

PULMONARY IMMUNITY TO RAGWEED IN A BEAGLE DOG MODEL OF ALLERGIC ASTHMA

Tamara K. Redman, Karin Rudolph, Edward B. Barr, Larry E. Bowen, Bruce A. Muggenburg, and David E. Bice □ Lovelace Respiratory Research Institute, Albuquerque, New Mexico, USA

□ *To create an allergy model in the dog, allergic Beagles with high levels of serum immunoglobulin E (IgE) and eosinophilia were bred; resulting puppies were sensitized to ragweed by intraperitoneal (IP) injection within 24 hours of birth through 22 weeks of age. At least 50% of the puppies developed high levels of serum IgE and eosinophilia. As young adults, 6 of these dogs, and 6 control age-matched, nonallergic, nonimmunized dogs were exposed by inhalation to ragweed twice at 13-day intervals, and a third time 45 days later. Total and ragweed-specific serum IgE and ragweed-specific serum IgG were increased significantly in allergic dogs relative to baseline. Allergic dogs had significantly greater levels of antibody specific for ragweed, as well as higher eosinophil counts in the bronchoalveolar lavage fluid, compared to nonallergic dogs. Airway reactivity to histamine in allergic, but not nonallergic dogs, increased significantly after aerosol exposure to ragweed. After a third exposure to ragweed, airway responses to histamine were elevated in the allergic dogs and remained high for at least 5 months. These results demonstrate the potential of the allergic dog model for investigating the underlying pulmonary immune mechanisms and therapeutic treatment of allergic asthma.*

Keywords airway reactivity, canine allergy model, eosinophils, IgE, pulmonary immunity

Allergic asthma is a chronic inflammatory disease of the entire lung [1, 2] characterized by lymphocytes [3–5], macrophages [4], eosinophils [3, 4], neutrophils and plasma cells [6], and mast cells [7] infiltrating the bronchial mucosa. This chronic inflammation leads to airway obstruction and hyperreactivity [8, 9], excessive mucous production [7], epithelial desquamation, goblet cell metaplasia and hyperplasia, and thickening of the submucosa [10]. The incidence and prevalence of asthma are increasing in the United States [11], as well as in other developed countries [12], but the reasons for this increase are unknown. However, several possible

Received 15 July 2000; accepted 27 November 2000.

The authors thank Ms. Selita Lucas for performing the ragweed aerosol exposures. This research was conducted by the National Environmental Respiratory Center with support from the U.S. EPA under Coop. Agree. CR826442-01-1 with LRRI. This report has not been subjected to the Agency's peer or policy reviews. Therefore, it does not necessarily reflect the views of the Agency. No official endorsement should be inferred.

Address correspondence to David E. Bice, Lovelace Respiratory Research Institute, P.O. Box 5890, Albuquerque, NM 87185-5890, USA. E-mail: dbice@lrri.org

explanations have been proposed, including increased exposure to environmental pollutants [13–17] and antigens [18], as well as decreased bacterial infections that may be important in developing protective (Th1) immune responses localized to the lungs [19–21]. Without bacterial infections, allergic Th2 immunity may be more likely to develop in response to inhaled allergens. Our data and the data of others indicate that allergic immune responses localized to the lungs cause asthma [22–25]. Although rodents can be used to study some mechanisms of asthma, our data indicate that rodents do not develop localized immune responses in the lungs as seen in the lungs of dogs, primates, and humans [26–29].

Understanding the factors that result in aberrant immunity leading to allergic asthma requires the use of an animal model demonstrating immune responses that reflect those observed in human allergic asthma. In addition to the development of localized pulmonary immunity as observed in humans, dogs develop clinically important allergic disease. Atopic dogs have been used successfully as a model for food allergy [30]. At Lovelace Respiratory Research Institute (LRRI), we have produced allergic Beagle dogs by breeding males and females having high levels of immunoglobulin E (IgE) and eosinophilia. As is commonly observed in humans, at least half of the offspring of allergic Beagles have high levels of IgE and eosinophilia and will develop allergic immunity after sensitization with allergen. These allergic dogs develop features and characteristics of allergic asthma similar to the responses observed in human asthmatics, including immune responses localized to the lungs [31]. The data presented here demonstrate the utility of allergic dogs for investigating the underlying pulmonary immune and cellular mechanisms of allergic disease.

