



For reprint orders, please contact: reprints@futuremedicine.com

Successful immunotherapy of canine flea allergy with injected Actinomycetales preparations

Aims: Can heat-killed, borate-buffered suspensions of *Gordonia bronchialis*, *Rhodococcus coprophilus* or *Tsukamurella inchoensis* be used to treat canine flea allergy? **Materials & methods:** Organisms cultured on Sauton's medium into stationary phase were autoclaved in borate-buffered saline and stored at 10 mg wet weight/ml. Intradermal injections of 0.1 ml containing 1 mg of bacilli were administered on the first and 20th days of the study. *G. bronchialis* and *R. coprophilus* were most effective in a pilot study of a small number of dogs with flea allergy. A larger number of affected dogs were then randomized to receive placebo or either of the two selected reagents. The extent and severity of allergic signs and symptoms were scored and blood samples were collected just before the first injection and 28 days after the second. **Results:** Both selected reagents reduced the extent and severity of lesions ($p < 0.001$) and reduced scratching. Eosinophil numbers were reduced ($p < 0.0001$) between the first and second assessment. **Conclusions:** Injections of *G. bronchialis* or *R. coprophilus* effectively reduce the signs and symptoms of flea allergy in dogs.

KEYWORDS: actinomycetes • clinical improvement • flea allergy • pruritus

Flea allergy dermatitis in dogs is a disorder occurring in animals sensitized to substances present in flea saliva. It is the most common hypersensitivity skin disorder of dogs in parts of the world where fleas are common [1]. The disease appears, especially during spring and summer, in dogs from 6 months of age and there does not seem to be a gender preference.

Fleas of several species are associated with the disorder (*Ctenocephalides felis*, *Ctenocephalides canis*, *Pulex simulans* and *Echidnophaga gallinacea*, the poultry sticktight flea), the commonest being *C. felis* [1,2]. The major allergen is known and is among the antigenic substances in flea saliva [2].

Flea-bites produce a papule that lasts for 72 h and later acquires a crusted appearance. The skin lesions are typically, but not always restricted to the lumbar region and the caudal and medial part of the legs, abdomen and flanks. Pruritus is the principal clinical symptom. In extremely hypersensitive dogs, extensive areas of alopecia, erythema, and self-trauma develop. Traumatic moist dermatitis occurring at sites easily scratched by the dog can also occur. As the disease becomes chronic, the dog may develop generalized alopecia, severe seborrhea, lichenification and hyperpigmentation [1].

Current treatment is based on the exclusion of fleas via treatment with ectoparasiticides such as fipronil and imidacloprid, prevention

of the development of young fleas by lufenuron or suppression of the clinical signs by anti-inflammatory corticosteroids. Better than any of these treatments would be correction of the hyper-reactivity, which could prevent the allergic dermatitis from occurring, especially in its delayed and chronic form.

Flea allergy dermatitis comprises features of both immediate, IgE-mediated, cutaneous basophil hypersensitivity and delayed, cell-mediated hypersensitivity [3]. Allergic dogs have a higher number of tissue mast cells before exposure to flea allergens compared with nonallergic dogs, and the number of IgE-positive cells is higher in allergic dogs both before and after flea exposure [4]. Raised levels of the cytokines IL-4, IL-5, IL-13, TNF- α and IFN- γ are found after exposure to flea allergens when the mRNA from biopsies and polymorphonuclear (PMN) cells of allergic and nonallergic dogs were compared [4], which is consistent with an excessive Th2 response. Early exposure to fleas and constant prolonged exposure to fleas can both reduce the likelihood of flea-allergy and it is suggested that an immunological approach in which tolerance is induced, or the nature of the immune response changed, would be helpful [3].

