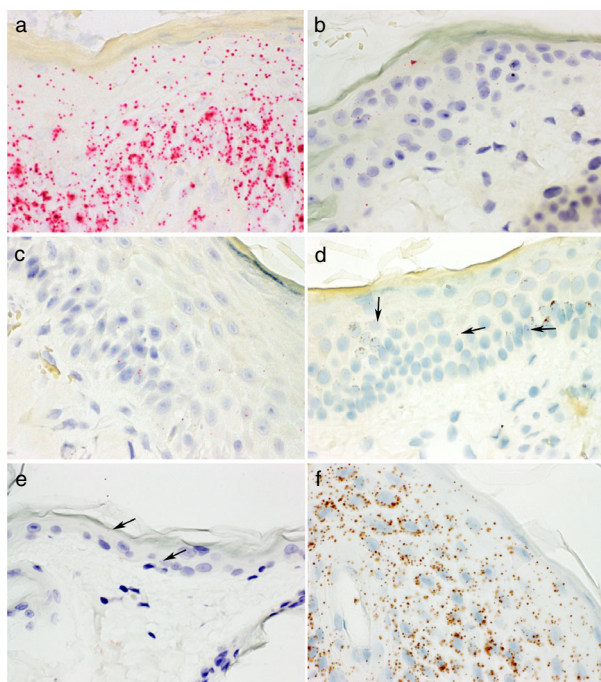


Figure 2. RNA *in situ* hybridisation (RNA-ISH) signals for RNAScope assays in the epidermis of feline skin.

(a) Oncostatin M receptor (OSMR)- β exhibited strong expression. (b) Interleukin (IL)-31RA and (c) IL-33 mRNA expression was weaker, but still observed in most sections. (d) IL-5 and (e) IL-31 signals were rarely observed or undetectable in some sections (arrows indicate hybridisation signals). (f) Signals from the hybridisation reactions with the positive control probe targeting PPIB indicated that RNA integrity was appropriate in all samples for RNA-ISH. Red dots indicate positive signals for (a–e), brown dots indicate positive signals for (f), $\times 400$. Hybridisation reactions with the negative control probe targeting DapB did not yield signals (not shown).

Table 2. RNA *in situ* hybridisation (RNA-ISH) scores expressed as mean $\pm$ SD

Target	Control ($n = 20$)	Allergic ($n = 21$)	Wilcoxon P -value
IL-31	0.075 $\pm$ 0.183	0.227 $\pm$ 0.329	0.1599
IL-31RA	1.025 $\pm$ 0.499	0.929 $\pm$ 0.350	0.2689
IL-33	0.632 $\pm$ 0.436	0.909 $\pm$ 0.464	0.0420
IL-5	0	0.114 $\pm$ 0.179	0.0285
OSMR	0.944 $\pm$ 0.539	2.545 $\pm$ 0.890	<0.0001

Comparisons yielding significant results are in bold.

Table 3. Cytokine and chemokine protein concentrations in feline serum samples

Cytokine	Healthy (n = 17)	Allergic dermatitis (n = 18)	Asthma (n = 18)
sFAS	129.55 ± 161.70 (94%)	138.37 ± 240.94 (88%)	130.18 ± 162.34 (72%)
Flt-3 ligand	150.02 ± 76.93 (94%)	168.97 ± 90.27 (100%)	155.07 ± 60.71 (100%)
GM-CSF	31.19 ± 40.20 (59%)	28.84 ± 50.32 (61%)	24.66 ± 31.89 (56%)
IFN- γ	575.70 ± 723.76 (94%)	416.63 ± 872.03 (94%)	452.29 ± 780.99 (100%)
IL-1 β	136.47 ± 143.47 (88%)	85.74 ± 140.26 (67%)	90.61 ± 129.04 (100%)
IL-2	85.55 ± 95.23 (88%)	65.84 ± 128.76 (72%)	80.45 ± 115.95 (67%)
IL-4	2,727.49 ± 3288.60 (94%)	2,005.09 ± 3961.02 (94%)	2,065.38 ± 3509.26 (100%)
IL-6	1,052.31 ± 1232.64 (94%)	708.60 ± 1300.65 (94%)	697.59 ± 1113.55 (88%)
IL-8	96.01 ± 78.50 (100%)	71.75 ± 88.69 (100%)	74.19 ± 81.21 (100%)
IL-12 p40	433.30 ± 296.19 (100%)	581.99 ± 627.33 (100%)	400.45 ± 206.74 (100%)
IL-13	66.48 ± 58.374 (94%)	60.92 ± 68.40 (100%)	60.57 ± 54.06 (100%)
IL-18	1,609.11 ± 1642.23 (94%)	1,127.64 ± 1819.76 (88%)	959.81 ± 1249.90 (100%)
IL-31	0.73 ± 1.95 (20%)	1.50 ± 3.14 (56%)	21.57 ± 84.01 (22%)
KC	16.61 ± 26.06 (82%)	36.91 ± 110.35 (83%)	38.80 ± 115.54 (94%)
MCP-1	6,890.39 ± 5640.70 (100%)	5,109.12 ± 5782.25 (78%)	5,321.13 ± 5603.20 (83%)
PDGF-BB	1,560.81 ± 2558.87 (94%)	843.81 ± 1368.12 (78%)	630.08 ± 510.57 (83%)
RANTES	94.90 ± 71.49 (100%)	104.05 ± 74.47 (100%)	79.12 ± 65.87 (100%)
SCF	338.53 ± 258.43 (100%)	277.15 ± 295.54 (100%)	318.60 ± 262.67 (100%)
SDF-1	3,459.99 ± 1685.02 (100%)	2,797.61 ± 1516.32 (100%)	2,909.02 ± 1213.19 (100%)
TNF- α	488.48 ± 644.46 (53%)	307.26 ± 687.22 (50%)	318.51 ± 596.99 (61%)