MATERIALS AND METHODS

Experimental Animals

Beagles were born and maintained at the LRRI closed dog colony. The dogs were housed in kennel buildings with indoor and outdoor runs. Floors were heated in the winter and cooled in the summer. Each dog was fed 350 g of dry dog food once a day (Wayne Mini Lab Dog Diet 8759; Teklad Premier Laboratory Diets, Madison, WI), and water was available at all times. Food was withheld for 18 hours prior to administering the anesthetic agent, isoflurane (Abbott Laboratories, North Chicago, IL).

Within 24 hours of birth, Beagle puppies were sensitized by intraperitoneal (IP) injection with 500 µg of ragweed allergenic extract (Ragweed Short, *Ambrosia artemisiifolia*, Bayer Corporation, Elkhart, IN) combined with aluminum hydroxide (Accurate Chemical and Scientific, Westbury, NY), followed by weekly IP injections through week 4, subcutaneous (SC) injections with 50 µg of ragweed combined with aluminum hydroxide weekly from

weeks 5 through 8, and biweekly from weeks 10 through 22. Six ragweed-sensitized and 6 age-matched, non-ragweed-sensitized control dogs were chosen for this study. All 12 dogs were exposed by inhalation to ragweed extract. An estimated 500 μg of ragweed protein (1.9 to 2.7 mg total particulate matter) was deposited in the lungs during 2 exposures 2 weeks apart, followed 45 days later by a third exposure.

All animal procedures for this study were reviewed and approved by the LRRI Institutional Animal Care and Use Committee. The Institute is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Inhalation Exposures

A diagram of the exposure system is illustrated in Figure 1. Inhalation exposures were done using a dynamic exposure system that allows continuous nose-only inhalation using a 6-port canine exposure system (In-Tox Products, Albuquerque, NM). The system consisted of an $88.9 \times 12.7 \times 20.30\text{-cm}$ cuboidal exhaust plenum and a 2.54-cm diameter inlet plenum that was located centrally and internal to the exhaust plenum. Six exposure ports were located symmetrically and equidistantly in pairs, 3 on each side of the exhaust plenum. An exposure cone was placed into each exposure port and sealed with o-rings at the exhaust plenum and inlet plenum interfaces. The system was operated at a volumetric flow rate of 22 L/min and a pressure of -0.3 to -0.5 inches water column. A constant supply of fresh aerosol was delivered to the inlet of the exposure cones and drawn past the breathing zone of the dogs by the exhaust plenum. Hence, the exposure cones functioned in a positive flow-by manner. Four sampling ports were available on the system. Three of them were located at the junction of each pair of exposure ports and the inlet plenum and were used to collect filter samples for determining aerosol concentration by gravimetric and chemical analysis. The fourth sampling port was located at the end of the inlet plenum and was used to collect cascade impactor samples for determination of particle size distribution.

Each dog was custom fitted with a mouthpiece specific for mouth-only inhalation exposures. The mouth pieces were made by applying dental impression material to $1.3 \times 10.2\text{-cm}$ sections of brass tubing and placing the tubes covered with dental impression material in the mouth of each anesthetized dog. After 2 to 3 minutes, the dental material solidified into the shape of the dog's palette, tongue, and bite pattern. Custom-made face masks constructed from gauze and platex mold rubber were placed over the muzzles of the dogs. Each face mask had a hole cut in the end to allow the brass tubing from the mouth piece to protrude approximately 1.2 cm while sealing around the outside of the brass tubing. The face masks

