Late-phase reactions to intradermal testing with *Dermatophagoides farinae* in healthy dogs and dogs with house dust mite-induced atopic dermatitis

Andrew Hillier, BVSc; Lynette K. Cole, DVM, MS; Kenneth W. Kwochka, DVM; Catherine McCall, DPhil

Objective—To determine the prevalence of late-phase reactions to intradermal testing with *Dermatophagoides farinae* in healthy dogs and dogs with atopic dermatitis and an immediate reaction to *D farinae*.

Animals—6 healthy dogs and 20 dogs with atopic dermatitis and immediate reactions to *D farinae*.

Procedure—Intradermal tests were performed with *D farinae* at 1:1,000 wt/vol and 1:50,000 wt/vol concentrations, and skin reactivity was evaluated after 0.25, 6, and 24 hours. Serum *D farinae*-specific IgE antibodies were assayed. Extent of lesions (atopy index) and pruritus (visual analogue scale) were evaluated in dogs with atopic dermatitis.

Results—Late-phase reactions were observed in healthy dogs at 6 hours (n = 2 dogs) and 24 hours (1) with the 1:1,000 wt/vol concentration, and at 6 hours (1) and 24 hours (1) with the 1:50,000 wt/vol concentration of allergen. Late-phase reactions in healthy dogs were only observed in dogs with an immediate reaction to *D farinae*. Late-phase reactions were observed in 11 of 20 dogs with atopic dermatitis at 6 and 24 hours with the 1:1,000 wt/vol concentration and in 10 of 20 at 6 and 24 hours with the 1:50,000 wt/vol concentration of allergen. There was no difference in mean atopy index, mean visual analogue scale of pruritus, or mean serum *D farinae*-specific IgE concentration of dogs with a late-phase reaction, compared to dogs without a late-phase reaction.

Conclusions and Clinical Relevance—Late-phase reactions may be observed after an immediate reaction to intradermal skin testing in healthy and allergic dogs but are more commonly observed in dogs with atopic dermatitis. (*Am J Vet Res* 2002;63:69–73)

The **intradermal test (IDT)** for detection of allergen hypersensitivity is characterized by an **immediate reaction (IR)** that occurs at 10 to 20 minutes and is followed hours later by a **late-phase reaction (LPR)**.[1] The IR and LPR are IgE-dependent reactions.[1-3]

Received Feb 5, 2001.
Accepted May 23, 2001.
From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 (Hillier, Cole, Kwochka); and Heska Corp, 1613 Prospect Pkwy, Ft Collins, CO 80525 (McCall).
Results of this study were reported at the 4th World Conference of Veterinary Dermatology, San Francisco, September 2000.
Supported by grants from The Ohio State University Canine Research Funds and Heska Corp, Ft Collins, Colo.

In humans, the LPR is reported to peak at 6 to 12 hours and then diminishes 24 to 48 hours after allergen injection,[1] although in 1 study[4], a peak at 24 hours but no significant difference in the LPR at 6, 24, and 48 hours was reported. Macroscopically, the LPR is characterized by edema, erythema, pruritus, and heat.[2] As the dose of allergen increases, the prevalence of a LPR after IDT approaches 100%, with reports of 10 to 150 times the dose of allergen being necessary to reliably induce a LPR, compared with the allergen dose required to induce an IR.[1,5,6] Individuals who have a detectable LPR are generally believed to have a higher degree of allergen hypersensitivity than individuals who only have an IR to allergen.[7] Although the precise clinical relevance of a LPR is still unknown, the histologic, immunohistochemical, and immunologic changes observed during the LPR suggest that it more closely resembles the changes associated with chronic allergic reactions than does the IR.[8-10]

Canine **atopic dermatitis (AD)** is an IgE-mediated hypersensitivity to environmental allergens.[11] Allergen-specific hypersensitivity in dogs is often detected by observation of the IR after IDT. There are few reports of the LPR in dogs[12-18]; LDR after IDT in dogs with spontanrous, clinical disease has been reported in only 2 studies with small numbers of dogs. To the authors' knowledge, parameters for the optimal performance, evaluation, and interpretation of LPR in dogs have not been reported.