Studies have shown that bacterial immunomodulators based on aerobic Actinomycetales such as *Mycobacterium vaccae* can switch off pre-existing Th2 preponderance and promote

Alicia Marro¹,
Mónica Pirles¹,
Laura Schiaffino¹,
Liliana Bin¹,
Héctor Dávila²,
Oscar A Bottasso²,
Graham McIntyre³,
Paul R Ripley³,
Cynthia A Stanford^{3,4}
& John L Stanford^{1,3,4}

¹School of Veterinary Sciences, Universidad Nacional de Rosario, Casilda, Argentina

²Instituto de Inmunología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Argentina

³BioEos Ltd, Kent, UK

⁴Centre for Infectious Diseases & International Health, Windeyer Institute of Medical Sciences, University College London, UK

*Author for correspondence: j.stanford@ucl.ac.uk

future
medicine part of fsg

Th1-mediated mechanisms, whatever the previous environmental immune priming of the individual [5]. Within this context, it has been reported that heat-killed *M. vaccae* exerted beneficial effects in mild/moderate atopic dermatitis in dogs [6].

Recent studies have identified a number of bacterial species closely related to mycobacteria, which are capable of exerting subtly different adjuvant or immunomodulatory activities when injected as suspensions of killed bacilli.

In order to establish whether the immune response of symptomatic dogs can be modulated to one in which excessive allergic responses could be eliminated, we have investigated preparations of species of three near-mycobacterial genera for their potential to alleviate the clinical signs of flea allergy dermatitis. An initial pilot study in dogs with flea allergy compared heat-killed preparations of the bacterial species *Gordonia bronchialis*, *Rhodococcus coprophilus* and *Tsukamurella inchonensis*. The first two preparations were found to be most effective and these were used in a larger, placebo-controlled study to treat dogs from individual households referred to the small animal clinic of the Veterinary School, Casilda, near Rosario, Argentina.

Materials & methods

■ Animals

Dogs, healthy apart from their skin problems, were examined by a clinic veterinarian. Those with a strict diagnosis of flea allergy dermatitis based on the presence of pruritus, erythema, papules or papular crusts, showing a typical distribution pattern, and evidence of fleas and/or their excreta, were recruited for the studies. On the basis of the initial clinical examination by experienced small animal dermatologists together with skin scrapings or smears, dogs showing signs of classical atopic dermatitis, active cutaneous bacterial or fungal infection and the presence of other ectoparasites were excluded from the trial. Other exclusion criteria included dogs treated with immunosuppressants, corticosteroids or other anti-inflammatory treatments over the preceding few weeks.

No campaign was needed to find the necessary numbers of animals since this is a common condition. Dogs entering the pilot study were mainly 'outdoor' dogs, recruited from a refuge or from private owners, whereas all those recruited into the main study were either 'indoor' or 'outdoor' dogs brought to the clinic by private owners. Owners and care providers were fully informed about the studies, although not of which reagent

their dog would receive, and agreed not to give any other treatments during the course of the investigations. The studies received ethical approval from two independent reviewers.

■ Reagents

The preparations tested were heat-killed cultures of the bacterial species *G. bronchialis* (BE-G101), *R. coprophilus* (BE-R102) and *T. inchonensis* (BE-T101) provided by BioEos Ltd, UK. The organisms were grown on Sauton's liquid medium, which is free from other antigens and compliant with all relevant transmissible spongiform encephalitis regulations. Culture was at 32°C and continued until the end of the logarithmic phase. Harvested bacterial cell mass was suspended in borate buffered physiological saline at pH 8 and autoclaved at 15 lbs/square inch at 121°C for 15 min. The suspensions were diluted to 10¹⁰ bacteria/ml with further borate buffer, and doses of 0.1 ml (containing 10⁹ bacilli, equivalent to 1 mg wet weight) were given by intradermal injection into the dorsum of the neck. In the main study, where a placebo was used, this was of borate-buffered saline.

■ The studies

For the pilot study, 20 dogs with flea allergy were recruited and subsequently 54 dogs were recruited for the main study. Treatments were allocated according to computer-generated random lists of blocks of four. Each animal received two treatments, the first just after the initial clinical assessment and the second 20 days later. In the pilot study seven dogs received *G. bronchialis*, eight received *R. coprophilus* and five received *T. inchonensis*. The first two of these were found the most effective and were investigated in the main study, together with a placebo.

In the main study, 20 dogs received *G. bronchialis*, 17 received *R. coprophilus* and 17 received placebo. As requested by the independent reviewers, in the main study each dog was treated with a single dose of fipronil (Frontline®, Merial, Dardo Rocha, Argentina) given by 'spot-on' application, to eliminate fleas, and a single subcutaneous injection of prednisolone (John Martin Veterinary Laboratory, Argentina), 1 mg/kg bodyweight, at the time of administering the first dose of test reagent, just after the first clinical assessment.

