

# Effect of Hyposensitization with Irrelevant Antigens on Subsequent Allergy Test Results in Normal Dogs

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**Abstract**—Five normal greyhounds were evaluated for hypersensitivity to various grasses, weeds, trees and fungi with both an intradermal allergy test and a commercial enzyme-linked immunosorbent assay (ELISA). All dogs were hyposensitized for 6 months with the same mixture of 14 allergens, according to the treatment schedule recommended by the commercial laboratory that provided the ELISA allergy test. Following hyposensitization with irrelevant antigens at a concentration of 1:200 w/v, dogs were reevaluated for hypersensitivity with the intradermal allergy test. No significant increase in intradermal reactions was found after 6 months of hyposensitization, and all dogs remained asymptomatic during the study period.

Hyposensitization of normal greyhounds with irrelevant aqueous antigens, administered according to one treatment schedule recommended following ELISA allergy testing, did not appear to cause false positive reactions on subsequent intradermal allergy tests or to induce clinical hypersensitivity. Further studies are required to determine if hypersensitivity to irrelevant antigens is induced in atopic dogs following hyposensitization with nonaqueous extracts or higher concentrations of aqueous antigens.

**Key Words:** Irrelevant antigens; Hyposensitization; Intradermal allergy testing; Dogs.

## INTRODUCTION

Intradermal (ID) allergy testing has been considered the best method for confirming a diagnosis of canine atopy and for selecting allergens for hyposensitization (1–3). Unfortunately, several disadvantages are associated with ID allergy testing. Anti-inflammatory drugs must be completely withdrawn prior to testing or false negative results may occur. The test cannot be performed on dogs with generalized dermatitis or dermatographism. Intradermal allergy testing is usually done at dermatology referral centers, since the procedure is time consuming and is not cost effective when performed infrequently. Test procedures have not been standardized by veterinary dermatologists, and results are based on subjective evaluation that requires experience to ensure accuracy.

*In vitro* allergy testing, which measures serum concentrations of allergen specific IgE, avoids many of

the disadvantages associated with ID allergy testing (4, 5). Both a radioallergosorbent test (RAST) and an enzyme-linked immunosorbent assay (ELISA) are commercially available for allergy testing of dogs in North America. These tests require only a serum sample, and thus are readily available to the private practitioner. Results are expressed quantitatively. Treatment recommendations and allergens for hyposensitization are provided by the commercial laboratory.

Some *in vitro* tests evaluate groups of antigens rather than individual antigens. Any or all of the antigens within a test group may be responsible for producing a positive reaction for the group; therefore, a positive group may contain antigens to which the animal has no hypersensitivity (i.e. irrelevant antigens). Since all the antigens within positive groups are included in the hyposensitization mixture, it is likely that atopic dogs will be treated with one or more irrelevant allergens. In addition, some *in vitro* allergy tests have been shown to have a high false positive rate (3, 4, 6, 7). Therefore, it is also likely that nonatopic dogs will receive hyposensitization therapy based on *in vitro* test results.

Hypersensitivity can be induced in nonsensitive humans and dogs by repeated parenteral exposure to an antigen (8–10). Nine nonatopic human volunteers were parenterally immunized with six injections of alum-absorbed rye grass allergen (8). All nine developed immediate skin reactivity 25 weeks after immunization was started, and five out of seven

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volunteers that were tested 8 years later demonstrated persistence of sensitivity. One patient developed clinical signs that were consistent with rye grass allergy. Immediate skin reactivity has also been induced in four nonatopic dogs following four weekly injections of ragweed emulsion at a concentration of 20,000 PNU.ml<sup>-1</sup> (9); however, challenge of one dog by inhalation and intravenous administration failed to produce clinical signs. In another study, positive intradermal reactions were induced in normal dogs by repetitive intradermal allergy testing with aqueous allergens (10). Seven dogs were intradermally tested with 35 antigens once weekly for 12 consecutive weeks, and all seven dogs developed multiple reactions to allergens that previously had yielded negative results.