The purpose of this study was to describe the prevalence of LPR after IDT in healthy dogs and in dogs with AD and an IR to *D farinae* with the IDT. Furthermore, we aimed to determine whether the presence or absence of a LPR was associated with allergen dose, severity of clinical signs, and serum allergen-specific IgE concentrations in dogs with AD.

Materials and Methods

Sample population

Healthy dogs—Adult pet dogs with no history of skin disease and no history of disease of any other organ system that may have an allergic cause were selected for inclusion in the study. No abnormalities were detected via full physical examination at the time of entry into the study. The owner's consent was granted before inclusion into the study.

Dogs with AD—Atopic dermatitis was diagnosed in adult pet dogs on the basis of suggestive historical features, typical clinical signs of disease, and rule-out of differential diagnoses.[19] Specifically, all dogs had a history of nonseasonal pruritus and were believed to have clinically important hyper-

sensitivity to house dust mites on the basis of an immediate reaction to *D farinae* at 1:50,000 wt/vol concentration on routine IDT with a regional panel of 55 allergens. *Sarcoptes scabeii* infestation was ruled out on the basis of history, clinical signs, negative results of superficial skin scrapings, or lack of response to appropriate treatment. A cutaneous adverse food reaction was ruled out by lack of response to the feeding of commercial or home-cooked elimination diets for a minimum of 4 weeks (typically 8 weeks). Dogs with bacterial or *Malassezia pachydermatis* infections of the skin were treated until resolution of the infection prior to entry into the study.

Intradermal test—Routine withdrawal of anti-inflammatory drugs was performed for all dogs prior to IDT.[19] The test was performed by use of standard protocol on the left lateral aspect of the thorax, and 0.05 ml of a positive control solution (histamine[a]), negative control solution (saline [0.9% NaCl] diluent[b]), and crude *D farinae*[c] at 1:1,000 wt/vol and 1:50,000 wt/vol (wt/vol) were injected at separate sites. During the course of the 24 hours of the study, the thorax was lightly wrapped with bandaging tape,[d] and the dogs wore a body suit[e] to prevent trauma to the IDT sites. The protective wrap and body suit were removed and replaced at the time of assessment for LPR.

The IR was evaluated 15 minutes after allergen injection. Reactions were graded on a scale of 0 to 4 on the basis of size of the erythema and wheal, where 4 was equivalent to the positive control and 0 was equivalent to the negative control. Reactions larger than the positive control were graded as 5. Reactions scored as ≥ 2 were considered to be positive.

The LPR was evaluated 6 and 24 hours after allergen injection. Reactions were graded on a scale of 0 to 4 on the basis of the size and intensity of erythema (0 = no reaction, 1 = extremely mild erythema, 2 = mild erythema, 3 = moderate erythema, 4 = severe erythema). Reactions graded as ≥ 1 were considered positive and an indication of a LPR.

Atopy index—The clinical condition of the dogs with AD was evaluated and an atopy index was obtained for each dog, as follows:

Atopy index = clinical sign score + visual analog scale (× 1.8)

The extent and severity of skin lesions were documented as the clinical sign score. Five lesion types (papules, alopecia, erythema, excoriations and crusts, and hyperpigmentation, lichenification, and scaling) were scored as 0 = absent, 1 = mild, 2 = moderate, and 3 = severe for each lesion in each of 4 body sites typically involved in canine AD (face, axilla, groin, and feet and distal portions of the limbs). The same scoring scheme was used for evaluation of the ears and ear pinnae for lesions of alopecia, erythema, hyperplasia, stenosis, and discharge. The maximum possible clinical sign score was 75 (5 body sites × 5 lesions × 3 maximum score).

Owners assessed the severity of pruritus during the 3 days prior to entry into the study on a visual analog scale of 0 to 10 in which 0 = no pruritus and 10 = constant pruritus. The visual analog scale score was multiplied by 1.8 so that the score for pruritus (maximum 18) accounted for approximately 20% of the atopy index, which is described for clinical scoring systems in human medicine.[20] The maximum possible atopy index score was 93.

Serum *D farinae*-specific IgE quantitation—A blood sample was collected to obtain serum from each dog immediately prior to IDT. Serum was submitted for quantitation of IgE antibodies against *D farinae* by use of an ELISA in which allergen-specific IgE is detected by use of a recombinant truncated version of the human Fc-εα chain of the high affinity IgE receptor.[f] The concentration of allergen-specific IgE was reported in **ELISA absorbance (EA)** units.