Any other dog at the home of a main study animal was given a dose of fipronil by the spot-on method and the floors, carpets, cushions and other areas frequented by the dog(s) were treated with a pyrethroid spray such as K-Othrine (25% deltamethrin).

At the times of the first injection and 28 days after the second injection, severity and distribution of lesions and the intensity of scratching were recorded. Assessment of the severity of clinical signs was made in two ways.

A simple scheme based on visual observation was recorded on a body chart before the first test injection and 28 days after the second injection had been given. Right and left areas were treated as separate sites, dorsal and ventral were treated as separate zones and scores were assigned to each lesion site, wherever they might occur, according to the type and severity of lesions:

- Papules were accorded as 1, 2 or 3 points;
- Papules with crusts were accorded as 2, 4 or 6 points;
- Crusts alone were accorded as 3, 6 or 9 points.

Scores were summed; for example in a dog with two abdominal affected sites (papules with crusts of 4 and 6 severity) and another lesion on the back (crusts alone of 6 severity) the score was 16.

Pruritus was classified into grades none (0), mild (1), moderate (2) or severe (3), according to the intensity of scratching. The scheme for assessment of pruritus was agreed between the owners and the investigators. Owners were instructed by the clinicians on how to grade pruritus according to the amount of scratching and comparisons between owners and clinicians assessments found that they did not differ.

In the main study, the severity of the clinical signs of flea allergy dermatitis were additionally assessed using the scoring system taken from the original Canine Atopic Dermatitis Extent and Severity Index (CADESI, version 3) [7]. CADESI consists of an assessment of the severity: none (0), mild (1), moderate (2–3) and severe (4–5), of the four principal signs: erythema, excoriation, lichenification and self-induced alopecia at each of 62 sites over the whole body surface. Pruritus was evaluated by both owners and veterinarians. To reduce bias in the main study, evaluations were made by members of the department who were unaware of the study groups in addition to those made by the study veterinarians. There was a close agreement between them as confirmed by the Spearman correlation. The data recorded and described was that of the study veterinarians.

In the main study, venous blood samples were collected immediately before the first treatment dose and 28 days after the second

treatment dose. Total white blood cell counts and the numbers of leukocytes, eosinophils, polymorphonuclear granulocytes, monocytes and lymphocytes were measured in both samples using automated methods, by a technician blinded to the study.

■ Statistical analysis

In the pilot study, an improvement of 60–70% in lesion scores with respect to the baseline score was obtained. According to this, a minimum number of 12 subjects per treatment group was considered necessary in the main study in order to obtain statistically significant differences with a confidence of 95%. Statistical comparisons were performed by the Kruskal–Wallis (using Dunn's post-test) and Mann–Whitney U tests. Related samples were analyzed by the Wilcoxon and McNemar tests. A p-value of <0.05 was considered significant.

Results

The results of the pilot study are shown in TABLES 1 & 2. The mean age of the *T. inchoensis* recipients was greater than that of either of the other groups, and, unlike the other groups, they included a preponderance of males. The groups undergoing treatment with *G. bronchialis* or *R. coprophilus* had greater initial lesion scores in comparison with the *T. inchoensis* recipients ($p < 0.01$). On subsequent assessment of the extent and severity of skin lesions 28 days after the second injection, the groups receiving *G. bronchialis* or *R. coprophilus* showed a significant reduction in skin lesion scores with respect to the initial evaluation. This was not the case for *T. inchoensis* recipient dogs whose lesion scores remained substantially unchanged. Pruritus scores improved between the first and second evaluations in all but one animal among the *G. bronchialis* and *R. coprophilus* recipients, whereas four out of five recipients of *T. inchoensis* failed to show improvement.

In the main study, there were no significant differences between groups with respect to size of dog, gender, age, extent and severity of lesions, or in numbers of cohabitant pets and indoor/outdoor maintenance. TABLE 3 gives the characteristics of the experimental groups, and the pruritus and lesional scores as given by the simpler scoring system. Five placebo-recipient dogs were lost to follow-up at the second evaluation. TABLE 4 records the intensity of pruritus as measured at the beginning of the study and 28 days following the second injection. Lesion

Table 1. Characteristics of experimental groups in the pilot study together with pruritus and lesion scores assessed by the simpler system.