Dogs that have failed to improve following hyposensitization with antigens selected on the basis of serologic test results are often referred for intradermal allergy testing. It is unknown if prior hyposensitization with irrelevant aqueous antigens will induce positive reactions on subsequent intradermal allergy tests and if these reactions would be clinically significant. The purpose of the study reported here was to determine if hypersensitivity is induced in normal dogs that are parenterally treated with aqueous allergens according to a standard hyposensitization schedule recommended by one commercial company providing ELISA testing (Arest<sup>®</sup>, Bioproducts DVM, Tempe, AZ, U.S.A.).

## MATERIALS AND METHODS

### *Dogs*

Five normal greyhounds, 8–9 years of age, that were being retired as blood donors were used in the study. These dogs had been kennelled at the Veterinary Teaching Hospital during the previous 5 years and had been observed to be asymptomatic for allergic skin disease. The dogs were housed indoors, except for 1 h. each day, when they were allowed to exercise in a large, outdoor pen. No abnormal findings were noted on physical examination. Dogs were evaluated for hypersensitivity reactions with an intradermal allergy test prior to hyposensitization and after 6 months of hyposensitization injections.

### *Intradermal allergy test*

The intradermal allergy tests were performed with 48 aqueous allergenic extracts (Greer Laboratories, Lenior, NC, U.S.A.) diluted with phosphate buffered saline to a concentration of 1000 PNU.ml<sup>-1</sup> (Table 1). House dust, rhizopus and wool were further diluted to 100, 250 and 500 PNU.ml<sup>-1</sup>, respectively. Intradermal testing was done on the lateral aspect of the trunk using physical restraint. A volume of 0.05 ml of each allergen was injected intradermally with a 27 gauge, 3/8 in. needle. Diluent and histamine (1:100,000 w/v) controls were included at the begin-

ning and end of each test. At 15 min after injection, the diameter of each wheal was measured in mm, and each wheal was subjectively graded from 0 to +4 based on size, erythema and turgidity. Reactions graded 0 and +4 were similar to those seen with saline and histamine controls, respectively. A +2 reaction was halfway between the reaction of positive and negative controls.

### *ELISA*

Prior to onset of the study, sera were submitted to a commercial laboratory for the measurement of allergen specific IgE (Arest<sup>®</sup>, Bioproducts DVM, Tempe, AZ, U.S.A.) using a previously reported micro-ELISA technique (11). Fourteen groups of antigenically related allergens were tested (Table 2). The laboratory recommended that class 0 be considered negative, class 1 borderline, and classes 2 through 5 positive. Based on ELISA scores, hyposensitization would have been recommended in all dogs. Highly positive scores (class 3 and 4) for several allergen groups were obtained in four out of five dogs.

### *Hyposensitization*

All dogs were hyposensitized with the same mixture of irrelevant antigens. The laboratory recommends that treatment mixtures contain no more than 16 allergens. Included in the treatment mixture were group 1 grasses (blue, redbud, timothy), group 5 herbs/shrubs (dock, plantain, cocklebur), group 7 trees (birch mix, box-elder, cottonwood, oak mix) and group 13 fungi (*alternaria*, *aspergillus*, *cladosporium*, *helminthosporium*). The total concentration of the treatment mixture was 1:200 w/v, which is equivalent to 2500 protein nitrogen units (PNU).ml<sup>-1</sup>. Each dog was hyposensitized according to the standard injection schedule recommended by the laboratory providing ELISA testing (Table 3). Each dog received 26 injections of the final treatment volume during a 6 month hyposensitization period (September through to March).

### *Statistical analysis*

Intradermal reactions 0 to +1 were considered negative and reactions  $\geq +2$  were considered positive. Dermal reactions to antigens included in the treatment mixture were compared before and after hyposensitization. Wilcoxon's signed rank test (12) was used to determine whether a significant difference ( $P < 0.05$ ) in dermal reactivity occurred following hyposensitization with these antigens. Intradermal reactions to antigens not included in the treatment mixture served as a control group. The number of positive reactions in the control group was compared before and after hyposensitization to determine if positive reactions were induced nonspecifically following hyposensitization or by repeated testing. A Fisher exact test (13) was used to determine if simi-