Statistical analyses—Nonparametric statistical testing (Mann-Whitney *U* test)[g] was used to determine whether differences in atopy index, visual analog scale of pruritus, and serum allergen-specific IgE concentrations between dogs with AD with a LPR and dogs with AD without a LPR were significantly ($P < 0.05$) different.

Results

The healthy group comprised 6 dogs with a mean age of 4.9 years (range, 1 to 12 years). The AD group comprised 20 dogs that met all inclusion criteria and had a mean age of 3.9 years (range, 1.5 to 8 years). Mean age at onset of clinical signs was 1.8 years (range, 3 months to 6 years), and mean duration of clinical signs was 2.2 years (range, 4 months to 5.5 years). One dog had a history of pruritic dermatitis of only 4 months' duration at the time of entry into the study. The nonseasonal nature of the dog's skin disease was confirmed 1 year later.

IR to *D farinae*—In the healthy group, 3 of 6 dogs had an IR to *D farinae* at 1:1,000 wt/vol, and 2 of 6 dogs had an IR at the 1:50,000 wt/vol concentration (**Table 1**). By definition for inclusion in the study, all 20 dogs in the AD group had an IR to *D farinae* at 1:50,000 wt/vol. All 20 dogs also had an IR at the 1:1,000 wt/vol concentration.

LPR to *D farinae*—Reactions detected 6 hours after injection of allergen were characterized by variable intensity and size of erythema. Induration was noticed at sites of reactions, but it was subtle in most instances. Evidence of edema was absent. There were no reactions at the sites of the positive and negative controls at the 6-hour evaluation.

In the healthy group, 2 of 6 dogs had a 6-hour LPR to *D farinae* at 1:1,000 wt/vol concentration, and 1 of 6 dogs had positive results at 1:50,000 wt/vol (**Table 1**). Late-phase reactions at 6 hours were only detected in dogs with an IR, and no LPR was detected in the 3 healthy dogs that did not have an IR to *D farinae*.

In the AD group, 11 of 20 dogs had a 6-hour LPR to *D farinae* at 1:1,000 wt/vol, and 10 of 20 dogs had positive results at 1:50,000 wt/vol (Table 1). Three of the 11 dogs with a 6-hour LPR at the 1:1,000 wt/vol concentration had negative results at the 1:50,000 wt/vol concentration. Two of the 10 dogs with a 6-hour LPR at the 1:50,000-wt/vol concentration had negative results at the 1:1,000 wt/vol concentration of allergen.

Reactions at 24 hours were similar in appearance to those described at the 6-hour time point. There were no reactions at the sites of the positive and negative controls. In the healthy group, 1 of 6 dogs had a 24-hour LPR to *D farinae* at both allergen concentrations; both were weak reactions (score, 1; Table 1). This dog had a 6-hour LPR to the 1:1,000 wt/vol concentration of allergen, but not the 1:50,000 wt/vol concentration.

In the AD group, 11 of 20 dogs had a 24-hour LPR to *D farinae* at the 1:1,000 wt/vol concentration; 3 of these 11 dogs had negative results at the 1:50,000

Table 1—Late-phase reactions (LPR) to intradermal injection of *Dermatophagoides farinae* in 6 healthy dogs and 20 dogs with house dust mite-induced atopic dermatitis (AD)

Time of LPR evaluation	Healthy dogs (No. of dogs)	Dogs with AD (No. of dogs)
6 hours		
D farinae 1:1,000 wt/vol		
IDT score		
0	4	9
1	0	3
2	1	5
3	0	0
4	1	3
D farinae 1:50,000 wt/vol		
IDT Score		
0	5	10
1	0	5
2	1	3
3	0	1
4	0	1
24 hours		
D farinae 1:1,000 wt/vol		
IDT Score		
0	5	9
1	1	2
2	0	5
3	0	0
4	0	4
D farinae 1:50,000 wt/vol		
IDT Score		
0	5	10
1	1	3
2	0	3
3	0	1
4	0	3

IDT = Intradermal test.

wt/vol concentration, and 9 of 11 had a 6-hour LPR at the 1:1,000 wt/vol concentration (Table 1).