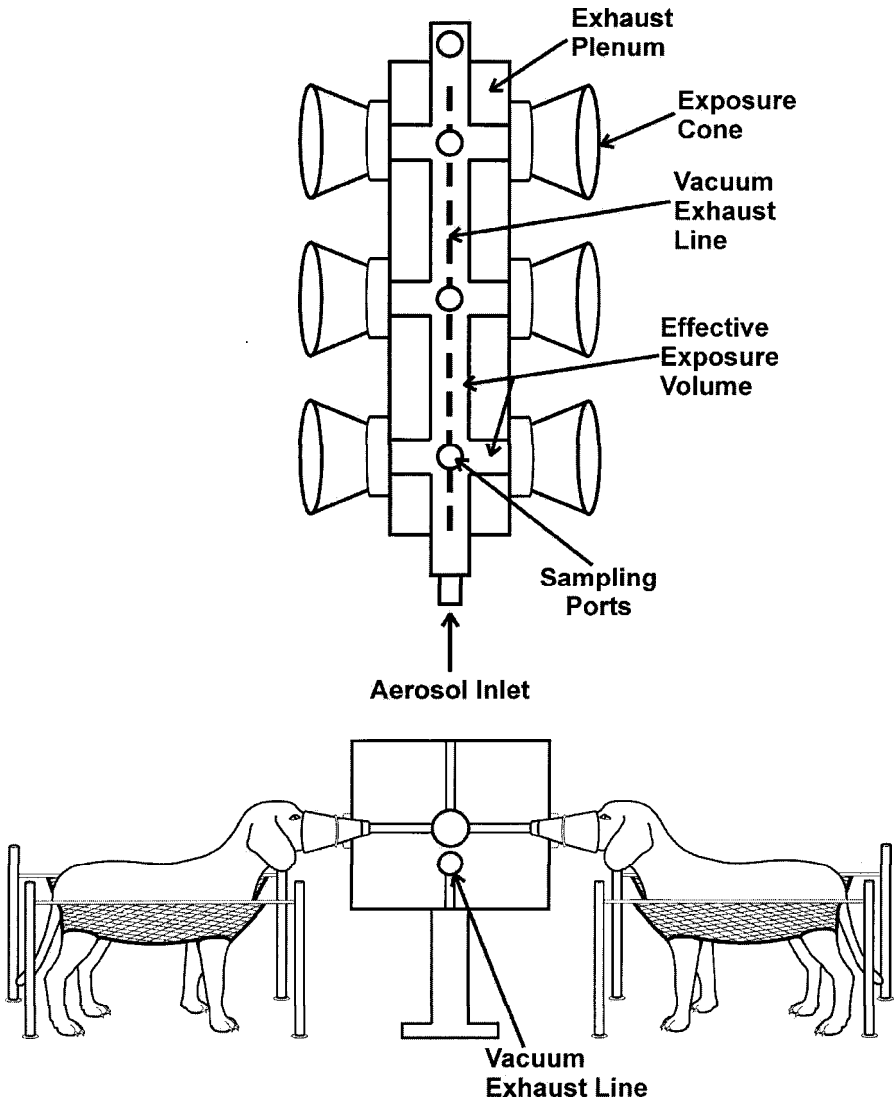


FIGURE 1 Diagram of the 6-port ragweed aerosol inhalation exposure system.

also served to occlude the dogs' nares, assuring mouth-only inhalation. Dental dam material was cut into 15.2 cm square sections, and a circular section was cut out of the center of each square. The dental dam material was then secured to the exposure cones. During the exposures, the dogs were suspended in canvas slings. The slings were mounted on tracks, which allowed the dogs to be gently directed forward so that a positive seal could be established between the facemasks and the dental dam on the exposure cone. Minimal training was required for the dogs to accept this exposure procedure.

