

Sensitization of Naive Beagles by Intradermal Injection of an *Ascaris* Antigen: Induction of a Model of Skin Allergy

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Ascaris suum-hypersensitive beagles represent a unique model of skin allergy. Despite its suitability, the need to test numerous dogs prior to the selection of natural positive responders causes many complications. We hypothesized that the model could be induced in adult beagles by primary sensitization. Ten dogs were included in an intradermal sensitization program using *ascaris* antigen and were thereafter tested for cutaneous reaction. After 2 weeks, 60% developed a positive reaction that lasted for months. We therefore present a straightforward method to establish the *Ascaris* canine model of allergy that will extend its availability for research in cutaneous allergy immunomodulation.

Keywords Atopic Dermatitis, *Ascaris suum* antigen, Canine Model, Intradermal Sensitization.

INTRODUCTION

Canine spontaneous hypersensitivity to *Ascaris suum* antigens (Ag) has been established as a model of allergy.^(1–6) Exposure of predisposed dogs to the *Ascaris suum* Ag⁽⁷⁾ induces an IgE-mediated reaction^(1,8,9) that is manifested in the skin as a wheal⁽⁶⁾ followed by an allergic inflammatory process⁽¹⁰⁾ similar to that observed in the airways after exposure to the Ag.^(2,11) Usually, the selection of dogs that are naturally hypersensitive to *ascaris* is done for research purposes on the basis of their response to a diagnostic intradermal (ID) injection of *ascaris* Ag (intradermal test or IDT).⁽⁵⁾ Despite the suitability

of the model, selection of hypersensitive dogs presents scientific complications (age, sex, and often breed heterogeneity) along with economic and practical considerations caused by the need to test numerous dogs prior to selection of positive responders. This process restricts accessibility of the model and represents a problem when trying to select a homogeneous population of dogs.

In addition to early and late effector mechanisms in the skin, ID injection of the purified Ag may induce a primary immunological response.^(12,13) The ability to induce sensitization in a canine model would improve the suitability, usefulness, and availability of the model for studies of the allergic response in skin. Interestingly, sensitization through subcutaneous injection of the allergen has been established in puppies with high levels of IgE.⁽¹⁴⁾ However, the immunological mechanisms underlying this subcutaneous approach may differ from those associated with native exposure to the allergen, since in both spontaneous human and canine atopic dermatitis, but not in the subcutaneous model, IgE-bearing Langerhans cells are seen in the epidermis.^(15,16) Although much work needs to be done to elucidate the early sensitizing events, ID injection may share more immunological mechanisms with percutaneous exposure.

Given the usefulness of *Ascaris suum* hypersensitive dogs compared with other models for research into the mechanisms and modulation of skin allergy, we evaluated primary sensitization of naive adult beagle dogs through dermal exposure to the *Ascaris suum* allergen Asc S1 as a straightforward method for the experimental induction of this canine model of skin hypersensitivity.

MATERIALS AND METHODS

Naive dogs

All animal procedures were approved by the *Ethics Committee for Animal Research of the Universitat Autònoma de Barcelona*. Fifty dogs from a colony of adult beagles were tested for their sensitivity to ID injection of the *Ascaris suum* Ag Asc S1 (Greer Laboratories, Lenoir, NC, USA) and a group of 1- to 2-year-old beagles was selected ($n=10$). The dogs were bred for experimental purposes at Isoquimen S.L. (Barcelona, Spain), where they were kept under very close veterinary surveillance and strict sanitary conditions. All the tested dogs were unresponsive to ID injection of ascaris Ag prior to initiating the study and therefore, qualified as naive for this particular Ag. The lack of response to ID injection of the Ag probably reflects the efficacy of the deworming program used at Isoquimen S.L. (as confirmed by the lack of ascaris parasites in feces). In this program, 1-, 3-, and 5-week-old puppies received 100 mg/7 kg pirantel pamoate (Canex; Pfizer, S.A. Madrid, Spain) followed on week 7 by 2 g/kg of the ascaricide piperacine citrate (Ascaricida-N; Pfizer, S.A.), and finally, when they reached 11 weeks old by a tablet (per 10 kg) of 50 mg pirantel

pamoat, 50 mg praziquantel, and 150 mg febantel (Drontal Plus, Bayer Química Farmacéutica, Barcelona, Spain).

Skin Sensitization Protocol

The 10 selected naive beagles underwent a skin sensitization protocol, the goal of which was to expose the dermis to the purified Ag. First, 2 ID injections of a high dose ascaris Ag (Asc S1) were administered. The injections were performed on day 0 and day 7 in the dorsal skin area. Primary sensitization was achieved by injecting 10 μ l of 100 μ g/ml Asc S1. The Ag was injected intradermally to avoid licking (therefore prevent sensitization through other routes) and to ensure that the entire dose of the protein allergen was in contact with the dermis for a sufficient time.

Diagnostic Intradermal Test

An intradermal test (diagnostic IDT) was performed to diagnose canine hypersensitivity to Asc S1, i.e., to discriminate between Ag-sensitive and insensitive dogs and to reveal the presence of Ag-specific IgE. Briefly, 10 μ L of purified Ag (10 μ g/ml) were injected in the dorsal area. The skin reaction at the Ag-injection site was always compared with a parallel negative control (ID saline injection). The intensity of the reaction to both the Ag and the saline control was assessed 20 min after ID injection. In addition to the screening of the Isoquimen colony described above, the diagnostic IDT was performed in the selected animals to assess sensitivity to the Ag prior to the injection (day -1), and 7 and 14 days after initiating the skin sensitization protocol. After 2 months, a new diagnostic IDT was performed to evaluate the sustainability of the hypersensitivity to the Ag.