Data are presented as mean concentrations (pg/mL) ± SD. The percentage of samples with quantified concentrations greater than the limit of detection is shown in parenthesis.

Noninterleukin abbreviations defined: sFas, soluble Fas; Flt-3 ligand, Fms-related tyrosine kinase 3 ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN- γ , interferon gamma; KC, keratinocyte-derived cytokine; MCP-1, monocyte chemoattractant protein 1; PDGF-BB, platelet-derived growth factor (two B subunits); RANTES, regulated upon activation, normal T-cell expressed and secreted; SCF, stem cell factor; SDF-1, stromal cell-derived factor 1; TNF- α , tumour necrosis factor alpha.

Discussion

To the best of the authors' knowledge, this is the first comprehensive evaluation of cytokine profiles in cats with allergic dermatitis or asthma. Additionally, this is the first study to describe the expression of IL-31 and related proteins in feline skin. The data provide further insight into the pathogenesis of feline allergies, which remains elusive.

Contrary to our hypothesis, neither the qPCR nor the RNA-ISH results demonstrated differences in systemic or cutaneous IL-31 expression between cats with allergic dermatitis and controls. While cutaneous IL-31 mRNA levels appeared higher in allergic samples, this was neither a significant nor a substantial difference. Similar to cutaneous mRNA expression, serum protein concentrations were low and could not be detected in a majority of samples. Interestingly, we were able to quantify IL-31 in more samples from cats with allergic dermatitis (56%) than healthy cats (20%). While our results indicated slightly higher concentrations of circulating IL-31 in allergic cats (mean 1.50 pg/mL) compared to healthy controls (0.73 pg/mL), this was not significant. Our results do not invalidate the contribution of IL-31 to pruritus in feline allergic dermatitis; however, they do not strongly support the use of IL-31 as a biomarker or therapeutic target for feline allergic dermatitis. Certainly, further research would be needed to confirm these results.

While the expression IL-31 may not be increased in cats with allergic dermatitis, we considered whether changes in the expression of IL-31 receptor subunits, IL-31RA and OSMR- β , could be important in the disease process. Overall, we did not find IL-31RA expression to be upregulated in allergic skin; only a small number of

samples (eight of 43) had levels that were detectable with qPCR and RNA-ISH indicated low levels with no statistical significant difference between allergic and control samples. However, RNA-ISH results showed significantly higher mRNA expression of OSMR- β in samples obtained from allergic animals.

OSMR- β is one of the subunits of the heterodimeric receptor complex for IL-31. Upon binding of IL-31, the IL-31 receptor complex induces signalling via the JAK/STAT, PI3K/AKT and several other pathways. These pathways have implications in cytokine secretion, epidermal barrier function, cell migration and differentiation, and, most notably, pruritus. Like IL-31, the IL-31 receptor complex is present on a wide range of cells, including keratinocytes, mast cells, eosinophils and peripheral neurons. In addition to skin, OSMR- β also has been found in other tissues such as the lungs and dorsal root ganglia.²⁹ Importantly, IL-31 is not the only ligand that can bind to OSMR- β . Research into the expression of its other ligands may reveal that they are potential targets for therapeutic intervention. For example, OSM is involved in skin inflammation³⁰ and, when injected into mice, induced expression of several genes including those coding for the Th2 cytokines IL-4, IL-5 and IL-13.³¹

In addition to evaluating the cytokine profile in cats with allergic dermatitis, we characterised the circulating cytokine profile in cats with asthma. Like allergic dermatitis, asthma traditionally has been associated with a Th2 response,³² and, as mentioned previously, IL-31 is thought to play a role in asthma in humans.^{33,34} In our study, none of the 20 evaluated cytokines were found to be present in significantly different concentrations. Many samples had protein concentrations which were below the LoD for several of the cytokines evaluated; perhaps

our ability to observe differences between our sample groups was affected by the sensitivity of these assays. Another reason that we may not have observed a clear Th2 skewed immune response could be the nature of our sample cohort. A strong Th2/Th22 response typically occurs in the acute phase of allergic dermatitis before switching to a predominantly Th1 response in the chronic phase.³⁵ More than half of the cats with allergic dermatitis had current flares (Table S2), but these data cannot be used to assume that the cats were in the acute phase of the immune response. While all of the cats affected by asthma did have current clinical signs, some cats had fairly rare coughing episodes (only once or twice per week). Only two cats had severe respiratory signs requiring urgent in-hospital management. While we did not identify the allergy-associated Th2 skewed cytokine profile, it is worth noting that the presented serum data also did not demonstrate a strong Th1 skewed cytokine profile, as exhibited by similar IFN- γ serum levels in all groups.