In the AD group, 10 of 20 dogs had a 24-hour LPR to *D farinae* at 1:50,000 wt/vol; 2 of these 10 dogs had negative results at 1:1,000 wt/vol, and 6 of 10 had a 6-hour LPR at 1:50,000 wt/vol (Table 1).

Atopy index—The atopy index of dogs in the AD group ranged from 6.6 to 43.2 (mean, 21). There was no significant difference in the mean atopy index of dogs with a LPR, compared with dogs without a LPR at 6 or 24 hours with either concentration of *D farinae* (**Table 2**).

Pruritus—For dogs in the AD group, the owners' assessment of pruritus during the 3 days prior to entry into the study ranged from 1 to 9 (mean, 4.9). There was no significant difference in mean visual analog scale score for dogs with a LPR, compared with the dogs without a LPR at 6 or 24 hours with either concentration of *D farinae* (Table 2).

Serum *D farinae*-specific IgE—The serum *D farinae*-specific IgE in all 20 dogs with AD and in 5 of 6 healthy dogs ranged from 7 to 2,166 EA units of IgE (mean, 489.4). Serum IgE concentrations were not measured in 1 of the healthy dogs. There was no significant difference in mean serum *D farinae*-specific IgE concentration of dogs with AD that had a LPR, compared with dogs that did not have a LPR at 6 or 24 hours witheither concentration of *D farinae* (Table 2).

Table 2—Comparison of clinical signs (atopy index), level of pruritus (visual analogue scale; VAS) and serum *D farinae*-specific IgE concentrations in 20 dogs with atopic dermatitis, with and without LPR 6 and 24 hours after intradermal injection of *D farinae*

Variable	Positive LPR	Negative LPR	*P* value
Mean atopy index			
6 hours			
D farinae 1:1,000 wt/vol	22.3	19.5	0.71
D farinae 1:50,000 wt/vol	19.8	22.3	0.53
24 hours			
D farinae 1:1,000 wt/vol	23.3	18.3	0.34
D farinae 1:50,000 wt/vol	22.8	19.2	0.63
Mean VAS			
6 hours			
D farinae 1:1,000 wt/vol	4.6	5.2	0.63
D farinae 1:50,000 wt/vol	4.3	5.5	0.35
24 hours			
D farinae 1:1,000 wt/vol	4.9	4.8	0.97
D farinae 1:50,000 wt/vol	5.3	4.5	0.58
Mean IgE concentrations*			
6 hours			
D farinae 1:1,000 wt/vol	523.5	423.4	0.76
D farinae 1:50,000 wt/vol	427.6	529.4	0.63
24 hours			
D farinae 1:1,000 wt/vol	680.5	231	0.34
D farinae 1:50,000 wt/vol	530.3	426.7	0.91

*Reported in ELISA absorbance units.

Discussion

In the study reported here, the prevalence of a LPR in dogs with spontaneous AD was 11 of 20 at 6 and 24 hours after allergen injection with the 1:1,000 wt/vol allergen concentration and 10 of 20 at 6 and 24 hours with the 1:50,000 wt/vol allergen concentration. In the healthy dogs, the prevalence of 6-hour LPR (2 of 6 at 1:1,000 wt/vol concentration and 1 of 6 at 1:50,000 wt/vol concentration) and 24-hour LPR (1 of 6 at each concentration of allergen) was lower, compared to dogs with AD.

Late-phase reactions have been reported in clinically normal dogs artificially sensitized to allergen,[12] clinically normal dogs that received intradermal injection with nonallergenic agents that may induce a LPR,[16-18] and dogs with spontaneous AD.[14,18] In a study of dogs artificially sensitized to ragweed, 4 of 5 dogs had a macroscopically visible LPR 6 hours after allergen injection.[12] In a study of dogs with spontaneous AD and *D farinae* hypersensitivity, 3 of 3 dogs had histopathologic evidence of a LPR after allergen and anti-IgE antibody injection, which peaked between 6 and 12 hours.[18] In a study of 2 dogs with AD, histologic evidence of a LPR was reported 3 hours after allergen injection.[14] Compound 48/80 and anti-IgE antibodies have been reported to induce histologic changes consistent with the LPR between 6 and 30 hours after intradermal injection in healthy dogs.[16-18] It is interesting to note that in 1 of the studies reporting reactivity to compound 48/80,[16] significant increases in skin-fold thickness, compared with phosphate-buffered saline solution, peaked at 15 to 30 minutes after intradermal injection but largely dissipated by 6 hours.[16] Thus, despite histologic evidence of a LPR, no macroscopically visible reaction was documented.