Assessment of Skin Reaction to Ag

The intensity of the immediate skin reaction (local edema) was expressed in terms of the wheal area (mm^2). Briefly, the study monitor used tracing paper to outline the wheal perimeter. The area of the wheal was then assessed using a scanning and imaging software system (Mip 4 Advanced, Consulting de Imagen Digital SL, Spain). To control for nonspecific skin reaction, the effect observed at the control saline-injected site was subtracted from the effect observed at the Ag-injected site and the net wheal area calculated. An area under 30 mm^2 was considered negative. Statistical comparisons were performed using the unpaired Student's *t*-test.

Beagle Clinical Evaluation

The selected dogs were under clinical observation throughout the study. Particular attention was paid to the clinical condition of the skin at the injection sites. Overall condition, including body temperature, heart and respiratory

rates, mucosal membranes, and lymph nodes, was assessed for potential disorders. Additionally, dog feces were analyzed by microscopy for ascaris eggs at various points during the study.

RESULTS AND DISCUSSION

No cutaneous reaction was observed at day -1 in any of the selected adult dogs, indicating that none had developed hypersensitivity to the Ag prior to initiation of the skin sensitization protocol. As early as the 7th day, 4 of the 10 dogs showed a clear reaction to the diagnostic IDT. A second sensitizing boost was then administered (day 7), and at day 14, 2 additional beagles showed clear skin reactions to the diagnostic IDT. Thus, 60% of the otherwise-naïve dogs developed skin reaction after local administration of the Ag. This result is particularly relevant in the light of a recent report showing that the IDT is a more sensitive assay of the presence of IgE than the direct assessment of specific serum IgE levels in an allergic dog model.⁽¹⁷⁾ Thus, in mechanistic terms, 60% of the dogs that had no specific sensitizing anti-Asc S1 IgE antibodies prior to the study, most probably produced these antibodies after dermal contact with the Ag.

Two dogs had no reaction whatsoever, either before or after primary sensitization. Two other dogs exhibited a very low response ($<30 \text{ mm}^2$), that was considered negative. Two groups could therefore be established: those that became hypersensitive and those that did not. Figure 1 depicts the average course of the skin reaction in the 6 positive responders and the 4 negative responders. Although further analysis will be necessary, it is likely that one of the differences between the two groups is their genetic background, possibly a predisposition to develop an IgE-oriented response.⁽¹⁸⁾ Given the inheritance trait of the atopic state, inbreeding of laboratory beagles may well be a major contributing factor to the high proportion of responding animals.

To confirm the sustainability of the induced hypersensitivity state, we showed that the 6 dogs that became positive responders on day 14 remained positive 2 months later. This finding indicates that these experimentally sensitized animals will be useful for mid- to long-term studies with only occasional boosts. It is important to note that the diagnostic IDT injections may themselves have contributed to boost the allergic response. Finally, clinical evaluation of the dogs throughout the study revealed no additional symptoms, lesions, or apparent signs of clinical disorders. Furthermore, no eggs or adult parasites were identified during the study.

CONCLUSION

We have induced primary sensitization in otherwise naïve adult dogs through the use of a straightforward method of dermal exposure to an Ag. The

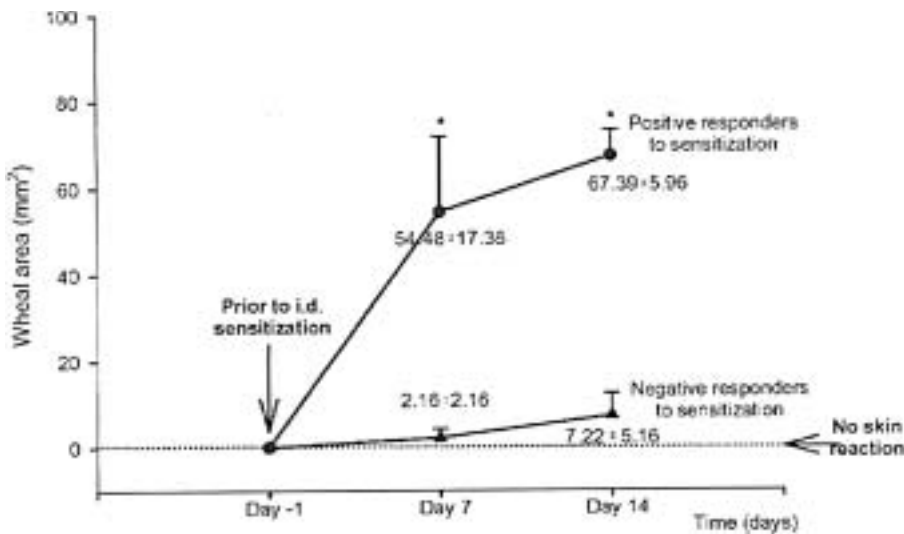


Figure 1: Average skin reaction to diagnostic intradermol test in the positive and negative responders. The average Ag-induced wheal area in dogs that responded positively to the primary sensitization (circle; mean $\pm$ SEM; $n=6$) is depicted compared with the reaction of the nonresponders (triangle; mean $\pm$ SEM). Comparisons of the wheal area of the two groups were made by unpaired Student's *t*-test (* $p < 0.05$).

proposed method overcomes the scientific, practical, and economic difficulties associated with the selective screening of dogs and precludes the need to initiate sensitization at birth.^(14,19) Therefore, these findings will broaden the value of the *Ascaris suum* Ag-hypersensitive canine model of cutaneous allergy by providing a way to generate hypersensitive dogs rather than select spontaneously sensitized animals.

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