Allergic dermatitis affecting cats has been known to be different from the disease affecting other species, particularly in terms of clinical patterns. In this study, we provide evidence indicating that the disease is distinct with respect to the immune system as well, suggesting the involvement of potentially unknown pathomechanisms in feline allergic dermatitis. While cats with allergic dermatitis do show an inflammatory infiltrate that is typical of allergic disease, our results do not suggest that these cells are actively overproducing the characteristic Th2 cytokines observed in other species. This is consistent with a previous study in which lesional and nonlesional skin samples from allergic cats and skin samples from healthy cats were subjected to qPCRs targeting 10 cytokines spanning both Th1 and Th2 response, and also did not find evidence of a Th2-skewed cytokine profile.²⁴ Similar to the serum samples, although all FFPE samples included in our study were obtained from cats that had current clinical flares of disease, it is possible this did not coincide with an acute immune response.

One limitation of our study was sample size; studies evaluating a larger sample cohort would be useful, particularly for the serum assays where substantial variation was observed. While our study aimed to evaluate cytokine profiles in cats with naturally occurring disease, future studies using samples obtained from an experimental cohort could provide additional information. This would allow for a more controlled population and inducing an allergic flare would allow characterisation of the immune response during acute and chronic phases. Additionally, our experiments to assess mRNA expression in the skin utilised FFPE tissues, which are not ideal in terms of RNA quality or quantity. Experiments using fresh or snap-frozen samples should be done to confirm the low mRNA expression for cytokines that we have described in the study herein. Finally, the method of RNA-ISH used in the present study utilises probes that were validated *in silico* by the manufacturer²⁸; further validation using cell lines known to express the molecules of interest or using additional methods to quantify the targets measured in this study would be useful to confirm the present findings.

In summary, our results suggest that cats with allergic dermatitis do not exhibit the traditional allergy-associated Th2 response, and that a strong Th1 response is not apparent. Although IL-31 mRNA expression does not appear heightened in allergic cats and was found in only low levels regardless of technique, this does not rule out that targeting molecules within the IL-31 pathway may still be valuable. In particular, analysis of protein expression of other molecules involved in this pathway, such as OSMR- β , in both the skin and circulation could be useful. Expression of these molecules in other tissues involved in pruritus signalling, for example the dorsal root ganglia, may add to our understanding of the role of IL-31 signalling in feline allergic dermatitis.

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Author contributions

Caitlin Older: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing-original draft, Writing-review & editing. **Ali-son Diesel:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing-review & editing. **Johanna Heseltine:** Conceptualization, Funding acquisition, Investigation, Resources, Writing-review & editing. **Amanda Friedeck:** Investigation, Resources. **Carolyn Hedke:** Investigation, Methodology, Resources. **Stephanie Pardike:** Investigation, Methodology, Resources. **Katrina Breitreiter:** Investigation, Resources, Writing-review & editing. **Michael Rossi:** Investigation, Resources, Writing-review & editing. **James E. Messamore:** Investigation, Methodology, Resources, Writing-original draft, Writing-review & editing. **Gary Bammert:** Investigation, Methodology, Resources, Writing-review & editing. **Andrea Gonzales:** Funding acquisition, Methodology, Resources, Supervision, Writing-review & editing. **Aline Rodrigues Hoffmann:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing-review & editing.

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Supporting Information

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

Appendix S1. Detailed methods for serum immunoassays.

Table S1. Signalment for FFPE samples.

Table S2. Signalment for serum samples.

Résumé

Contexte – La pathogénie de la dermatite allergique féline (FAD) n'est pas claire, avec plusieurs différences par rapport à la dermatite allergique de l'homme et du chien.

Hypothèses/Objectifs – Etudier le niveau d'expression des cytokines chez les chats sains et les chats atteints de dermatite allergique ou d'asthme.

Sujets – Des biopsies cutanées fixées dans le formol et montées dans la paraffine de 22 chats avec dermatite allergique et 21 chats sans dermatite allergique ont été utilisées pour tests cutanés. Le serum a été obtenu de 17 chats sains, 18 chats avec dermatite allergique et 18 chats avec diagnostic présumé d'asthme.