Bronchoalveolar Lavage

Bronchoalveolar lavage fluid (BALF) samples were collected as described previously [32]. Briefly, dogs were sedated with acepromazine (Phoenix Pharmaceutical, St. Joseph, MO) subcutaneously (2 mg in 200 μ L). About 30 minutes later, the dogs were anesthetized with isoflurane, and an endotracheal tube was inserted. A fiberoptic bronchoscope (5.5 mm OD, Model BF4B2, Olympus, Lake Success, NY) was advanced to the right cardiac lobe and wedged in a segmental bronchus. The subtended segment was lavaged by instillation and withdrawal of 5 10-mL aliquots of normal saline (0.9% NaCl) solution (B. Brown Medical, Inc., Irvine, CA). Cells were separated from the BALF by centrifugation at $200 \times g$ for 10 minutes at 4°C. The cell pellets were washed twice in RPMI 1640 (Hyclone, Logan, UT), suspended in 5 mL of 10% fetal calf serum (Sigma, St. Louis, MO) in RPMI 1640, counted using a cell counter (Model ZBI, Coulter Electronics, Hialeah, FL), and evaluated for cell type as described below in Evaluation of Immunity. The BALF was aliquoted, frozen at -80°C , and assayed for total IgE and ragweed-specific antibody at a later time.

Serum Collection

Serum was obtained from whole blood drawn from the jugular vein. The blood was allowed to clot, and the serum was collected following centrifugation at $1500 \times g$ for 15 minutes at 4°C. Frozen aliquots were stored at -80°C for subsequent evaluation of total IgE and ragweed-specific IgG and IgE.

Evaluation of Immunity

The levels of ragweed-specific IgG and IgE in serum and BALF were measured by enzyme-linked immunoassay (ELISA). Each well in 96-well polyvinyl chloride plates (Dynex, Chantilly, VA) was coated with 100 μ L of 5 μ g/mL ragweed extract in phosphate-buffered saline (PBS, pH 7.2; Sigma.) and incubated overnight at 4°C. The next day, the wells were washed manually 3 times with PBS–0.05% Tween-20 (Sigma). The remaining binding sites were blocked with 200 μ L/well of 2.5% skim milk (Difco Laboratories, Detroit, MI) in PBS, pH 7.4, and incubated for 1 hour at room temperature. Samples were diluted in 2.5% skim milk, and 100 μ L was added to each well. The plates were incubated for 1 hour at 37°C. After washing the wells manually 3 times, 100 μ L of goat anti-dog IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD), diluted 1:1000, or monoclonal mouse anti-dog IgE (antibody D9 provided by Dr. Doug DeBoer, University Wisconsin School of Veterinarian Medicine, Madison, WI), diluted 1:2000

in 2.5% skim milk, was added to each well, and the plates were incubated for 1 hour at 37°C. The plates were washed 3 times. Either 100 µL of rabbit anti-goat IgG conjugated to horseradish peroxidase (for detecting dog IgG specific for ragweed, Kirkegaard and Perry Laboratories) or donkey anti-mouse IgG conjugated to horseradish peroxidase (for detecting IgE specific for ragweed, Jackson Laboratories, Westgrove, PA), diluted 1:2000 in 2.5% skim milk, was added to each well. The plates were then incubated for 1 hour at 37°C. After 5 manual washes, 100 µL of 2, 2'-azino-di(3-ethylbenzthiazoline sulfonate) at a concentration of 0.6 g/L in glycine buffer plus 0.02% H₂O₂ in citric acid buffer (ABTS peroxidase substrate system, Kirkegaard and Perry Laboratories) was added to each well. The plates were incubated at 37°C for 30 minutes for ragweed-specific IgG and between 30 and 60 minutes for ragweed-specific IgE. Color development was stopped by adding 100 µL of 5% sodium dodecyl sulfate in water (Kirkegaard and Perry Laboratories) to each well. Plates were read at an optical density of 405 nm (Thermomax microtiter plate reader, Molecular Devices, Sunnyvale, CA) with subtraction of blank values.