Characteristic	Experimental group		
	<i>G. bronchialis</i>	<i>R. coprophilus</i>	<i>T. inchonensis</i>
Mean age in years ($\pm$ SD)	4.2 (2.6)	2.6 (1.1)	6.4 (5.5)
Gender	5 female and 2 male	5 female and 3 male	1 female and 4 male
Pruritus score			
First evaluation	2.29 (0.49)	2.5 (0.75)	2.8 (0.45)
Second evaluation	1.14 (0.69) [†]	1.0 (0.53) [†]	2.6 (0.55)
Lesion score			
First evaluation	21.5 (9.36)	13.2 (5.7)	5.2 (1.1)
Second evaluation	8.57 (7.45) [‡]	3.62 (5.04) [§]	5.2 (1.1)

[†]Significantly different within-group comparison from the first evaluation ($p < 0.05$, McNemar test for related categorical variables).

[‡]Significantly different within-group comparison from the first evaluation ($p < 0.02$, Wilcoxon signed rank test).

[§]Significantly different within-group comparison from the first evaluation ($p < 0.01$, Wilcoxon signed rank test).

G. bronchialis: *Gordonia bronchialis*; R. coprophilus: *Rhodococcus coprophilus*; SD: Standard deviation; T. inchonensis: *Tsukamurella inchonensis*.

scores and pruritus were reduced in all groups, presumably as a result of the initial fipronil and steroid administered to each animal on the first day of the study. However, pruritus reduced to a much greater extent in both *G. bronchialis* and *R. coprophilus* groups compared with the placebo group. A total of 14 out of 20, and 15 out of 17 dogs, respectively, in these groups had zero pruritus scores at the second assessment, compared with none out of 12 in the placebo group.

Lesion scores, as assessed by the CADESI score system, are summarized in TABLE 5. Highly significant reductions in lesion scores occurred in both test treatment groups whereas in the placebo group the reduction was marginal and not statistically significant.

TABLE 6 gives the results for the total white blood cell counts and differential blood counts. At the first evaluation, all animals showed a marked eosinophilia, by chance least in the placebo group. By the second evaluation, both

test treatment groups had highly significant reductions in eosinophils ($p < 0.0001$), whereas the placebo group remained unchanged. Both lymphocyte and total PMN counts increased significantly in the test treatment groups (*G. bronchialis*: lymphocytes $p < 0.0065$, PMN $p < 0.0001$; *R. coprophilus*: lymphocytes $p < 0.025$, PMN $p < 0.008$). These changes were not seen in the placebo-recipient animals.

An assessment of the safety of the treatments was performed by an assessor, external to the study, who examined the local reactions and spoke with the owners. Locally and generally, injections of the actinomycete preparations and placebo were well tolerated, and there were no signs of pain or discomfort associated with the injections. A nodular swelling a few mm in diameter, localized at the sites of the actinomycete injections had disappeared by the time of the second evaluation. Otherwise, there was no difference between the treatments and no systemic adverse events were observed.

Table 2. Graded pruritus scores from the pilot study.

Pruritus score	Experimental group					
	<i>G. bronchialis</i>		<i>R. coprophilus</i>		<i>T. inchonensis</i>	
	First evaluation	Second evaluation	First evaluation	Second evaluation	First evaluation	Second evaluation
0	0	1	0	1	0	0
1	0	4	1	6	0	0
2	5	2	2	1	1	2
3	2	0	5	0	4	3
Total	7	7	8	8	5	5

Within-group comparisons (McNemar test): scores significantly improved between the first and second evaluations in the *G. bronchialis* and *R. coprophilus* groups ($p < 0.05$). Changes in the *T. inchonensis* group were minimal. Comparisons between groups could not be made because of the low numbers.

G. bronchialis: *Gordonia bronchialis*; R. coprophilus: *Rhodococcus coprophilus*; T. inchonensis: *Tsukamurella inchonensis*.

Table 3. Characteristics of experimental groups in the main study together with pruritus and lesion scores assessed by the simpler system.