Matériels et méthodes – L'expression d'ARNm cutané a été évaluée par PCR quantitative [interleukine

(IL)-31 et IL-31 Récepteur A] et hybridation d'ARN in situ (ISH) [IL-5, IL-31, IL-31RA, IL-33 et Oncostatin M receptor (OSMR)- β]. Les concentrations de protéine IL-31 ont été évaluées dans le serum avec ELISA. Le niveau sérique de 19 cytokines additionnelles a été évalué par panel Luminex.

Résultats – L'expression d' IL-31, IL-31RA, IL-5 et IL-33 mRNA était soit exprimée en faibles quantités ou non détectables dans la plupart des échantillons. A contrario, l'expression OSMR- β était significativement plus élevée dans la peau des chats allergiques versus chats sains ($P < 0.0001$). Bien que l'IL-31 sérique ait été détecté dans un plus grand nombre de chats avec dermatite allergique que de chats sains, et que les concentrations semblaient plus élevées chez les chats avec allergies, ces différences n'étaient pas statistiquement significatives. Les chats atteints d'asthme ont aussi montré des concentrations plus élevées non significatives d'IL-31 dans le serum.

Conclusions et importance clinique – Nos résultats suggèrent que les maladies allergiques félines peuvent présenter différents patho-mécanismes des maladies allergiques touchant d'autres espèces. Ces données sont utiles pour guider le développement futur des cibles pouvant avoir un rôle dans la pathogénie des maladies allergiques félines allergiques.

Resumen

Introducción – la patogenia de la dermatitis alérgica felina (FAD) no está clara, con varias diferencias con la dermatitis alérgica en perros y seres humanos.

Hipótesis/objetivos – Estudiar los niveles de expresión de citoquinas en gatos sanos y gatos afectados por dermatitis alérgica o asma.

Animales – para los ensayos cutáneos se utilizaron biopsias de piel fijadas con formalina e incluidas en parafina de 22 gatos con dermatitis alérgica y 21 gatos sin dermatitis alérgica. Se obtuvo suero de 17 gatos sanos, 18 gatos con dermatitis alérgica y 18 gatos con diagnóstico presuntivo de asma.

Métodos y materiales – Se evaluó la expresión de RNAm cutáneo con PCR cuantitativa [receptor A de interleuquina (IL) -31 e IL-31] e hibridación in situ de RNA (ISH) [IL-5, IL-31, IL-31RA, IL-33 y receptor de oncostatina M (OSMR)- β]. Las concentraciones de proteína IL-31 se evaluaron en suero con un ensayo inmunoabsorbente ligado a enzimas. Se evaluaron los niveles séricos de 19 citoquinas adicionales usando un panel Luminex.

Resultados – la expresión de RNAm de IL-31, IL-31RA, IL-5 e IL-33 se expresó en cantidades bajas o no fue detectable en la mayoría de las muestras. Por el contrario, la expresión de OSMR- β fue significativamente mayor en la piel de gatos alérgicos que en gatos sanos ($P < 0.0001$). Aunque se detectó IL-31 sérica en un mayor número de gatos con dermatitis alérgica que en gatos sanos, y las concentraciones parecieron ser más altas en gatos con alergias, esta diferencia no fue estadísticamente significativa. Los gatos afectados por el asma también exhibieron concentraciones no significativamente más altas de IL-31 en el suero.

Conclusiones y relevancia clínica – Nuestros resultados sugieren que las enfermedades alérgicas felinas pueden presentar diferentes patomecanismos que las enfermedades alérgicas que afectan a otras especies. Estos hallazgos son útiles para orientar el desarrollo terapéutico adicional hacia objetivos que pueden tener un papel en la patogénesis de la enfermedad alérgica de la piel felina.

Zusammenfassung

Hintergrund – Die Pathogenese der feline allergischen Dermatitis (FAD) ist unklar, wobei einige Unterschiede zur allergischen Dermatitis von Hunden und Menschen bestehen.

Hypothese/Ziele – Ein Überblick über die Zytokin Expression von gesunden Katzen und Katzen mit allergischer Dermatitis oder Asthma.

Tiere – In Formalin-fixierte und in Paraffin-eingebettete Hautbiopsien von 22 Katzen mit allergischer Dermatitis und 21 Katzen ohne allergische Dermatitis wurden für die Haut-Assays verwendet. Es wurde Serum von 17 gesunden Katzen, von 18 Katzen mit allergischer Dermatitis, und von 18 Katzen mit einer vermeintlichen Diagnose von Asthma entnommen.

Methoden und Materialien – Die mRNA Expression in der Haut wurde mittels quantitativer PCR [Interleukin (IL)-31 und IL-31 Rezeptor A] und RNA *in situ* Hybridisierung (ISH) [IL-5, IL-31, IL-31RA, IL-33 und Oncostatin M Rezeptor (OSMR)- β] evaluiert. IL-31 Proteinkonzentrationen wurden im Serum mittels Enzyme-linked Immunosorbent Assay evaluiert. Serumwerte von 19 weiteren Zytokinen wurden mittels Luminex Panel evaluiert.