TABLE 1. Allergens (Greer Laboratories, Lenion, NC, U.S.A.) included in the intradermal test\*

|                            |                              |
|----------------------------|------------------------------|
| 1. Histamine               | 27. Eastern cottonwood       |
| 2. Saline                  | 28. Easter oak mix           |
| 3. Flea                    | 29. Eastern sycamore         |
| 4. Bermuda                 | 30. Hickory mix              |
| 5. Fescue                  | 31. Pine mix                 |
| 6. Johnson                 | 32. Red cedar                |
| 7. Kentucky blue           | 33. Red mulberry             |
| 8. Orchard                 | 34. Sweet gum                |
| 9. Perennial rye           | 35. Three maple mix          |
| 10. Red top                | 36. <i>Alternaria tenuis</i> |
| 11. Sweet vernal           | 37. <i>Aspergillus</i> mix   |
| 12. Timothy                | 38. <i>Fusarium</i> mix      |
| 13. <i>Chenopodium</i> mix | 39. <i>Helminthosporium</i>  |
| 14. Cocklebur              | 40. <i>Cladosporium</i>      |
| 15. Dock-Sorrel mix        | 41. <i>Mucor</i> mix         |
| 16. English plantain       | 42. <i>Penicillium</i> mix   |
| 17. Goldenrod              | 43. <i>Rhizopus arrhizus</i> |
| 18. Ragweed mix            | 44. Cotton linters           |
| 19. Rough pigweed          | 45. Epidermal mix            |
| 20. American beech         | 46. House dust               |
| 21. American Elm           | 47. Kapok                    |
| 22. Ash mix                | 48. Mixed feathers           |
| 23. Birch mix              | 49. Pyrethrum                |
| 24. Black walnut           | 50. Sheep wool               |
| 25. Black willow           | 51. Histamine                |
| 26. Box elder              | 52. Saline                   |

\*The histamine control was a 1:100,000 w/v solution. House dust, *Rhizopus* and wool were tested at a concentration of 100 PNU.ml<sup>-1</sup>, 250 PNU.ml<sup>-1</sup> and 500 PNU.ml<sup>-1</sup>, respectively. All other allergens were tested at a concentration of 1000 PNU.ml<sup>-1</sup>.

TABLE 2. Components of allergen groups tested by ELISA\* and the number of five normal greyhounds positive ELISA scores†

| ELISA Group        | Components  | +ELISA scores | ELISA Group    | Components  | +ELISA scores |
|--------------------|---|---------------|----------------|---|---------------|
| 1. Grasses         | Blue<br>Redtop<br>Timothy                             | 4             | 8. Trees       | Ash<br>Elm<br>Hickory<br>Sycamore   | 3             |
| 2. Grasses         | Bermuda<br>Johnson<br>Orchard                         | 4             | 9. Trees       | Hazelnut<br>Hackberry<br>Mulberry   | Cedar 3       |
| 3. Grasses         | Rye<br>Fescue<br>Vernal                               | 4             | 10. Trees      | Hemlock<br>Pine<br>Walnut   | 3             |
| 4. Ragweed         | Ambrosia  | 4             |                |   |               |
| 5. Herb/<br>Shrubs | Dock<br>Plantain<br>Cocklebur                         | 5             | 11. Dust Mite  | <i>Dermatophagoides</i>   | 5             |
|                    |   |               | 12. Epidermals |   |               |
| 6. Herb/<br>Shrubs | Lamb's quarter<br>Pigweed<br>Mexican firebush<br>Sage | 4             | 13. Fungi      | <i>Alternaria</i><br><i>Aspergillus</i><br><i>Cladosporium</i><br><i>Helminthosporium</i> | 0             |
| 7. Trees           | Birch mix<br>Box-elder<br>Cottonwood<br>Oak mix       | 3             | 14. Fungi      | <i>Penicillium</i><br><i>Rhizopus</i><br>Johnson grass<br>smut<br><i>Stemphylium</i>      | 4             |

\*Arest® N. Atlantic/Ohio Valley Screen, Bioproducts DVM, Tempe, AZ, U.S.A.