In contrast, in 2 studies[13,15] where observations for a macroscopically visible LPR were made, no such reaction was recorded in any dogs. In 1 study, 0 of 29 dogs without allergy and 0 of 24 dogs with clinical signs of allergy had a LPR at 4, 24, or 48 hours in response to storage mite extract, despite the presence of an IR to storage mites in 4 of 29 healthy dogs and 18 of 24 dogs with allergy.[13] Similarly, in the other study, 0 of 24 healthy Beagles had a LPR to *D farinae* at 48 hours, despite an IR in 11 of 24 (46%) of these dogs.

In humans, the prevalence of LPR after allergen injection approaches 100% as the allergen dose increases.[1] In a study involving 20 subjects with atopic rhinitis, development of a macroscopic cutaneous LPR required a 150× higher dose of allergen than that required for an IR.[5] These investigators concluded that all atopic individuals are capable of mounting a LPR if challenged with sufficient allergen. In our study, there was no significant difference in the prevalence of the LPR at either time point between the 2 doses of allergen, despite a 50-fold difference in allergen dose. It is possible that an even higher concentration of *D farinae* than the 1:1,000 wt/vol concentration used in our study, or microscopic evaluation of IDT sites may be needed to reveal increased prevalence of LPR above that observed with allergen at the 1:50,000 wt/vol concentration. It is interesting, however, that the mean score of the 25 recorded LPR (all dogs at 6 and 24 hours) with the 1:1,000 wt/vol concentration of *D farinae* was 2.4, compared with the mean score of the 22 recorded LPR (all dogs at 6 and 24 hours) with the 1:50,000 wt/vol concentration of 2.0. In addition, 8 of 25 (32%) LPR with the 1:1,000 wt/vol concentration of allergen had a score of 4, compared with 4 of 22 (18%) LPR with the 1:50,000 wt/vol concentration. These findings are suggestive of stronger and more intense reactions with the higher allergen concentration.

Higher concentrations of allergen have been cited as a cause for irritant-induced false-positive reactions on IDT.[19,21] We believe that the 1:1,000 wt/vol concentration of allergen extract used in our study was unlikely to have acted as an irritant, because 3 of 6 healthy dogs had no reaction at any time point at this concentration, and 2 of 6 healthy dogs that had a reaction at this concentration of allergen also had a reaction to the 1:50,000 wt/vol concentration of allergen, indicating true hypersensitivity. In addition, in a recent study[18] it was also reported that 3 of 3 clinically normal adult research Beagles had no IR with IDT with *D farinae* allergen at 1:1,000 wt/vol concentration.[18]

In humans, individuals who have a LPR are generally believed to have a higher degree of allergen hypersensitivity, compared with individuals who only have an IR to allergen.[7] In our study, there was no significant difference in the mean clinical score atopy index, mean level of pruritus (VAS), or mean serum *D farinae*-specific IgE concentrations between dogs with AD and a LPR and dogs with AD and no LPR at either time point with either concentration of allergen. Thus, neither dogs with more severe disease nor dogs with higher allergen sensitivity were more likely to have a LPR in our study.

The results of our study raise the question of the clinical relevance of the LPR in dogs with allergen hypersensitivity. Results of studies in humans indicate that the macroscopic, histologic, and immunologic changes evident in the LPR are similar to those seen in chronic allergic inflammatory lesions, and the LPR has thus been proposed as a superior model of chronic atopic disease, compared with the IR.[8-10] Results of early studies[12,14] of the histologic features of the LPR in dogs and a more recent study[18] of the cellular infiltrate indicate that the LPR in dogs follows a pattern similar to that seen in human skin. In humans and dogs, these changes can be induced in clinically allergic patients after IDT with allergen but also in clinically normal patients after anti-IgE antibody injection,[3,17,18] indicating that the LPR is not necessarily a phenomenon restricted to patients with allergic disease. We confirmed in the study reported here that clinically normal dogs with *D farinae* hypersensitivity can also mount a LPR that is visible at 6 and 24 hours after allergen injection. Thus, we are unable to speculate on the clinical importance of a macroscopically visible LPR. However, it is of interest that only 1 healthy dog had a 24-hour LPR, and this reaction was a weak reaction (grade 1 at both allergen concentrations). In contrast, 21 of 40 (53%) possible LPR at 24 hours in the dogs with AD did occur, and the mean score for these reactions was 2.5. Of these 21 LPR, 16 (76%) were scored 2 or higher. Thus, moderate to strong LPR at 24 hours may be suggestive of clinically relevant hypersensitivity.