Ergebnisse – IL-31, IL-31RA, IL-5 und IL-33 mRNA wurde in den meisten Proben entweder nur in niedrigem Ausmaß oder im nicht messbaren Bereich gefunden. Im Gegensatz dazu war die OSMR- β Expression in der Haut von allergischen im Gegensatz zu gesunden Katzen signifikant höher ($P < 0.0001$). Obwohl IL-31 Werte im Serum in einer größeren Anzahl von Katzen mit allergischer Dermatitis als bei gesunden Katzen gefunden wurden und die Konzentrationen bei Katzen mit Allergien höher zu sein schienen, war dieser Unterschied statistisch nicht signifikant. Katzen mit Asthma zeigten ebenso unbedeutend höhere IL-31 Konzentrationen im Serum.

Schlussfolgerungen und klinische Bedeutung – Unsere Ergebnisse zeigen, dass die allergischen Erkrankungen von Katzen andere Pathomechanismen als die allergischen Krankheiten anderer Spezies

aufweisen. Diese Ergebnisse sind nützlich, indem sie die weitere therapeutische Entwicklung in die Richtung der Ziele lenken, die eine Rolle bei der Pathogenese der allergischen Hauterkrankung von Katzen spielen könnten.

要約

背景 – 猫アレルギー性皮膚炎 (FAD) の病因は不明であり、犬や人のアレルギー性皮膚炎とはいくつかの違いがある。

仮説・目的 – 本研究の目的は、健康猫およびアレルギー性皮膚炎や喘息に罹患した猫におけるサイトカインの発現レベルを調査することであった。

供試動物 – アレルギー性皮膚炎の猫22頭およびアレルギー性皮膚炎のない猫21頭のホルマリン固定パラフィン包埋皮膚生検を皮膚アッセイ法に使用した。血清は、健康猫17頭、アレルギー性皮膚炎の猫18頭、喘息と推定診断された猫18頭から採取した。

材料と方法 – 皮膚の mRNA 発現を定量的 PCR [インターロイキン (IL)-31 および IL-31 受容体 A] および RNA in situ ハイブリダイゼーション (ISH) [IL-5, IL-31, IL-31RA, IL-33 および オンコスタチン M 受容体 (OSMR)-β] で評価した。血清中の IL-31 タンパク質濃度は、酵素結合免疫吸着法で評価した。さらに19種類のサイトカインの血清中の濃度をLuminexパネルを用いて評価した。

結果 – IL-31, IL-31RA, IL-5, IL-33 の mRNA 発現は、ほとんどのサンプルで少量しか発現していないか、検出されなかった。一方、OSMR-βの発現は、アレルギー猫および健康猫の皮膚で有意に高かった ($P < 0.0001$)。血清中の IL-31は、健康猫よりもアレルギー性皮膚炎の猫の方が多く検出され、アレルギー猫では濃度が高いようであったが、統計学的に有意差はなかった。喘息罹患猫も、血清中の IL-31の濃度が有意に高くなかった。

結論と臨床的妥当性 – 我々の結果は、猫のアレルギー疾患が、他の種が罹患するアレルギー疾患とは異なる病態メカニズムを示す可能性を示唆している。これらの知見は、猫のアレルギー性皮膚疾患の病因に関与している可能性のある標的に向けて、さらなる治療法の開発を導くのに有用である。

摘要

背景 – 猫過敏性皮膚炎(FAD)の发病机制尚不清楚, 与犬和人过敏性皮炎存在一些差异。

假设/目的 – 调查患有过敏性皮炎或哮喘的健康猫和猫的细胞因子表达水平。

动物 – 对22只过敏性皮炎猫和21只无过敏性皮炎猫进行皮肤试验, 将皮肤活检组织进行福尔马林固定、石蜡包埋。血清取自17只健康猫、18只过敏性皮炎猫和18只推定诊断为哮喘的猫。

方法和材料 – 使用定量PCR[白介素(IL)-31和IL-31受体A]和RNA原位杂交(ISH)[IL-5、IL-31、IL-31RA、IL-33和制瘤素M受体(OSMR)-β]评价皮肤mRNA表达。采用酶联免疫吸附试验评价血清中的IL-31蛋白浓度。使用Luminex试剂盒评价19种其他细胞因子的血清水平。

结果 – 在大多数样本中, IL-31、IL-31RA、IL-5和IL-33 mRNA表达量较低或检测不到。相比之下, 与健康猫相比, 过敏猫皮肤中OSMR-β表达显著增高($P < 0.0001$)。尽管与健康猫相比, 在更多过敏性皮炎猫中检测到血清IL-31, 并且在过敏猫中的浓度似乎更高, 但该差异无统计学显著性。在血清中, 哮喘猫也表现出不显著更高浓度的IL-31。

结论和临床相关性 – 我们的结果表明, 猫过敏性疾病表现出的发病机制可能与其他物种的过敏性疾病不同。这些发现有助于指导进一步开发治疗方案, 以达到可能在猫过敏性皮肤病发病机制中发挥作用的靶点。

Resumo

Contexto – A patogênese da dermatite alérgica felina (DAF) não é clara, apresentando várias diferenças em comparação com a dermatite alérgica em cães e humanos.