†ELISA scores 101 to 2000 EA units (classes 2 to 5) are considered positive.

TABLE 3. Hyposensitization schedule recommended by a commercial laboratory\* providing ELISA allergy testing

| Day | Amount of injection†<br>(ml) |
|-----|------------------------------|
| 1   | 0.2                          |
| 2   | 0.4                          |
| 3   | 0.6                          |
| 4   | 0.8                          |
| 5   | 1.0                          |

\*Bioproducts DVM, Inc., Tempe, AZ, U.S.A.

†Total concentration of treatment mixture was 1:200 w/v.

After day 5, treatment is continued by administering 1.0 ml weekly. Clinical response may be assessed after 25–30 subcutaneous injections.

TABLE 4. Antigens inducing a positive intradermal reaction before and after hyposensitization with 14 antigens\* obtained from a commercial laboratory offering ELISA allergy testing.

| Dog | Before hyposensitization | After hyposensitization |
|-----|--------------------------|-------------------------|
| 1   | cotton, housedust        | none                    |
| 2   | dock†                    | fescue                  |
| 3   | wool                     | none                    |
| 4   | none                     | none                    |
| 5   | none                     | <i>cladosporium</i> †   |

\*Arest® allergen groups 1, 5, 7, 13; Bioproducts DVM, Tempe, AZ, U.S.A.

†Denotes antigens included in the hyposensitization mixture.

lar variability in dermal reactivity was exhibited by antigens in the treatment mixture and antigens in the control group following hyposensitization.

## RESULTS

No significant difference in dermal reactivity to antigens included in the treatment mixture occurred following hyposensitization. Negative intradermal reactions were observed following 69 out of 70 injections of antigens included in the treatment mixture (14 antigens × five dogs) both before and after hyposensitization. A single +2 intradermal reaction was produced both before and after hyposensitization by two different antigens in the treatment mixture (Table 4).

Similar variability in dermal reactivity was observed with the control group of antigens before and after hyposensitization. Of 170 intradermal injections of antigens not included in the treatment mixture (34 antigens × five dogs), three +2 reactions were recorded before hyposensitization and one +2 reaction was recorded after hyposensitization (Table 4).

None of the dogs exhibited any clinical signs of allergic skin disease during the 6 month treatment period.

## DISCUSSION

A total of four positive intradermal reactions occurred prior to hyposensitization in three dogs despite their lack of clinical signs. False positive or irrelevant intradermal reactions have been reported by other investigators, and are thought to represent irritant reactions, prior or subclinical hypersensitivity, or individual variation in normal skin threshold concentration for allergens (2, 14, 15). Threshold concentration of each antigen should be used for intradermal testing, and is the maximum concentration for which a minimum number ( $\leq 2\%$ ) of normal dogs will exhibit a positive reaction (14). None of the four allergens produced a positive reaction following hyposensitization, even though only one was included in the hyposensitization mixture. Lack of reproducibility in 4% of duplicate antigen injections has been previously reported (16), and could explain the difference in intradermal test results before and after hyposensitization. The number of positive reactions did not increase following hyposensitization, and no more positive reactions were produced by antigens included in the treatment mixture than by control antigens. These findings indicated that hyposensitization according to this protocol did not induce positive intradermal reactions.

Previous studies have suggested that positive intradermal reactions, and even clinical hypersensitivity, may be induced by hyposensitization with irrelevant antigens (8–10). The increasing popularity of serologic allergy tests has increased the likelihood that both atopic and nonatopic animals will be treated with irrelevant allergens, and has raised concern about the potential for inducing hypersensitivity. The study reported here did not substantiate this concern. Hyposensitization of normal greyhounds with irrelevant aqueous allergens at a 1:200 w/v dilution for 6 months did not cause a significant increase in dermal reactivity on subsequent intradermal allergy tests. No clinical signs of allergic skin disease developed in any of the dogs during the 6 month study period.

