Airway Responses of Dogs with Ragweed and Ascaris Hypersensitivity*

Roy Patterson, M.D.; C. J. Mellies, M.D.; Joseph F. Kelly, M.D.; and Kathleen E. Harris, B.S.

The occurrence and induction of immediate-type airway responsiveness to standardized ascaris antigen (SAA) was studied in dogs. Two of nine dogs with significant cutaneous reactivity to SAA had respiratory responses on initial aerosol challenge. Of the remaining seven dogs, six developed respiratory responses to SAA within three aerosol exposures. The respiratory responsiveness is of long duration, persisting at least four months, when the animals are exposed biweekly. Reaginic antibody against AA can be detected in the respiratory secretions of dogs sensitive to ascars and, although this increases after antigen exposure, the increase in titer was not markedly different from the increase in serum titer. In contrast, no reaginic antibodies were detected in the RS of dogs allergic to ragweed, with respiratory responses. Respiratory function abnormalities in the canine respiratory response to ascars were accompanied by changes in pulmonary resistance, which may precede other abnormalities during the development of respiratory responsiveness.

Immunologically mediated, immediate-type airway responses due to reaginic antibodies in dogs and rhesus monkeys provide systems for study of these reactions under conditions of controlled antigen delivery. Studies of the characteristics of the reaginic antibodies responsible for these reactions indicate that they are canine and rhesus analogues of human reaginic antibodies of the IgE class. Animals with this respiratory reactivity provide a means of study of the physiology of the response, the effect of pharmacologic agents, the reactive mast cells obtained by biopsy from the bronchial mucosa or the free mast cells and basophilid cells which appear in the lumen of the bronch. Dogs with immediate-type respiratory responses to nematode antigens have provided a particularly useful subject for study of reflex mechanisms involved in airway responses.

The respiratory reactivity to environmental antigens such as ragweed pollen antigen occurring as a result of environmental exposure has been described in dogs. This is the only species other than man in which defined studies have correlated clinical disease and exposure to environmental aeroallergens. In both dogs and monkeys, the respiratory reactivity to antigen may be of long duration. It has been present for up to 3 years in monkeys and 13 years in a dog sensitive to ragweed.

Allergy to ragweed occurs in dogs but has not been found in monkeys. Although respiratory responses to ascars antigen occur in monkeys, this reactivity occurs in a limited population of these animals and the degree of allergic sensitivity necessary for the respiratory responses has not been induced by aerosolization or injection of ascars antigen. The respiratory reactivity in these monkeys frequently disappears in spite of repeated respiratory antigenic challenge.

In contrast, we observed that respiratory responses to ascars antigen could be induced in dogs in whom airway responses did not occur at the time of initial antigen challenge. Because this canine respiratory response to ascars antigen provides the most readily available experimental animal system for study of a reagin-mediated, immediate-type airway response, the current studies were done to determine the occurrence of this response in a group of dogs, the time required for induction of a response in those dogs initially unresponsive and whether reaginic antibodies could be found in the respiratory secretions of these dogs. The subjects were selected only on the basis of cutaneous reactivity to purified ascars antigen which occurs in a high percentage of unselected dogs.

Materials and Methods

Animals

For ascars studies, dogs of mixed breed in apparent good health were used. They were selected for this study only on the basis of cutaneous reactivity to 1:1,000 or 1:10,000 dilutions of purified ascars antigen. This degree of reactivity occurs in a majority of dogs. Dogs allergic to ragweed were animals with clinical sensitivity to environmental ragweed pollen, positive cutaneous reactivity to ragweed antigen and immediate-type respiratory response to aerosolized ragweed antigen following controlled laboratory exposure.

Antigens

A purified preparation of standard ascars antigen (SAA) was prepared from a saline extract of a homogenate of Ascaris suum by a cross-linked dextran gel (Sephadex) G 50 and G 75 fractionation using the method of Hogarth-Scott. The SAA was standardized by cutaneous testing in dogs sensitive to ascars. The purified SAA used in these experiments contained 0.70 mg N/ml dilution of AA were made.

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Reprint requests: Dr. Patterson, 303 East Chicago Avenue, Chicago 60611

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in sterile 0.15 M phosphate buffered NaCl, pH 7.35. Ragweed antigen was an extract of *Ambrosia elatior* obtained from Hollister-Stier Laboratories. Ragweed antigen is expressed as weight/volume concentrations. A 1:50 concentration was used for aerosol challenge. Serial tenfold dilutions beginning with a 1:1,000 concentration were used for skin testing.

**Cutaneous Testing Procedures**

Active cutaneous testing was done following intravenous injection of the dog with 5 ml of 0.5 percent Evans blue dye. The dogs were next injected intracutaneously with 0.1 ml of serial tenfold dilutions of SAA. Passive cutaneous skin tests were done by injecting 0.1 ml of dilutions of serum or respiratory secretions intracutaneously into normal recipients. For studies of reaginic antibodies, these recipients were dogs that did not react to a $10^{-2}$ dilution of AA. Passive transfer studies for antiragweed reagin were done in recipients with no cutaneous reactivity to RW antigen. Passively sensitized sites were challenged 48 hours later by injection of the test site with 0.1 ml of the respective antigen.