Characteristic	Experimental group		
	<i>G. bronchialis</i>	<i>R. coprophilus</i>	Placebo
Mean age years ($\pm$ SD)	4.7 (2.9)	5 (3.3)	4.4 (2.5)
Gender	10 female and 10 male	8 female and 9 male	7 female and 5 male
Pruritus score			
First evaluation	2.3 (0.47)	2.6 (0.49)	2.5 (0.52)
Second evaluation	0.35 (0.59)	0.12 (0.33)	1.83 (0.39)
Lesion score			
First evaluation	7.14 (2.7)	6.92 (2.61)	6.82 (3.64)
Second evaluation	2.8 (2.21)*	2.3 (2.3)*	7.4 (2.99)*

*Significantly different from the first evaluation (within-group comparisons [Wilcoxon signed rank test]; $p < 0.001$).

*Significantly different from the *G. bronchialis* and *R. coprophilus* groups (between-group comparisons [Kruskal–Wallis analysis of variance]; $p < 0.001$).

G. bronchialis: *Gordonia bronchialis*; *R. coprophilus*: *Rhodococcus coprophilus*; SD: Standard deviation.

Discussion

The pilot study, carried out to select the most effective interventions, and the main study of the two selected preparations showed that the treatments resulted in significant reduction in the allergic response, indicated both by a reduction in lesion severity and a reduction in scratching as a measure of reduced pruritus. Although there were some methodological differences between the two studies, both produced substantially the same result.

In the main study, a reduction in lesion scores of over 80% was achieved, with comparable improvement in pruritus in dogs receiving either *G. bronchialis* or *R. coprophilus*. Five placebo-group dogs were lost to follow-up and it may be speculated that the poor response to ‘treatment’ perceived by the owners of placebo dogs may have been a factor, in which case lesions and pruritus scores in this group may have been even poorer. Alternatively, it is possible,

although unlikely, that the five dogs improved spontaneously in response to the action taken by owners to reduce the flea challenge without other treatment and for this reason the dogs were not returned to the clinic.

All animals in the main study had received treatment with an injection of corticosteroid at the start of the study, but lesion and pruritus scores were highly significantly lower than those of the placebo controls at the clinical evaluation 28 days after the second administration of the test preparations. At this time, placebo scores were little different from scores at the start of the study. Since both test and placebo dogs received these same treatments, this should not influence the observed differences between the treatment groups. These results compare favorably with those obtained in a study of cyclosporine A or methylprednisolone given by mouth daily for up to 12 weeks, in which mean reductions in CADESI scores of 45–62% were reported [8].

Table 4. Graded pruritus scores from the main study.

Pruritus score	Experimental group					
	<i>G. bronchialis</i>		<i>R. coprophilus</i>		Placebo	
	First evaluation	Second evaluation	First evaluation	Second evaluation	First evaluation	Second evaluation
0	0	14	0	15	0	0
1	0	5	0	2	0	2
2	14	1	6	0	6	10
3	6	0	11	0	6	0
Total	20	20	17	17	12	12

Within-group comparisons (Wilcoxon signed rank test): scores were significantly improved in all groups at the second evaluation. *G. bronchialis*: $p < 0.0001$; *R. coprophilus*: $p < 0.0005$; placebo: $p < 0.01$.

Comparisons between groups (Kruskal–Wallis analysis of variance): there were no significant differences between groups at the first evaluation. After treatment, *G. bronchialis* and *R. coprophilus* groups differed from the placebo group ($p < 0.001$).

G. bronchialis: *Gordonia bronchialis*; *R. coprophilus*: *Rhodococcus coprophilus*.

Table 5. Canine Atopic Dermatitis Extent and Severity Index lesional scores in the main study.