This study used normal dogs rather than atopic dogs or nonatopic, pruritic dogs that typically would be tested for atopy in a clinical situation. Atopic dogs or dogs with other types of allergic skin disease may be more easily sensitized through repeated antigen exposure. The hyposensitization mixture used in this study contained 2500 PNU.ml<sup>-1</sup> (1:200 w/v), although a more concentrated treatment mixture (1:40 w/v or 12,500 PNU.ml<sup>-1</sup>) is also recommended by the same laboratory for unresponsive cases. Other hyposensitization protocols use treatment solutions containing 10,000–20,000 PNU.ml<sup>-1</sup>. It is possible that more concentrated hyposensitization mixtures may induce positive intradermal reactions or clinical hypersensitivity to irrelevant antigens.

Variations in experimental design between this study and previous studies may explain conflicting

results. Turkeltaub found that a persistent hypersensitivity was induced in nonatopic human volunteers following parenteral immunization with rye grass (8). Alum-absorbed rye grass allergen was used in that study, while aqueous antigens were used in the present study. It is unknown whether alum-absorption of the allergen enhances IgE response. Turkeltaub also assessed the degree of sensitivity to rye grass by measuring endpoint dilutions. The volunteers developed a skin reaction to an endpoint concentration of  $\leq 10^{-2}$   $\mu\text{g.ml}^{-1}$ , whereas patients naturally allergic to rye grass pollen showed skin reactions to  $\leq 10^{-4}$   $\mu\text{g.ml}^{-1}$ . In the present study endpoint dilutions were not used; instead, dogs were tested with the standard antigen concentration (1000 PNU.ml<sup>-1</sup>) used to identify atopic animals in the United States. Developing hypersensitivity may have been identified by using higher test concentrations of the antigen, provided this concentration did not cause irritant reactions. Arkins found that intradermal reactivity was induced in nonatopic dogs after four injections of ragweed emulsion (9). A necrotizing granulomatous lesion occurred at injection sites in one dog, and this dog also developed the highest skin-sensitizing antibody titre. An inflammatory reaction to the emulsion similar to that produced by Freund's adjuvant may have augmented the immune response in these dogs (9). However, Schmeitzel found that repetitive intradermal allergy testing with aqueous antigens also induced positive intradermal reactions in normal dogs (10). Although aqueous antigens were used in both Schmeitzel's study and the present study, the route of antigen administration was different and may have affected the immune response. Finally, antigens used for hyposensitization in the present study were obtained from a different source than those used for intradermal testing. Different antigenic determinants that do not cross-react on intradermal testing may have been present in the different allergen preparations.

In conclusion, hyposensitization with irrelevant aqueous antigens at a concentration of 1:200 w/v did not appear to cause false positive reactions on subsequent intradermal allergy tests or to induce clinical hypersensitivity in normal greyhounds. Dogs that have shown no clinical improvement following hyposensitization with this protocol probably can be reliably evaluated with intradermal allergy testing. It should be noted, however, that repeated exposure to antigens under certain circumstances may induce positive intradermal reactions or even clinical hypersensitivity; therefore, exposure to irrelevant antigens should be limited. The present study is limited by the low number of dogs tested and by the use of greyhounds that are not predisposed to atopic disease. Further studies are required to determine if hypersensitivity to irrelevant antigens would be induced in atopic dogs following exposure to nonaqueous extracts or to higher concentrations of aqueous antigens.

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**Résumé**—Cinq Greyhound sains ont été utilisés pour étudier l'hypersensibilité à des pollens, des moisissures des arbres et des herbacées en utilisant des intradermoréactions et des tests ELISA du commerce. Tous les animaux ont été désensibilisés pendant 6 mois avec le même mélange de 14 allergènes, en suivant le protocole recommandé par le laboratoire qui commercialise le test ELISA. Après hyposensibilisation à ces antigènes à une concentration de 1/200 p/v, les chiens ont été réévalués par des intradermoréactions. Aucune différence significative avant et après traitement n'a été notée et tous les chiens sont restés asymptomatiques durant la période de l'essai.

