IFN-γ^+ TCR$\gamma\delta^+$ T cells. Importantly, intranasal application of an α-hemolysin–deficient strain, which presents a safer variant of *E coli* O83, had equally beneficial effects as the WT strain. This study contributes to further characterization of cellular and molecular pathways involved in regulation of AAI by probiotic bacteria.

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Supported by Austrian Science Fund (FWF) grants SFB 4612 (to U.W. and I.S.), SFB 4609 (to U.S. and C.K.), and Doctoral Program MCCA DK W1248-B13 (to C.Z., P.S., U.W. and I.S.) and DK W1248 (to U.S. and C.K.), and OeAD grants CZ 15/2015 (to C.Z., P.S., M.D., H.K., and I.S.) and CZ 13/2016 (to C.Z., P.S., M.D., M.K., and I.S.).

Disclosure of potential conflict of interest: M. S. Wilson is an employee of Genentech and owns Genentech stock. P. Sebo is a founder and shareholder of Revabiotech SE, which develops a next generation of whole cell pertussis vaccines. U. Wiedermann is member of the SAB and supporting CSO of the biotech company Imugene, Australia, developing B cell cancer vaccines and is also member of the Austrian Advisory Board of Immunizations of the government of Health in Austria. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online August 17, 2018.
https://doi.org/10.1016/j.jaci.2018.07.029

Fel d 1 and Fel d 4 levels in cat fur, saliva, and urine

To the Editor:

Cats are popular household pets and commonly cause allergies. Several cat allergens have been identified, including Fel d 1, an uteroglobin. More commonly, animal allergens are lipocalins, and the cat lipocalin allergen Fel d 4 has been identified.[1]

Recently, Fel d 1– and Fel d 4–specific IgE was assessed in patients with pet allergy.[2] Of those with cat allergy, 94% had increased levels (>0.35 kU/L) of Fel d 1, and 49% had increased levels of Fel d 4. Older studies cited by Zahradnik and Raulf[3] reported that Fel d 1 is produced in sebaceous and salivary glands and can be extracted from fur, skin, and saliva.[4] Intact male cats were found to have more fur Fel d 1 than female cats. There appears to be no information on age- or breed-related differences in Fel d 1 nor is there information about sites of Fel d 4 production. Therefore Fel d 1 and Fel d 4 levels in fur, urine, and saliva were evaluated and compared in a cohort of domestic cats to determine which phenotypes would distinguish cats with higher levels of either antigen.

This study was performed in collaboration with a local cat hospital and enrolled cats undergoing routine procedures requiring anesthesia. Canadian Council on Animal Care guidelines were followed. After obtaining the owners' consent, a mixed cohort of 26 male and female cats older than 5 months that were neutered and intact and of different breeds was recruited. Cats with dental (abscess or cavity) or skin diseases or other health problems were excluded.

Samples of 100 mg or more fur cut close to the skin by using clippers were collected from either front leg at the site of anesthesia administration. Before use of anticholinergic medication and after anesthesia, 0.25 to 0.50 mL of saliva was pipetted from the cheek pouches. Approximately 2.5 mL of urine was collected by means of catheterization performed by a veterinarian or delegate. For analysis, fur samples were placed in clean plastic bags, stored at 4°C, and shipped at room temperature. Saliva and urine were placed in sterile cryovials, stored at −20°C, and shipped in dry ice. Fel d 1 and Fel d 4 were eluted, 3 aliquots from each sample were analyzed by means of ELISA, and the average was reported (Indoor Biotechnologies, Charlottesville, Va). Medians and ranges were calculated. Comparisons were made with a nonparametric test (Mann-Whitney). There was no attempt to account for multiple comparisons.

The 26 cats studied came to the clinic for procedures that required anesthesia, mainly dentistry and spaying (see Table E1 in this article's Online Repository at www.jacionline.org). They were 5 months to 12 years old, half were female, about half were of the domestic short-hair variety, and the majority (65%) had been neutered. Fur was collected from 26 cats, urine was collected from 20 cats, and saliva was collected was from 17 cats. No adverse events were recorded from sample collection. Fel d 1 and Fel d 4 levels in fur, urine, and saliva are shown in Fig 1.