Lesion score	Experimental group					
	<i>G. bronchialis</i>		<i>R. coprophilus</i>		Placebo	
	First evaluation	Second evaluation	First evaluation	Second evaluation	First evaluation	Second evaluation
Mean score (SD)	22.45 (11.0)	3.25 (4.25)	25.5 (30.0)	1.29 (2.9)	19.7 (5.1)	18.2 (9.8)
Number	20	20	17	17	12	12

Within-group comparisons (Wilcoxon signed rank test): difference in scores between the first and second evaluation. *G. bronchialis*: $p < 0.001$; *R. coprophilus*: $p < 0.001$; placebo: not significant. Comparisons between groups (Kruskal–Wallis analysis of variance): there were no significant differences between groups at the first evaluation. At the second evaluation the *G. bronchialis* and *R. coprophilus* groups differed from the placebo group ($p < 0.001$). *G. bronchialis*: *Gordonia bronchialis*; *R. coprophilus*: *Rhodococcus coprophilus*; SD: Standard deviation.

The simple hematological studies recorded in TABLE 6 show the changes that would be expected in animals in which allergic symptoms have been alleviated. In both groups receiving the active treatments, there are similar and significant falls in eosinophil counts with small increases in lymphocytes and PMNs. Such changes did not take place in dogs of the placebo group.

The effectiveness of preparations of heat-killed Actinomycetales in the control of flea allergy seems surprising at first sight. However, organisms of this order of bacteria contain highly active immunomodulatory adjuvants in their cell walls, with subtle differences between species [9]. To focus the immunomodulatory effect on the sites of allergic responses, the bacterial preparations also contain short chains of amino acids from their heat-shock proteins, which are cross-reactive with similar amino acid chains in stress proteins of animal mitochondrial origin [10]. These proteins are expressed on cells stressed by the allergy. It is likely that the efficacy of immunotherapy in the dog flea model, similar to that of *M. vaccae* on bronchial

asthma in man [11], is to regulate the delayed tissue response and not the process of histamine release immediately following contact with the allergen, in this case, flea saliva.

The allergic process in response to flea bites is a complicated one. First, there is the direct effect of the flea saliva triggering local irritation with histamine release and a small area of erythema occurring in all bitten animals. In the allergic animal, there is a raised level of IgE that binds to eosinophils and mast cells migrating to the site of the flea bite, resulting in a further aggregation of eosinophils, degranulation of which results in increased local inflammation and in further damage to cells, inducing their expression of stress proteins. In the more severe cases, the accumulation of inflammatory cells and fluid exudate results in induration within a few hours, with increased itching and often tenderness and local pain. The expression of stress proteins on the surface of injured cells is thought to result in an autoimmune type of response around cells killed by the action of antibody and complement, with accumulation

Table 6. Total white blood cell count and differential giving percentages of different cell types ($\pm$ standard deviations) from the main study.

Cell type	Experimental group					
	<i>G. bronchialis</i>		<i>R. coprophilus</i>		Placebo	
	First evaluation	Second evaluation	First evaluation	Second evaluation	First evaluation	Second evaluation
Eosinophils	16.5 $\pm$ 6.83	5.6 $\pm$ 2.68	12.5 $\pm$ 5.23	4.81 $\pm$ 2.23	8.17 $\pm$ 3.43	8.17 $\pm$ 5.08
Lymphocytes	24.7 $\pm$ 5.96	28.5 $\pm$ 4.9	22.19 $\pm$ 8.44	25.06 $\pm$ 6.81	25.08 $\pm$ 5.7	26.17 $\pm$ 6.18
Polymorphs	54.35 $\pm$ 8.06	61.55 $\pm$ 5.1	61.5 $\pm$ 7.48	66.56 $\pm$ 6.04	62.33 $\pm$ 8.13	61.75 $\pm$ 8.09
Monocytes	4.2 $\pm$ 0.38	4.05 $\pm$ 0.35	3.86 $\pm$ 0.52	3.65 $\pm$ 0.73	4.13 $\pm$ 0.81	3.33 $\pm$ 0.42
Total WBCs	10,225 $\pm$ 2622	10,447 $\pm$ 4403	12,036 $\pm$ 6217	11,650 $\pm$ 5174	10,918 $\pm$ 3792	11,919 $\pm$ 3675