Hipótese/Objetivos – Estudar os níveis de expressão de citocinas em gatos saudáveis e gatos afetados por dermatite alérgica ou asma.

Animais – Amostras de biópsia parafinadas e fixadas em formol oriundas de 22 gatos com dermatite alérgica e 21 gatos sem dermatite alérgica foram utilizadas para ensaios cutâneos. Soro foi obtido de 17 gatos saudáveis, 18 gatos com dermatite alérgica, e 18 gatos com diagnóstico presuntivo de asma.

Métodos e materiais – A expressão cutânea de mRNA foi avaliada por PCR quantitativa de [interleucina (IL) -31 e receptor de IL-31 A] e hibridação in situ (ISH) [IL-5, IL-31, IL-31RA, IL-33 e receptor de Oncostatina M (OSMR) -β]. As concentrações de proteína IL-31 foram avaliadas no soro com um ensaio imunossorvente ligado à enzima. Níveis séricos de 19 citocinas adicionais foram avaliados usando um painel Luminex.

Resultados – A expressão de mRNA para IL-31, IL-31RA, IL-5 e IL-33 foi indetectável ou em baixas quantidades na maioria das amostras. Em contraste, a expressão OSMR-β foi significativamente maior na pele dos gatos alérgicos versus saudáveis ($p < 0,0001$). Embora o soro IL-31 tenha sido detectada em um número maior de gatos com dermatite alérgica do que gatos saudáveis, e concentrações aparentemente maiores em gatos com alergias, essa diferença não foi estatisticamente significativa. Os gatos afetados pela asma também exibiram concentrações maiores de IL-31 no soro, mas não significativas.

Conclusões e relevância clínica – Nossos resultados sugerem que as doenças alérgicas felinas podem exibir diferentes patomecanismos de doenças alérgicas que afetam outras espécies. Esses achados são úteis para orientar ainda mais o desenvolvimento terapêutico em relação aos alvos que possam ter um papel na patogênese da doença da pele alérgica felina.

Table S1. Signalment for FFPE samples.

Sample	Sample group	Age	Sex	Breed	Diagnosis	Histopathological findings
D1	Allergic	4y	SF	DSH	Allergic dermatitis	Mild-to-moderate, multifocal, superficial-to-deep perivascular dermatitis with epidermal hyperplasia, ulceration and serocellular crusting.
D2	Allergic	5y	CM	DSH	Allergic dermatitis	Eosinophilic and mastocytic dermatitis, moderate-to-marked, with epidermal hyperplasia, and multifocal erosion and serocellular crusting.
D3	Allergic	3y	CM	DSH	Allergic dermatitis	Moderate-to-marked, multifocal, chronic, eosinophilic superficial-to-deep perivascular dermatitis with epidermal hyperplasia, dermal fibrosis and serocellular crusting (one biopsy).
D4	Allergic	2y	SF	DSH	Allergic dermatitis	Mild perivascular mastocytic dermatitis, with epidermal hyperplasia, and serocellular crusting.
D5	Allergic	14m	SF	DSH	Allergic dermatitis	Moderate, multifocal, chronic eosinophilic and mastocytic perivascular-to-diffuse dermatitis with epidermal hyperplasia, and focal ulceration with superficial cocci bacteria.
D6	Allergic	13m	CM	DSH	Allergic dermatitis	Mastocytic and eosinophilic perivascular dermatitis, chronic suppurative, with epidermal hyperplasia and spongiosis, and focal ulceration with serocellular crusting.
D7	Allergic	2y	CM	DSH	Allergic dermatitis	Marked, diffuse, chronic mastocytic and eosinophilic dermatitis with epidermal hyperplasia, oedema, mucinosis and erosions.
D8	Allergic	1y	CM	DSH	Allergic dermatitis	Mild, multifocal, subacute, perivascular dermatitis, mastocytic and eosinophilic with epidermal hyperplasia, dermal fibrosis, serocellular crusting and superficial bacteria.
D9	Control	3y	CM	Bengal	Allergic dermatitis	Severe, multifocal, chronic, eosinophilic superficial and deep dermatitis, perifolliculitis and furunculosis with epidermal hyperplasia and ulceration.
D10	Allergic	12y	SF	DSH	Allergic dermatitis	Diffuse, severe, eosinophilic and mastocytic dermatitis with multifocal ulceration and fibrosis
D11	Allergic	2y	SF	DSH	Allergic dermatitis	Diffuse, severe, eosinophilic dermatitis with flame figures
D12	Allergic	1y 8 m	SF	DSH	Allergic dermatitis	Moderate, multifocal, eosinophilic, mastocytic and histiocytic superficial dermatitis with epidermal hyperplasia and serocellular crusting.
D13	Allergic	5y	CM	DSH	Allergic dermatitis	Marked, diffuse, chronic mastocytic and eosinophilic superficial dermatitis with epidermal hyperplasia and serocellular