**Respiratory Aerosol Challenge**

Techniques used for the controlled delivery of antigen to the lower respiratory tract were those previously described. Briefly, dogs were anesthetized with pentobarbital and a cuffed endotracheal tube inserted. Delivery of antigen to the bronchi was accomplished by a Bird Mark 7 respirator with an in-line nebulizer. The same amount of antigen was delivered in separate experiments by controlling the number of inhalations, and the concentration of antigen.

**Bronchial Lavage**

Dogs were anesthetized with pentobarbital and the tracheas visualized by direct bronchoscopy. The bronchial tree was lavaged with 100 ml of sterile 0.15 M NaCl. Thirty to 40 ml of the saline and respiratory secretions were reserved from each wash.

**Respiratory Secretions (RS)**

The bronchial washings were concentrated in dialysis tubing to a volume of 1 ml. The RS from different washings were standardized to approximately the same concentration by both the optical density as determined by a Beckman DU spectrophotometer at 500 nm and by their IgA content. For this procedure, rabbit anticanine IgA was prepared and Mancini radial immunodiffusion plates were poured. Prior to use, the RS samples were concentrated or diluted to the same IgA ring diameter as a standard RS used for comparison using techniques previously described.

**Measurement of Lung Function**

The following parameters of respiration were measured in this study: frequency of respiration (*f*); peak expiratory flow rate (PEFR); expiratory time/inspiratory time ratio (E/I R); tidal volume (TV); minute volume (MV); respiratory resistance (PR); and compliance (C). The systems used have been described in detail. The computation of PR and C was performed by an on-line analog computer which has been used for studies of this type in dogs.

**RESULTS**

**Initial Responsiveness, Induction of Respiratory Responsiveness and Duration of Responsiveness to Ascaris Antigen Aerosol Challenge**

Dogs selected by cutaneous titration to SAA and having cutaneous dilution titers of $10^{2}$ to $10^{4}$ received repeated aerosol challenge consisting of 15 breaths of a 1:20 dilution of SAA. The results (Table 1) show that two of nine dogs had a respiratory response to aerosol challenge at initial exposure to the aerosolized antigen. Although seven dogs showed negative respiratory responses to the initial aerosol challenge, six of these seven dogs had positive respiratory responses at the second or third aerosol exposure to SAA. One dog did not develop respiratory reactivity in spite of repeated delivery of antigen to the respiratory tract. The respiratory reactivity which was present initially or which developed as a result of aerosol exposure was persistent in all animals except one. This dog (dog 1, Table 1) had negative respiratory response on the 13th and 15th week of the experiment. She was recognized as being pregnant, although this was not part of the experimental protocol. Subsequent to the delivery in week 16, respiratory responses to aerosolized AA were again positive. Whether the response in this animal became negative due to the pregnancy cannot be established from this single subject.

**Pulmonary Function Measurement Following Aerosol Challenge with SAA**

Previous studies of respiratory responses to SAA in dogs showed an increase in *f*, E/I R, and a decrease in PEFR. Similar changes occur in monkeys, and additional studies have shown an increase in

<table>
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<th>Dog No.</th>
<th>Initial Cutaneous Titer</th>
<th>Week 1</th>
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<th>Week 5</th>
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*Reciprocal of highest serial tenfold dilution of ascaris antigen.

**Dog pregnant**

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in PR and a decrease in TV and C. The development of a positive respiratory response to aerosolized SAA in dog 7 (Table 1) is shown in Figure 1. A negative response (Fig 1A) shows minimal changes in all parameters of pulmonary function, but less than 50 percent change in any parameter and no consistent change in the direction of positive or negative. In the second challenge of dog 7 (Fig 1B) a marked change in PR occurred without characteristic changes in other parameters. This is interpreted as a positive response because of the PR change. The characteristic severe responses are shown in Figure 1C and Figure 1D, in which increases of PR, E/I R and f of more than 50 percent and decreases of PEFR, TV and C of more than 50 percent are seen.

The increasing abnormalities of pulmonary function following four successive aerosol challenges of dog 9 (Table 1) are shown in Figure 2. Here, a negative response (Fig 2A) is followed by increasingly abnormal responses (Fig 2B 2C and 2D) without a marked increase in PR preceding the other changes.

Two responses of dog 8 (week 5 and 7, Table 1) are shown in Figure 3. These show that, although the abnormalities of pulmonary function described

Figure 1. Pulmonary function parameters of dog 7 (Table 1) following successive aerosol challenges with ascaris antigen. A, first challenge; B, second challenge; C, third challenge; D, fourth challenge.

Figure 2. Pulmonary function parameters of dog 9 (Table 1) following successive aerosol challenges with ascaris antigen. A, first challenge; B, second challenge; C, third challenge; D, fourth challenge.
above all occur and are in the same direction of negativity or positivity, rates of recovery may vary between different dogs and different experiments in the same dog. In addition, peak abnormalities of pulmonary function may not occur simultaneously as shown by the differences in time when the peak abnormalities of PR and F occur (Fig 3A, B).