Within-group comparisons (Wilcoxon signed rank test) between the first and second evaluation: *G. bronchialis*: eosinophils $p < 0.0001$, lymphocytes $p < 0.0065$, polymorphs $p < 0.0001$; *R. coprophilus*: eosinophils $p < 0.0001$, lymphocytes $p < 0.025$, polymorphs $p < 0.008$. Comparisons between groups (Kruskal–Wallis analysis of variance): at the first evaluation, the placebo group differed significantly in the percentage of eosinophils from the other groups ($p < 0.005$) and the *G. bronchialis* group had a significantly lower percentage of polymorphs than did the other groups ($p < 0.02$). At the second evaluation, the *G. bronchialis* and *R. coprophilus* groups had significantly fewer eosinophils than did the placebo group ($p < 0.0001$ and $p < 0.0015$, respectively). *G. bronchialis*: *Gordonia bronchialis*; *R. coprophilus*: *Rhodococcus coprophilus*; WBC: White blood cell.

of further cells and exudate several hours after the initiating flea-bite. Both the initial allergic and secondary autoimmune responses found in animals, in which the allergic response has become chronic, are thought to result from local release of types 1 and 2 cytokines, which together cause local cell damage and necrosis [12].

Downregulation of Th2 cells, probably associated with Tregs [13], reduces levels of type 2 cytokines so that an enhanced Th1 response is much less tissue damaging [12]. Thus the test treatments should relieve the acute allergic and inflammatory aspects of the pathology assuming that dogs behave in a similar manner to humans.

Dysregulated Th2 responses can lead to a variety of pathologies, including allergic reactions, which are excessive reactions to stimuli to which other animals make a normal response. Flea allergy dermatitis in dogs is a good example, as is the condition known as 'sweet-itch' in horses, which is a seasonal allergy to bites of midges belonging to the genus *Culicoides* and which also responds to treatment with actinomycete preparations, in that case *T. inchoensis* [14].

Although the cause of atopic dermatitis is as uncertain in dogs as it is in humans, the mechanism of pathology is likely to be similar to that of other insect-bite allergies. Hence, the published evidence of an improvement in atopic eczema in dogs [8] and humans [15,16] in response to injections of *M. vaccae*, paves the way towards the marked beneficial results we record following injections of species belonging to closely related genera. The benefits observed following injections of *G. bronchialis*

or *R. coprophilus* are likely to reflect similarities in adjuvant activity modulating the immune response.

Conclusion

The results provide a most promising basis for the treatment and/or prevention of flea allergy, a common, distressing and intractable disease, although further studies are needed to optimize treatment schedules. Our results suggest that dogs with flea allergy dermatitis may benefit from treatment with two doses of either *G. bronchialis* or *R. coprophilus* at 20-day intervals, as employed in our studies, with further injections given every few months to prevent recurrence. The efficacy of such a longer-term treatment schedule remains to be investigated.

Future perspective

A good clinical practice trial of injections with *G. bronchialis* in the treatment of flea allergies in dogs is almost completed, and given a good result, this may become the treatment of choice for this troublesome condition.

The promising preliminary findings, together with those published elsewhere on the efficacy of preparations of *T. inchoensis* against allergy to midge bites in equines [14], suggest that the approach may be applicable to insect bite allergies in other species, such as the cat in which flea allergy is also an important condition.

If similar benefits can be obtained with oral preparations in man, then the approach could be of value as part of 'travel medicine' for those fearing troublesome reactions to mosquito, sand fly or other insect bites.

Executive summary

- Flea allergy dermatitis is a common and distressing disease in many dogs.
- Current treatment of removal of fleas and suppression of the immune response is by no means ideal.
- Scoring systems were adopted for assessing progress in the two studies.
- The pilot study compared two intradermal injections of three bacterial preparations; one of *Gordonia bronchialis*, another of *Rhodococcus coprophilus* and the third of *Tsukamurella inchoensis*.
- *G. bronchialis* and *R. coprophilus* were the most effective according to the pilot study.
- *G. bronchialis* and *R. coprophilus* were included in the main study and were randomized together with a placebo-recipient group.
- Both scoring systems (one was the Canine Atopic Dermatitis Extent and Severity Index [CADESI] system) produced broadly the same results, with both active treatments resulting in marked resolution of lesions and of pruritus.
- Differential blood counts on samples taken at the beginning of the study and at the major assessment on day 28 after the second treatment injection, showed a marked reduction in eosinophils and small increases in lymphocytes and polymorphonuclear granulocytes.
- The results give promise for an improved treatment for the disease and further studies are justified.