Total IgE in serum and BALF was measured by ELISA as described [32]. Polystyrene 96-well microtiter plates (Corning Glass Works, Corning, NY) were coated with 100 µL of dog serum diluted 1:1000 in 0.1 M sodium carbonate buffer (pH 9.6) or 100 µL of undiluted BALF and were incubated overnight at 4°C. After 5 manual wash cycles with 0.15 M NaCl–0.05% Tween-20, the remaining binding sites were blocked by adding 200 µL of 0.4% bovine serum albumin (Sigma) in PBS solution. After incubation at room temperature for 2 hours, the D9 mouse anti-dog IgE monoclonal antibody was added to each well at a dilution of 1:2000. The microtiter plates were then incubated for 2 hours at 37°C. After washing 5 times, 100 µL of donkey anti-mouse IgG horseradish peroxidase–conjugated antibody (Jackson Laboratories) was added to each well at a dilution of 1:1000. The plates were incubated for 2 hours at 37°C. After 5 manual washes, the peroxidase substrate was added to each well, and color development was detected as described above for ragweed-specific dog IgG and IgE.

Reference serum obtained as a pool from many allergic dogs testing positive for contact sensitivity to allergic ragweed extract was included in each assay. The pool of standard serum was assigned a value of 100 IgE U/mL and was used to generate a standard curve from which absorbance values of unknown samples were obtained.

Cytology was evaluated from cells obtained from BALF. Cells were washed twice as described above, counted, and used to prepare cytocentrifuge slides using a Shandon cytocentrifuge (Shandon Southern Products, Astmoor, England). The cytocentrifuge smears were then stained using a modified Wright-Giemsa stain (Diff-Quik, Baxter Healthcare, McGraw Park, IL) for differential counting of 200 cells. The numbers of eosinophils, neutrophils,

macrophages, lymphocytes, and mast cells were determined according to standard morphologic criteria [33].

Measurement of Airway Reactivity

Airway reactivity was evaluated by a modification of the method described by Collie and colleagues [32]. Dogs were sedated with acepromazine subcutaneously (2 mg in 200 μ L). About 30 minutes later, the dogs were anesthetized with isoflurane delivered by face mask. The dogs were then intubated, ventilated on a Harvard pump (Harvard Apparatus, Holliston, MA) and maintained on isoflurane throughout the procedure. Ventilation was maintained with 2% isoflurane at a tidal volume of about 15 mL/kg of body weight. Respiratory rate was set to 13 to 15 breaths per minute, and the end-tidal CO_2 ranged from 4 to 5% (LB2, medical gas analyzer; Beckman Instruments, Palo Alto, CA). An esophageal balloon catheter was positioned in the caudal thoracic portion of the esophagus at a point where fluctuations in esophageal pressure were maximal. Transpulmonary pressure was measured by connecting the esophageal catheter to one side of a differential pressure transducer (Model MP45, Validyne, Northridge, CA); the other side was connected to a port at the proximal end of the endotracheal tube. Respiratory flow was measured using a pneumotachograph (Fleisch No. 1) connected to the endotracheal tube. Outputs from flow and transpulmonary pressure transducers were conditioned, amplified with a Buxco amplifier (Buxco Electronics, Sharon, CT) as necessary, and converted from analog to digital form using appropriate hardware (AT-MIO-16, National Instruments, Austin, TX). Custom-designed computer software (LabView 3.0.1, National Instruments) was used to facilitate integration of the flow signal to yield volume and to derive the pulmonary resistance (R_L). R_L was calculated using the method of least squares linear regression [34, 35].

After the mechanical indices stabilized, airway responsiveness was assessed by obtaining dose-response curves of responses to aerosols of water, histamine, and ragweed solutions. Solutions of histamine diphosphate (Sigma) were prepared according to accepted guidelines [36] at concentrations of 0.3, 1.0, 3.0, and 10 mg/mL in water. Similarly, ragweed was prepared at concentrations of 0.01, 0.03, 0.10, 0.30, 1.00, and 3.00 mg/mL. Water was used as the diluent for histamine and ragweed because it completely evaporates, leaving no precipitate during the process of aerosolization. Aerosols were generated using a jet nebulizer (No. 950, Hospitak, Lindenhurst, NJ) connected directly to the endotracheal tube. Five breaths of vehicle (water), histamine, and ragweed solutions, standardized to an end-inspiratory transpulmonary pressure of 15 cm of H_2O , were allowed, and the response was assessed until peak resistance was reached. Values of R_L were allowed to recover within 10% of baseline values prior to subsequent

challenge or until a plateau was reached. The response was evaluated by calculating the percent change in peak R_L following inhalation of histamine or ragweed compared to baseline. Baseline levels were considered as 0% change.