Isolated LPR have been observed in humans after a skin test did not elicit an IR,[1] but the cause and importance of such reactions is unclear. Because all of the dogs with AD in our study had an IR, we are unable to speculate on the cause or importance of such isolated reactions in dogs.

The LPR in humans is reported to be an IgE-dependent phenomenon.[1-3] The observation that 3 healthy dogs in our study that did not have *D farinae* hypersensitivity (no IR and low serum IgE concentrations) and did not have a LPR supports this hypothesis in dogs. The presence of an IR to *D farinae* in 3 healthy dogs in our study confirmed the reported occurrence of subclinical hypersensitivity to this dust mite,[15,22,23] and we also detected a LPR in 2 of these dogs.

In most of the dogs in our study, a 6-hour LPR was followed by a LPR at 24 hours. In addition, most 24-hour LPR were preceded by a 6-hour LPR. Isolated LPR were observed at both times, and we speculate that this is a reflection of the complex nature of allergic responses in which allergen sensitivity, effector cell reactivity, and end-organ responsiveness to mediators are important variables in each animal and determine the presence and strength of a reaction.

Macroscopically visible LPR were seen. Reactions seen at 6 and 24 hours were characterized by erythema, which varied from mild and barely detectable to large areas of intense redness. The LPR also had various degrees of induration, although this feature was not as obvious as the erythema. Edema, which is a feature of the IR to IDT,[21,24] was notably absent. These findings are in agreement with reported descriptions of the macroscopic appearance of the LPR in dogs.[12,18] In those studies, as in ours, the diffuse edema report-

ed with the LPR in humans[2] was absent. Changes in skin-fold thickness have been reported at sites of the LPR in dogs.[16,18] In 1 study[16] the visible lesion is not described, whereas in another study[18] the lesions are described as erythematous patches (which by definition are not raised above the surrounding normal skin) with a 1- to 2-mm increase in skin-fold thickness measured with calipers.

The LPR is purported to be a good model of chronic allergic disease. As such, the observation of pruritus (a hallmark of AD) at the site of the LPR would further support this belief. We were unable to observe the dogs for pruritus, because they wore body suits during the study. It has been reported that self-trauma may be a cause for false-positive IR after IDT.[21]

The potential clinical importance of the LPR and whether the observation of a LPR in patients undergoing IDT is of any further benefit in the diagnosis of specific allergen hypersensitivity in dogs with AD are presently unknown.

[a]Histatrol, Center Labs, Port Washington, NY.
[b]07030 Sterile diluent for allergenic extracts, Greer Laboratories, Lenoir, NC.
[c]B51 *Dermatophagoides farinae*, Greer Laboratories, Lenoir, NC.
[d]Vetrap, 3M Animal Care Products, St Paul, Minn.
[e]Dog torso sling suit, Alice King Chatham Medical Arts, Hawthorne, Calif.
[f]Allercept ELISA, Heska Corp, Ft Collins, Colo.
[g]SAS statistical software, version 6.12, SAS Institute Inc, Cary, NC.

References

1. Peters SP, Zangrilli JG, Fish JE. Late phase allergic reactions. In: Middleton E, Reed CE, Ellis EF, et al, eds. *Allergy principles and practice*. St Louis: Mosby, 1998;342–355.

2. Solley GO, Gleich GJ, Jordon RE. The late phase of the immediate wheal and flare skin reaction. *J Clin Invest* 1976;58:408–420.

3. Dolovich J, Hargreave FE, Chalmers R, et al. Late cutaneous allergic responses in isolated IgE-dependent reactions. *J Allergy Clin Immunol* 1973;52:38–46.