In fur, Fel d 1 levels (median, 12.24 μg/g; range, 5.49-20.72 μg/g) were significantly greater than Fel d 4 levels (median, 0.09 μg/g; range, 0.03-0.19 μg/g; $P < .001$). Conversely, Fel d 4 levels were greater than Fel d 1 levels in saliva (median of 7.62 μg/mL [range, 1.41-17.42 μg/mL] vs 2.45 μg/mL [0.87-5.11 μg/mL], respectively; $P = .039$). In urine, Fel d 1 (median,

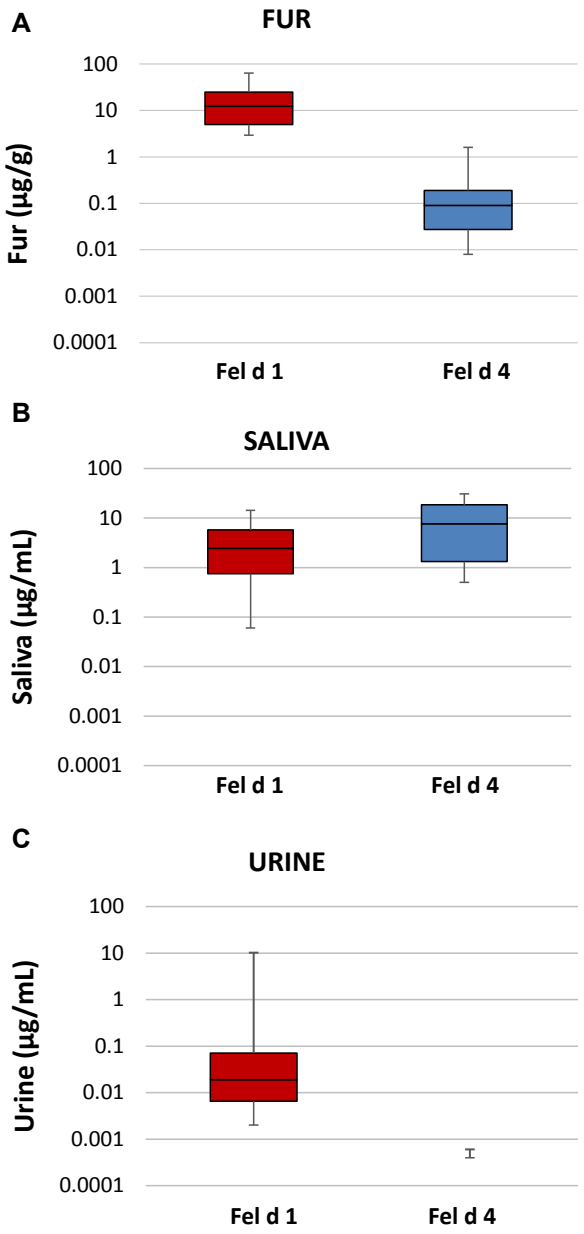

FIG 1. Box and whisker plots of Fel d 1 and Fel d 4 levels by anatomic location. **A,** Fur (n = 26). **B,** Urine (n = 20). **C,** Saliva (n = 17). *Box*, Twenty-fifth and 75th percentiles; *bars*, minimum and maximum values; *solid line*, median values. Fel d 4 levels were less than Fel d 1 levels in fur and greater in saliva. All but 2 urine Fel d 4 samples had levels of less than the limit of detection, which was used when calculating the median. The limits of detection for Fel d 1 are 0.08 µg/g for fur and 0.002 µg/mL for saliva and urine, and there is a less than 0.01% cross-reactivity with natural Can f 1 allergen. Fel d 4 limits are 0.008 µg/g for fur and 0.0004 µg/mL for saliva and urine and less than 0.01% cross-reactivity with natural Fel d 1 allergen.