Data Analysis

Data obtained from evaluating ragweed-specific immunoglobulin, total IgE, cell cytologies from lung lavage, and airway reactivity measurements were compared with repeated measures analysis of 1-way analysis of variance using the GraphPad Prism Software (GraphPad Software, San Diego, CA). Only values at $P < .05$ were considered significant.

RESULTS

Systemic Immunity

Antibody responses were measured in both sensitized (allergic) and nonsensitized (nonallergic), age-matched dogs after exposure to ragweed by inhalation. At all time points, ragweed-specific serum IgG was significantly greater in allergic compared to nonallergic dogs (Figure 2A). Antibody levels in allergic dogs following the second aerosol exposure to ragweed differed significantly compared to baseline levels on days 13, 21, 24, and 26 (Figure 2A). Ragweed-specific serum IgE and total serum IgE were significantly greater in allergic compared to nonallergic dogs following aerosol challenge (Figures 2B and C). There were also significant differences in ragweed-specific and total serum IgE levels in allergic dogs following the second aerosol exposure to ragweed compared to baseline levels at all time points (Figures 2B and C). No change in antibody activity following ragweed exposures compared to baseline levels was detected in serum from control, nonimmunized dogs.

Pulmonary Immunity

Similar to serum, ragweed-specific IgG was significantly greater in BALF from allergic compared to nonallergic dogs on days 1 and 4 following the second ragweed aerosol exposure, and increased over time (Figure 3A). There was a trend for total IgE in BALF to be greater in allergic compared to nonallergic dogs after the second ragweed aerosol challenge, although this was not statistically significant (Figure 3B). We were unable to measure anti-ragweed IgE in BALF. It is possible that specific IgE could be measured by concentrating the BALF.