						crusting
D14	Allergic	2y	CM	DSH	Allergic dermatitis	Multifocal to coalescing, moderate-to-severe, mastocytic and eosinophilic dermatitis.
D15	Allergic	8y	SF	DSH	Allergic dermatitis	Severe eosinophilic dermatitis and panniculitis, perivascular-to-diffuse, with epidermal hyperplasia and multifocal ulceration.
D16	Allergic		CM	Mixed	Allergic dermatitis	Diffuse, severe, mastocytic and eosinophilic dermatitis with ulceration
D17	Allergic	5y	SF	DSH	Allergic dermatitis	Multifocal, moderate, mastocytic and eosinophilic dermatitis with multifocal epidermal ulceration.
D18	Allergic		CM	DSH	Allergic dermatitis	Diffuse, severe, eosinophilic dermatitis with luminal folliculitis and furunculosis
D19	Allergic	5y	SF	DSH	Allergic dermatitis	Multifocal to coalescing, severe, mastocytic and eosinophilic dermatitis with multifocal flame figures and ulceration.
D20	Allergic	4y	CM	Bengal	Allergic dermatitis	Moderate, multifocal, chronic, superficial, perivascular dermatitis with epidermal hyperplasia, ulceration, serocellular crusting and fibrosis.
D21	Allergic	3y	SF	DLH	Allergic dermatitis	Severe, diffuse, eosinophilic dermatitis; Focal, chronic, mild granulomatous panniculitis.
D22	Allergic	2.5y	CM	DSH	Allergic dermatitis	Severe, multifocal, chronic eosinophilic, mastocytic and lymphocytic superficial to deep dermatitis with epidermal ulceration and serocellular crusting.
H1	Control	4y	CM	DSH	Follicular cysts, infundibular type (epidermal inclusion cysts)	
H2	Control	8y	F	DSH	Basal cell tumour	
H3	Control	11y	CM	DLH	Basal cell tumour	
H4	Control	13y	SF	DSH	Basal cell tumour	
H5	Control	13y	SF	DLH	Basal cell tumour	
H6	Control	10y	SF	DSH	Basal cell tumour	
H7	Control	5y	SF	Bengal	Infundibular follicular cyst	
H8	Control	13y	CM	Siamese	Tichoblastoma, spindle type	
H9	Control	17y	CM	DLH	Basal cell tumour	
H10	Control	4y	CM	DSH		
H11	Control	5	SF	DSH	fibrosarcoma	
H12	Control	12y	SF	DSH	Interemdiante grade spindle	

					cell sarcoma	
H13	Control	13y	CM	Oriental shorthair	Apocrine adenocarcinoma	
H14	Control	10y	SF	DSH	Squamous cell carcinoma	
H15	Control	14y	SF	Forest Norwegian	Suture reaction (granulomatous dermatitis and panniculitis)	
H16	Control	13y	CM	DSH	Mild myocyte atrophy	
H17	Control	16y	CM	DSH	Microchip-associated fibrosarcoma	
H18	Control	4y	CM	DSH	Bone lesion	
H19	Control	8y	SF	DMH	Focal cutaneous xanthoma near tubulopapillary mammary adenoma	
H20	Control	10y	SF	DSH	Collagenous hamartoma	
H21	Control	8y	SF	DSH	Suture granulomas and fibrosis	

Y, years; m, months; CM, complete male; SF, spayed female; F, female; M, male; DSH, domestic short hair; DMH, domestic medium hair; DLH, domestic long hair

Table S2. Signalment for serum samples.

Sample	Sample group	Age (years)	Sex	Breed	Flare?
D1	AD	6	M	DSH	Yes
D2	AD	6	SF	DSH	No
D3	AD	5	CM	DSH	No
D4	AD	4.5	M	DSH	Yes
D5	AD	2	CM	DSH	No
D6	AD	2	SF	DSH	Yes
D7	AD	7	SF	DSH	Yes
D8	AD	8	CM	Siamese	Yes
D9	AD	5	CM	DSH	No
D10	AD	2	SF	DSH	Yes
D11	AD	10	CM	DSH	Yes
D12	AD	12	SF	Siamese	Yes
D13	AD	4	CM	Siamese	Yes
D14	AD/AA	7	SF	DSH	Yes
D15	AD	10	SF	DSH	No
D16	AD	5	CM	DSH	Yes