0.02 µg/mL; range, 0.065-0.071 µg/mL) and Fel d 4 levels (<0.4 µg/mL, the limit of detection) were low.

The effect of sex and neutering on Fel d 1 and Fel d 4 levels is shown in Table I. In fur both antigens were unrelated to sex and neutering. In saliva there was a potential reciprocal relationship between Fel d 1 and Fel d 4 in neutered and nonneutered animals; however, the numbers were small, and only Fel d 4 levels in neutered female animals were significant ($P = .039$).

TABLE I. Fel d 1 and Fel d 4 levels in fur and saliva according to sex and neutering

	Fel d 1, median (25th-75th percentile)	Fel d 4, median (25th-75th percentile)
Fur (µg/g)		
All female cats (n = 13)	10.08 (4.3-16.13)	0.12 (0.04-0.21)
Neutered female cats (n = 7)	10.1 (4.0-14.03)	0.05 (0.03-0.15)
Nonneutered female cats (n = 6)	10.1 (6.8-34.7)	0.22 (0.21-0.42)
All male cats (n = 13)	12.72 (8.7-37.35)	0.08 (0.02-0.12)
Neutered male cats (n = 10)	10.89 (6.95-33.11)	0.09 (0.06-0.12)
Nonneutered male cats (n = 3)	14.5 (13.6-37.97)	0.02 (0.01-0.12)
Saliva (µg/mL)		
All female cats (n = 10)	3.3 (0.83-6.86)	9.71 (3.32-16.95)
Neutered female cats (n = 5)	0.59 (0.22-2.22)	17.42 (11.79-19.56)*
Nonneutered female cats (n = 5)	5.01 (3.67-7.93)	2.2 (0.79-6.71)*
All male cats (n = 7)	3.30 (1.46-4.84)	6.71 (1.32-19.71)
Neutered male cats (n = 5)	3.3 (1.46-4.84)	1.41 (1.22-12.04)
Nonneutered male cats (n = 2)	0.76 (0.41-1.10)	17.05 (11.88-22.21)

In fur Fel d 1 levels exceeded Fel d 4 levels, and there were no differences based on sex or neutering. In saliva Fel d 4 levels exceeded Fel d 1 levels, and there appeared to be a reciprocal relation between Fel d 1 and Fel d 4 levels based on neutering, which was significant only for neutered versus nonneutered female cats.
*$P = .039$.

There were no age-related differences in either Fel d 1 or Fel d 4 levels in fur or saliva (see Fig E1 in this article's Online Repository at www.jacionline.org), even though the youngest cats were not neutered.

Hair length was used as a surrogate for breed in this study, and although fur Fel d 1 levels were greatest in short-hair cats (15.32 vs 9.22 µg/g and 5.2 µg/g for long- and medium-hair cats, respectively), statistical significance was not reached (see Table E2 in this article's Online Repository at www.jacionline.org).

This study confirms earlier findings that Fel d 1 is present in cat fur and saliva and extends this observation to Fel d 4. Furthermore, the data suggest that saliva is the main source of Fel d 4. Given the low fur levels of Fel d 4, it is likely deposited during grooming rather than from sebaceous secretions, as with Fel d 1.

Urine is not a significant source of Fel d 1 (0.02 µg/mL). However, levels were greater in the 3 intact male cats (median of 5.15 µg/mL [range, 4.12-7.69 µg/mL] vs 0.013 [range, 0.007-0.026 µg/mL], $P = .012$), making it possible for litter boxes to be a source of this allergen in the home.

Lipocalins represent the largest group of mammalian inhalant allergens.[5] The lipocalin superfamily includes allergens, such as Fel d 4, the major dog allergen Can f 1, and the mouse major urinary protein Mus m 1. These and others have been identified in salivary glands or saliva[6-8] and urine[3,7] of several species. Our observation that Fel d 4 is present in cat saliva is consistent with these observations.