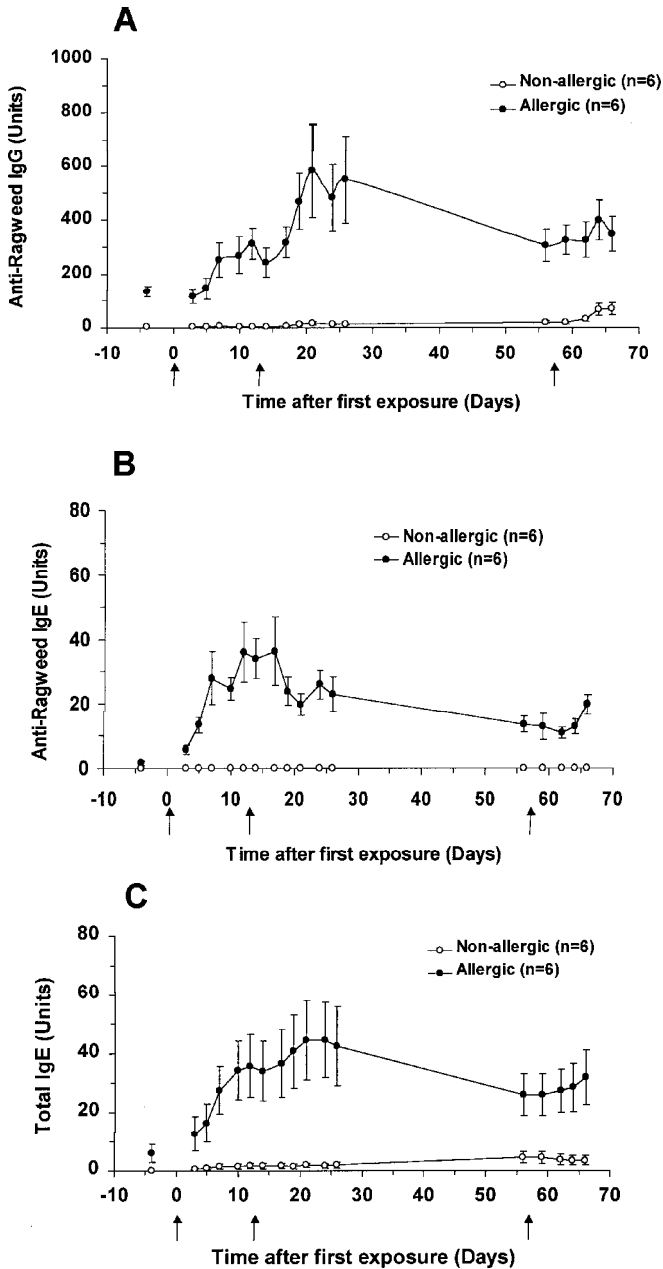


FIGURE 2 Serum antibody and immunoglobulin responses in allergic and nonallergic dogs following ragweed aerosol exposure; ragweed-specific serum IgG (A), ragweed-specific serum IgE (B), and total serum IgE (C). Data are presented as the mean $\pm$ standard error of the mean at different time points following exposure. A significant difference ($P < .05$) in antibody levels following ragweed exposure compared to baseline levels is presented in the text. Arrows indicate times of ragweed exposure by inhalation. All responses in allergic dogs are significantly greater than in nonallergic dogs.

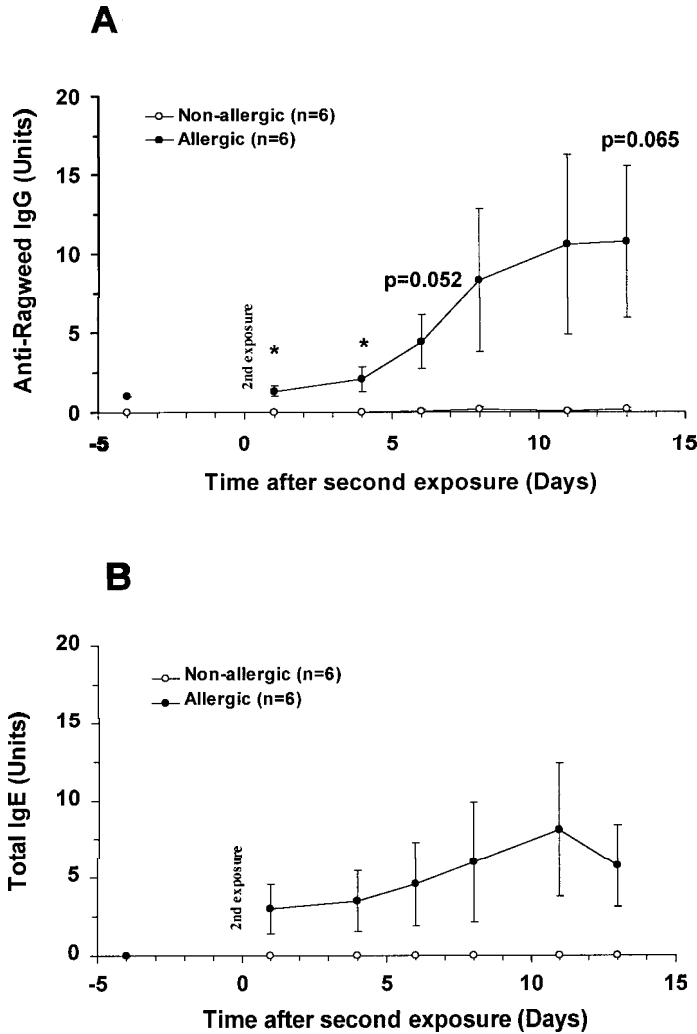
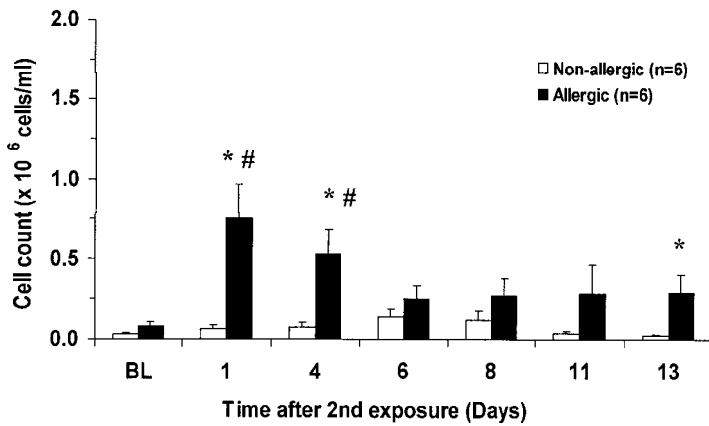