L'hyposensibilisation chez des Greyhounds sains avec des antigènes auxquels ils ne sont pas sensibles, en suivant le protocole recommandé par le laboratoire producteur, ne semble pas entraîner de fausses réactions intradermiques positives ni une hypersensibilité clinique. D'autres études sont nécessaires pour savoir si cette sensibilisation peut être induite chez des animaux atopiques, avec des extraits non aqueux et à de plus fortes concentrations. [Codner, E. C., Lessard, P., Effect of hyposensitization with irrelevant antigens on subsequent allergy test results in normal dogs (Effet d'une hyposensibilisation à l'aide d'allergènes sur les résultats des tests allergologiques chez des chiens sains). *Veterinary Dermatology*, 1992; 3: 209–214].

**Zusammenfassung**—Fünf gesunde Greyhounds wurden auf allergische Reaktionen gegenüber verschiedenen Gräsern, Unkräutern, Bäumen und Pilzen sowohl mit einem Intradermaltest als auch mit einem kommerziellen ELISA-Test getestet. Alle Hunde wurden über 6 Monate mit derselben Mischung aus 14 Allergenen "desensibilisiert". Hierzu wurde ein Therapieschema verwendet, das von dem Labor, das den ELISA-Test durchgeführt hatte, empfohlen worden war. Nach der "Desensibilisierung" mit nicht relevanten Allergenen in einer Konzentration von 1:200 w/v wurden die Hunde erneut mit einem Intradermaltest getestet. Nach der "Desensibilisierung" über sechs Monate gab es keinen signifikanten Anstieg bei den intradermalen Reaktionen. Alle Hunde blieben während der Untersuchungszeit symptomfrei.

"Desensibilisierung" von gesunden Greyhounds mit nicht relevanten wässrigen Antigenen, die nach dem empfohlenen Therapieschema nach einem ELISA-Allergietest angewendet worden waren, schienen keine falsch-positiven Reaktionen im nachfolgenden Intradermaltest hervorzurufen und keine klinische Allergie auszulösen. Weitere Untersuchungen sind erforderlich, um zu klären, ob eine Allergie gegenüber nicht relevanten Antigenen bei atopischen Hunden nach Desensibilisierung mit wässrigen Extrakten oder mit höheren Konzentrationen von wässrigen Allergenen induziert werden kann. [Codner, E. C., Lessard, P. Effect of hyposensitization with irrelevant antigens on subsequent allergy test results in normal dogs (Auswirkung einer Desensibilisierung mit nicht relevanten Antigenen auf das Ergebnis des nachfolgenden Allergietests). *Veterinary Dermatology*, 1992; 3: 209–214].

**Resumen**—Cinco perros galgos de apariencia clínica normal, se evaluaron con tests de alergia a varias hierbas, árboles y hongos, por medio de inyecciones intradérmicas y también con ensayos comerciales de enzima de inmuoabsorbencia ligada (ELISA). A todos los perros se les administró tratamiento de hiposensibilización por un curso de 6 meses con una mezcla de 14 alergenos, según el protocolo recomendado por el laboratorio comercial proveedores del test ELISA. Después del tratamiento inmunosupresivo con una concentración de antígenos irrelevantes de 1:200 w/v, los animales se volvieron a evaluar por medio del test de inyecciones intradérmicas. Después de 6 meses de terapia inmunosupresiva no se observaron cambios significativos en las reacciones cutáneas, y todos los animales permanecieron asintomáticos durante el periodo de estudio.

El tratamiento inmunosupresivo de perros galgos con antígenos en solución acuosa administrados de acuerdo con el protocolo recomendado después del test ELISA, no produjo reacciones falsas positivas en tests intradérmicos posteriores, o reacciones de hipersensibilidad alguna. Para determinar si la posibilidad de hipersensibilidad con antígenos irrelevantes es inducida en perros que padecen atopia después del tratamiento inmunosupresivo con extractos antigénicos de tipo no acuoso, o mayores concentraciones de antígenos acuosos, más investigaciones son necesarias. [Codner, E. C., Lessard, P. Effect of lyposensitization with irrelevant antigens on subsequent allergy test results in normal dogs (Efecto del tratamiento inmunosupresivo con antígenos irrelevantes en los resultados de tests de alergia llevados a cabo posteriormente, en perros clínicamente normales). *Veterinary Dermatology*, 1992; 3: 209–214].