D17	AD	10	SF	DSH	No
D18	AD	4	SF	DSH	Yes
H1	H	11	SF	DLH	N/A
H2	H	3	SF	DSH	N/A
H3	H	2	F	DSH	N/A
H4	H	2	F	DSH	N/A
H5	H	1.5	SF	DMH	N/A
H6	H	5.5	CM	DSH	N/A
H7	H	2.5	SF	Persian cross	N/A
H8	H	5	SF	DSH	N/A
H9	H	5	SF	DLH	N/A
H10	H	5.5	CM	Russian blue mix	N/A
H11	H	3	SF	DSH	N/A
H12	H	2	M	Persian	N/A
H13	H	7	M	Sphynx	N/A
H14	H	3	SF	DSH	N/A
H15	H	6	SF	DSH	N/A
H16	H	2	CM	DSH	N/A
H17	H	6	CM	DSH	N/A
R1	AA	10	M	DSH	N/A
R2	AA	9	SF	DSH	N/A
R3	AA	8	SF	DSH	N/A
R4	AA	4	CM	Siamese	N/A
R5	AA	9	CM	DSH	N/A
R6	AA	5	SF	DSH	N/A
R7	AA/AD	4	CM	DSH	N/A
R8	AA	2	CM	DSH	N/A
R9	AA	14	F	DSH	N/A
R10	AA	11	SF	DLH	N/A
R11	AA	10	CM	DSH	N/A
R12	AA	3	CM	DSH	N/A
R13	AA	6	SF	DSH	N/A
R14	AA	4	CM	DSH	N/A
R15	AA	11	CM	DSH	N/A
R16	AA	2	SF	Manx	N/A
R17	AA	8	CM	DSH	N/A
R18	AA	12	CM	DSH	N/A

AD, allergic dermatitis; AA, allergic asthma; H, healthy. Rest of key as for Table S1

Appendix S1. Detailed methods for serum immunoassays.

IL-31 serum immunoassay

The Simoa Feline IL-31 assay is a two-step digital immunoassay to measure the quantity of feline IL-31 in feline serum using the Quanterix Simoa HD-1 Analyzer and Single Molecule Array (Simoa) technology. In the first assay step, serum, anti-feline IL-31-coated paramagnetic capture beads, and biotinylated detector antibodies are combined. Feline IL-31 molecules present in the sample are captured by the anti-feline IL-31-coated capture beads and labelled with biotinylated detector antibody. After washing, a conjugate of streptavidin- β -galactosidase (S β G) is mixed with the capture beads. S β G binds to the biotinylated detector antibodies, resulting in enzyme labelling of captured feline IL-31. Following a second wash, the capture beads are resuspended in a resorufin β -D-galactopyranoside (RGP) substrate solution and transferred to the Simoa Disc Array. Individual capture beads then are sealed within microwells in the array. If feline IL-31 has been captured and labeled, the β -galactosidase hydrolyses the RGP substrate into a fluorescent product that provides the signal for measurement. A single labelled feline IL-31 molecule results in sufficient fluorescent signal in 30 s to be detected and counted by the Simoa optical system. At low feline IL-31 concentration, the percentage of bead-containing wells in the array that have a positive signal is proportional to the amount of Feline IL-31 present in the sample. At higher feline IL-31 concentration, when most of the bead-containing wells have one or more labelled feline IL-31 molecule(s), the total fluorescence signal is proportional to the amount of feline IL-31 present in the sample. The concentration of Feline IL-31 in unknown samples is interpolated from a standard curve using a 4PL regression model. Data output is transcribed into a signal metric: Average Enzymes per Bead (AEB). The standard curve for this assay is 0.021–40 pg/mL.

Multiplex serum immunoassay

Feline Cytokine/Chemokine Magnetic Bead Panel Premixed 19 Plex, FCYTOMAG-20K-PMX from Millipore Sigma was used to assess the samples on a Luminex FLEXMAP 3D instrument. Two lyophilised quality controls were reconstituted just before use which included target analyte concentrations that varied between analytes. Standard curve and quality controls were assayed in serum matrix to account for matrix effects. Serum samples were thawed at room temperature (RT) just before assay, shaken, spun at 3,500 rpm for 5 min and then diluted in assay buffer 1:5. Standards, controls and samples were incubated overnight at 4°C with shaking. Plates were washed three times on an automated plate washer containing a magnetic separator. Biotinylated detection antibodies were added to standards, controls and samples, and incubated for 1 h at RT with shaking. Streptavidin-phycoerythrin was added to all standards, controls and samples, and plates were allowed to incubate for 30 min at RT with shaking. Plates were washed three times on an automated plate washer containing a magnetic separator. Beads were resuspended in sheath fluid and shaken for approximately 5 min at RT. Plates were then assayed on the Luminex FLEXMAP 3D instrument. Each bead region was identified by fluorescent dye emission of the magnetic bead and captured analyte was detected by fluorescence emission of streptavidin-labelled phycoerythrin bound to biotinylated detection antibodies. Standards, quality controls and samples were analysed in duplicate. Standard curves were regressed using a five parameter logistic curve with response as the weighting factor ($1/y^2$). All samples that were found above the limit of quantitation were repeated with higher dilution.