Salivary Fel d 4 levels appeared to be greater in neutered than intact female cats and in intact compared with neutered male cats (Table I); only the former was statistically significant. Expression of another salivary lipocalin, male-specific submandibular gland protein, can be hormonally controlled and is increased in neutered female hamsters.[8] This might also be true for cat salivary Fel d 4.

Sex-related differences in fur Fel d 1 levels were not seen (Table I), likely because 10 of 13 male cats were castrated. The 3 intact male cats at 5, 6, and 12 months of age were sexually mature.

However, only the 6-month-old had increased fur Fel d 1 levels (61.44 μg/g). Therefore the data do not confirm or refute previous reports that intact male cats have greater fur Fel d 1 levels.

It is a common belief that certain cat breeds are hypoallergenic. Hair length as a surrogate for breed did not explain differences in Fel d 1 levels. Siebers et al[9] also found that sex, fur color, and fur length were not related to Fel d 1 levels in reservoir dust from homes. Although 23% of cats had fur Fel d 1 levels of greater than 30 μg/g in this study, it is possible that other factors, such as day-to-day variation or lack of grooming, might have led to these higher levels.

In conclusion, we observed cat-to-cat variability of Fel d 1 levels in fur of domestic cats unrelated to breed, sex, or age. This is contrary to other observations for Fel d 1.[3] The data suggest that hormonal status can affect salivary Fel d 4 levels in female cats and urinary Fel d 1 levels in male cats. These findings deserve further investigation. In addition, it would be of interest to compare levels of Fel d 4 and Fel d 1 in homes with cats. Given the low levels of Fel d 4 in cat fur, it is likely that levels in houses will also be low but might be still be sufficient to induce levels of specific IgE in patients with cat allergy.

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This research was funded by Red Maple Trials.

Disclosure of potential conflict of interest: S.M.K., J.K., B.S., J.Y., and W.H.Y. are principals of Red Maple Trials. J.M. and N.S. are employees of Red Maple Trials. D.B. is the veterinarian-owner of the Merivale Cat Hospital; he was reimbursed for his work in the study.

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Available online August 31, 2018.
https://doi.org/10.1016/j.jaci.2018.07.033

Personalized omalizumab treatment improves clinical benefit in patients with chronic spontaneous urticaria

To the Editor:

Omalizumab is a humanized anti-IgE mAb that was found to be highly efficacious in several randomized clinical trials, which led to licensing for chronic spontaneous urticaria (CSU) by the US Food and Drug Administration in 2014.[1-3] Omalizumab targets free IgE at the site of the Fc region of IgE (FcεRI), which prevents free IgE from binding to the high-affinity receptor FcεRI on mast cells and basophils. Possible mechanisms of action in patients with CSU include neutralization of IgE autoantibodies in so-called "autoallergic" patients and the gradual downregulation of the FcεRI receptor in so-called "autoimmune" patients who have IgG antibodies directed against the high-affinity receptor FcεRI.[4,5]

A major limitation of treatment with omalizumab in patients with CSU concerns the per-label fixed dosing schedule, without any options to adapt or tailor the therapy to the needs of the individual patient. Moreover, treatment duration in the registration studies was limited to a maximum of 6 months, and the next steps (ie, dose maintenance, downdosing, or discontinuation) remain unclear and are not discussed in international guidelines.

The objective of this study was to retrospectively evaluate the effectiveness of omalizumab in patients who were treated according to a predefined algorithm. In this personalized approach, we explored (1) the potential of updosing in patients who did not achieve complete remission (CR) with the standard treatment regimen, (2) a gradual downdosing schedule for patients who achieved CR, and (3) relapse rates after gradual downdosing. Patients initially received 300 mg of omalizumab every 4 weeks for 12 weeks per label. Four weeks after the third dose, the effect was evaluated with the validated Urticaria Activity Score (UAS-7) questionnaire. In case of CR (UAS-7 = 0), a downdosing schedule was initiated that entailed progressively increasing the dosing interval by 1 week every visit up to 8 weeks, as long as the patient remained in CR. Treatment was then discontinued if the patient remained in CR after these 4 additional administrations.