4. Barata LT, Ying S, Meng Q, et al. IL-4 and IL-5-positive T lymphocytes, eosinophils, and mast cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Allergy Clin Immunol* 1998;101:222–230.

5. Frew AJ, Kay AB. The pattern of human late-phase skin reactions to extracts of aeroallergens. *J Allergy Clin Immunol* 1988;81: 1117–1121.

6. Reshef A, Kagey-Sobotka A, Adkinson NF, et al. The pattern and kinetics in human skin of erythema and mediators during the acute and late-phase response. *J Allergy Clin Immunol* 1989;84:678–687.

7. Boulet LP, Roberts RS, Dolovich J, et al. Prediction of late asthmatic responses to inhaled allergens. *Clin Allergy* 1984;14:379–385.

8. Leung DYM. Immunopathology of atopic dermatitis. *Springer Semin Immunopathol* 1992;13:427–440.

9. Leung DYM. Pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 1999;104:S99–S108.

10. Zweiman B. The late-phase reaction: role of IgE, its receptor and cytokines. *Curr Opin Immunol* 1993;5:950–955.

11. Urticaria, angioedema, and atopy. In: Reedy LM, Miller WH, Willemse T, eds. *Allergic skin diseases of dogs and cats*. 2nd ed. London: WB Saunders Co, 1997;25–49.

12. Becker AB, Chung KF, McDonald DM, et al. Cutaneous mast cell heterogeneity: response to antigen in atopic dogs. *J Allergy Clin Immunol* 1986;78:937–942.

13. Vollset I, Larsen HJ, Mehl R. Immediate hypersensitivity in dogs induced by storage mites. *Res Vet Sci* 1986;40:123–127.

14. Thomsen MK, Kristensen F, Elling F. Species specificity in the generation of eicosanoids: emphasis on leukocyte-activating factors in the skin of allergic dogs and humans. In: Ihrke PJ, Mason IS, White SD, eds. *Advances in veterinary dermatology*. Oxford, England: Pergamon Press, 1993;2:63–78.

15. Bourdeau P, Blumstein P, Guenoden R. Positive skin reactions to allergenic challenge in healthy dogs: part 1: intradermal skin testing. In: Kwochka KW, Willemse T, von Tscharner C, eds. *Advances in veterinary dermatology*. Oxford, England: Butterworth Heinemann, 1998;3:444–445.

16. Mason IS, Lloyd DH. Evaluation of compound 48/80 as a model of immediate hypersensitivity in the skin of dogs. *Vet Dermatol* 1996;7:81–83.

17. DeBoer DJ, Cooley AJ. Use of induced cutaneous immediate-type hypersensitivity reactions to evaluate anti-inflammatory effects of triamcinolone topical solution in three dogs. *Vet Dermatol* 2000;11:25–33.

18. Olivry T, Dunston SM, Murphy KM, et al. Characterization of the inflammatory infiltrate during IgE-mediated late phase reactions in the skin of normal and atopic dogs. *Vet Dermatol* 2001;12:49–58.

19. Scott DW, Miller WH, Griffin CE. Immunologic skin diseases. In: Scott DW, Miller WH, Griffin CE, eds. *Muller and Kirk's small animal dermatology*. 5th ed. Philadelphia: WB Saunders Co, 1995;484–626.

20. European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. *Dermatology* 1993;186:23–31.

21. Allergy testing. In: Reedy LM, Miller WH, Willemse T, eds. *Allergic skin diseases of dogs and cats*. 2nd ed. London: WB Saunders Co, 1997;83–115.

22. Willis EL, Kunkle GA, Esch R, et al. Intradermal reactivity to various insect and arachnid allergens among dogs from the southeastern United States. *J Am Vet Med Assoc* 1996;209:1431–1434.

23. Hillier A, Kwochka KW, Pinchbeck LR. Reactivity to intradermal injection of *Dermatophagoides farinae*, *D pteronyssinus*, house dust mite mix, and house dust in dogs suspected to have atopic dermatitis: 115 cases (1996–1998). *J Am Vet Med Assoc* 2000;217:536–540.

24. Demoly P, Michel F-B, Bousquet J. In vivo methods for study of allergy skin tests, techniques, and interpretation. In: Middleton E, Reed CE, Ellis EF, et al, eds. *Allergy principles and practice*. 5th ed. St Louis: Mosby, 1998;430–439.