**Studies of Reaginic Antibodies in Respiratory Secretions (RS) and Serum (S) and Comparative Titers in RS and S Before and After Repeated Aerosol Challenge with Antigen**

Dogs received aerosol challenge with antigen on alternate weeks, with bronchial lavage to obtain RS on the weeks when there was no challenge with antigen. Serum samples were obtained simultaneously with the RS samples. Passive cutaneous transfer studies with RS samples concentrated to the same OD and IgA content were obtained from four dogs prior and subsequent to the development of respiratory responsiveness to SAA. RS and serum samples from dogs with R sensitivity were obtained prior and subsequent to aerosol challenge with RW antigen, although these animals demonstrated respiratory responses to RW on initial aerosol challenge with antigen. The results (Table 2) demonstrate that reaginic antibodies against SAA can be detected in the RS of dogs sensitive to ascaris. In contrast, no reaginic antibodies were detected in the RS of dogs sensitive to ragweed, although the serum reaginic antibody titers of these were of similar magnitude to those sensitive to ascaris.

One objective of this study was to determine if the respiratory responsiveness to SAA which occurs in these animals was accompanied by a differential increase in reaginic antibody in RS above that occurring in serum. This could suggest that local production of reaginic antibodies in the respiratory

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<th>Challenging Antigens</th>
<th>Ascaris</th>
<th>Ragweed</th>
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<tr>
<td>Dog No.</td>
<td>1  2  3  6</td>
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<td>Response to aerosol challenge with antigen</td>
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<td>late**</td>
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<td>Direct cutaneous skin test titers‡</td>
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<tr>
<td>late**</td>
<td>3 3 3 4</td>
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*Results of initial aerosol challenge with antigen or reaginic antibody titers prior to aerosol exposure to antigen

**Results of exposure to aerosol challenge with antigen or reaginic antibody titers after four exposures to antigen (Table 1)

†Expressed as log of highest serial twofold dilution of serum or respiratory secretion giving positive transfer.

‡Expressed as log of highest serial tenfold dilution of ascaris or ragweed antigen giving positive skin test.
tract could explain the development of respiratory responsiveness in these animals. The results of the studies (Table 2) demonstrate that, although there was an increase in the reaginic antibody in RS of the three dogs that developed respiratory responsiveness to SAA, this was only one, two or three dilutions, respectively. There was an increase in serum reaginic antibody titer of one or two dilutions in two of the dogs that developed respiratory responsiveness to SAA. Thus, suggestive but not definite evidence for differential increase in RS reaginic antibodies in response to aerosol exposure to SAA was obtained. Serum reaginic antibodies in dogs allergic to ragweed, with exposure by aerosol increased one to two dilutions in two of three dogs but no reaginic antibodies in the RS of these dogs was demonstrable.

**DISCUSSION**

The studies reported here demonstrate the availability of dogs with respiratory responsiveness to ascaris for experimental study. If the dogs with significant cutaneous reactivity to ascaris, which exists in a significant percentage, do not respond to aerosolized ascaris on initial challenge, all but one dog responded on subsequent challenges. There is a significant species difference between dogs and rhesus monkeys in this respect. We have observed that certain rhesus monkeys with cutaneous reactivity to SAA like dogs, will respond to aerosolized ascaris challenge. If they do not respond on initial challenge, repeated aerosolization of ascaris antigen does not induce respiratory responses in rhesus monkeys as it does in dogs. In addition, certain rhesus monkeys which respond initially to aerosolized ascaris lose this respiratory response in spite of periodic challenge. There is currently no explanation for this species difference.

We have provided evidence that the respiratory tract of the dog responds with local antibody synthesis of the IgG class in addition to an IgA type of response when the animals were stimulated by aerosolized keyhole limpet hemocyanin. An explanation for the induced respiratory response to SAA in these experiments could be a local immune response of reaginic antibody in the dog lung, following aerosolization of SAA. Although the results reported here show an increase in titer of reaginic antibody to SAA in RS, the minimal rise in RS reaginic antibody as compared with serum reaginic antibody did not justify a conclusion that there was a differential response in the respiratory tract. It was expected that the dogs with respiratory responses to ragweed would have reaginic antibody against ragweed antigen. This was not found in RS of any of the three dogs studied, although their serum reaginic antibody titers were comparable to the serum reaginic antibody titers against ascaris in three of six samples.

Abnormalities of pulmonary function following ascaris antigen challenge in dogs are similar in time of onset and character to those described in rhesus monkeys. Similar function studies in dogs sensitive to ragweed are not reported here because the system of analysis requires anesthetized animals. We have observed that aerosolized ragweed antigen to anesthetized dogs sensitive to ragweed may result in respiratory arrest and the value and limited availability of these dogs is such that these experiments have not been performed.

**REFERENCES**

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