Patients were advised to continue treatment with updosed H1 antihistamines for at least 12 weeks after stopping omalizumab. If symptoms reoccurred before the next scheduled administration, the dose interval was shortened so that the length of the next dosing interval equaled the previous symptom-free period. The dose interval of patients who relapsed during the first downdosing attempt was extended again as soon as they stayed symptom free for 4 weeks.

Patients with a partial response (PR; minimum decrease of 9.5 points) initially continued with the same dose of 300 mg every 4 weeks for 1 to 3 doses depending on the severity of symptoms. For those with CR, after this extended period of per-label treatment, tapering was started in the same way as for patients who achieved CR after 3 doses. For patients with a persistent PR, either the dose was increased to 450 mg every 4 weeks or the interval was decreased to 3 weeks if symptoms worsened during the fourth week. Doses were increased up to 600 mg every 3 to 4 weeks when needed to reach CR. When CR was reached, first the dose was downtitrated stepwise to the per-label dose of 300 mg every 4 weeks, if possible, before starting the tapering schedule.

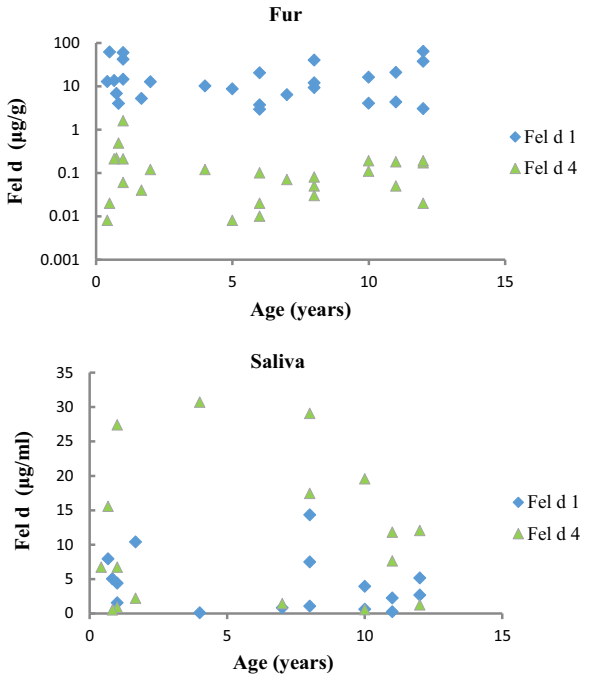

FIG E1. Fel d 1 and Fel d 4 levels versus age in cat fur and saliva. Neither antigen varies with age. In fur Fel d 4 levels are less than Fel d 1 levels, and there is no overlap. In saliva there is considerable overlap, and the greater median values for Fel d 4 are due to a subset of cats with high levels.

TABLE E1. Cat demographic data

Sex	
Male	13
Female	13
Age (y)	
Mean (SD)	5.6 (4.3)
Minimum	0.42
Maximum	12
Weight (kg)	
Mean (SD)	4.54 (1.46)
Minimum	2.47
Maximum	7.53
Breed (no.)	
Domestic short hair	14
Domestic medium hair	3
Domestic long hair	9
Neutered (no.)	
Yes	17
No	9
Procedures (no.)	
Dental cleaning	10
Spaying	9
Tests*	5
Ear cyst removal	1
Grooming	1

Twenty-six cats were enrolled in the study. The cats were mostly adults and neutered. They were anesthetized for the procedures, which were predominantly spaying and dental cleaning.

*Tests include biopsy, blood draw, and imaging.

TABLE E2. Fur Fel d 1 and Fel d 4 levels show no relation to hair length on a per-gram basis

	Fel d 1 (μg/g)	Fel d 4 (μg/g)
Domestic short hair (n = 14)	15.32 (8.12-46.21)	0.12 (0.0225-0.19)
Domestic medium hair (n = 3)	5.2 (4.04-39.80)	0.05 (0.045-0.08)
Domestic long hair (n = 9)	9.22 (4.0-13.02)	0.1 (0.05-0.12)