Acknowledgements

The authors would like to thank BioEos Ltd., UK, for providing the test reagents. They would also like to thank all the owners of dogs for their interest and care of their participant dogs, and veterinary colleagues at Casilda for their clinical observations.

Financial & competing interests disclosure

G McIntyre, OA Bottasso, CA Stanford and JL Stanford are shareholders in BioEos Ltd. and PR Ripley is a consultant to BioEos Ltd. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial

conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Bibliography

- 1 Scott D, Miller W, Griffin C. *Small Animal Dermatology (6th Edition)*. WB Saunders, PA, USA, 627–632 (2001).
- 2 McDermott MJ, Weber E, Hunter S *et al.* Identification, cloning, and characterisation of a major cat flea salivary allergen (Cte f 1). *Mol. Immunol.* 37(7), 361–375 (2000).
- 3 Halliwell RE, Preston JF, Nesbitt JG. Aspects of the immunopathogenesis of flea allergy dermatitis in dogs. *Vet. Immunol. Immunopathol.* 17(1–4), 483–494 (1987).
- 4 Wuersch K, Brachelente C, Doherr M *et al.* Immune dysregulation in flea allergy dermatitis – a model for the immunopathogenesis of allergic dermatitis. *Vet. Immunol. Immunopathol.* 110 (3–4), 311–323 (2006).
- 5 Stanford JL, Stanford CA, Grange JM. Environmental echoes. *Sci. Prog.* 84(Pt 2), 105–124 (2001).
- 6 Ricklin Gutzwiller ME, Reist M, Peel JE *et al.* Intradermal injection of heat-killed *Mycobacterium vaccae* in dogs with atopic dermatitis: a multicentre pilot study. *Vet. Dermatol.* 18(2), 87–93 (2007).
- 7 Olivry T, Marsella R, Iwasaki T *et al.* International task force on canine atopic dermatitis. Validation of CADESI-03, a severity scale for clinical trials enrolling dogs with atopic dermatitis. *Vet. Dermatol.* 18(2), 78–86 (2007).
- 8 Steffan J, Alexander D, Brovedani F *et al.* Comparison of cyclosporin A with methylprednisolone for treatment of canine atopic dermatitis: a parallel, blinded, randomised controlled trial. *Vet. Dermatol.* 14(1), 11–22 (2003).
- 9 Lund PA, Large AT, Kapatai G. The chaperonins: perspectives from the *Archaea*. *Biochem. Soc. Trans.* 31(Pt 3), 681–685 (2003).
- 10 Stanford JL, Stanford CA, Stansby G *et al.* The common mycobacterial antigens and their importance in the treatment of disease. *Curr. Pharm. Des.* 15(11), 1248–1260 (2009).
- 11 Camporota L, Corkhill A, Long H *et al.* The effects of *Mycobacterium vaccae* on allergen-induced airway responses in atopic asthma. *Eur. Respir. J.* 21(2), 287–293 (2003).
- 12 Rook GAW, Hernandez-Pando R, Dheda K, Teng Seah G. IL-4 in tuberculosis: implications for vaccine design. *Trends Immunol.* 25(9), 483–488 (2004).
- 13 Sakaguchi S, Miyara M, Costantino CM, Hafler DA. Foxp3⁺ regulatory T cells in the human immune system. *Nat. Rev. Immunol.* 10(7), 490–500 (2010).
- 14 Littlewood JD, O'Brien JK, Stanford JL *et al.* Management of *Culicoides* hypersensitivity (sweet-itch) in equids using killed bacterial preparation BE-T101: a randomised, double-blind, placebo-controlled study. Presented at: *World Association of Veterinary Dermatology Congress 2008*. Hong Kong, China, 19–22 November 2008.
- 15 Arkwright PD, David TJ. Intradermal administration of a killed *Mycobacterium vaccae* suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease. *J. Allergy Clin. Immunol.* 107(3), 531–534 (2001).
- 16 Davies M, Symons C, Shaw S *et al.* An open study to assess the efficacy, clinically and immunologically, of *M. vaccae* vaccine in patients with atopic dermatitis. *J. Dermatolog. Treat.* 17(2), 74–77 (2006).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.