FIGURE 3 Ragweed-specific IgG (A) and total IgE (B) in BALF from allergic and nonallergic dogs following ragweed aerosol challenge. Data are presented as the mean $\pm$ standard error of the mean at different time points following exposure. A significant difference ($P < .05$) in antibody levels in allergic compared to nonallergic dogs is indicated by *.

Allergic dogs had higher eosinophil counts in the BALF following ragweed aerosol challenge than nonallergic dogs, with significant differences detected on days 1, 4, and 13 (Figure 4A). However, compared to pre-exposure baseline numbers, eosinophil counts on days 1 and 4 were significantly different in allergic dogs (Figure 4A). There were no significant differences in the numbers of neutrophils in the BALF in allergic and nonallergic dogs before and after ragweed inhalation (Figure 4B), but there were significant differences on day 1 in the number of neutrophils in allergic

A Eosinophils



B Neutrophils

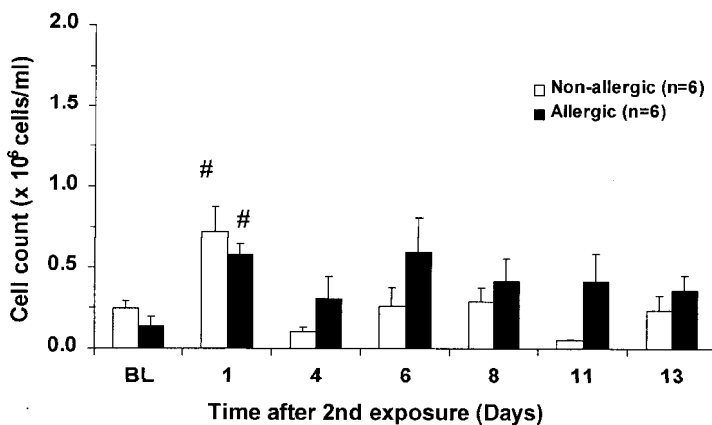


FIGURE 4 Eosinophil (A) and neutrophil (B) counts in BALF from allergic and nonallergic dogs following ragweed aerosol challenge. Data are presented as the mean $\pm$ standard error of the mean at different time points following exposure. A significant difference ($P < .05$) in cell counts following ragweed exposure compared to baseline levels is indicated by #. A significant difference ($P < .05$) in cell counts in allergic compared to nonallergic dogs is indicated by *.

compared to nonallergic dogs (Figure 4B). In addition, no difference was detected in the numbers of macrophages, lymphocytes, and mast cells in allergic compared to nonallergic dogs (data not shown).

Airway Responses

Allergic dogs were found to have increased resistance in response to histamine after the second ragweed challenge compared to baseline

reactivity (Figure 5A). This response was dose dependent and increased with increasing histamine concentration; however, the increase was not significant. In nonallergic dogs, the increase in R_L with increasing histamine doses was not different before and after 2 ragweed aerosol exposures (Figure 5B).

In addition to measuring responses to histamine challenge, R_L in response to ragweed challenge was determined. Although in allergic dogs there was no significant difference in R_L before and after 2 ragweed aerosol exposures, R_L tended to increase during challenge with increasing doses of ragweed (Figure 6A). Nonallergic dogs did not have measurable increases in R_L after ragweed aerosol exposure, and R_L was also not measurable during challenge with increasing doses of ragweed (Figure 6B). Pulmonary

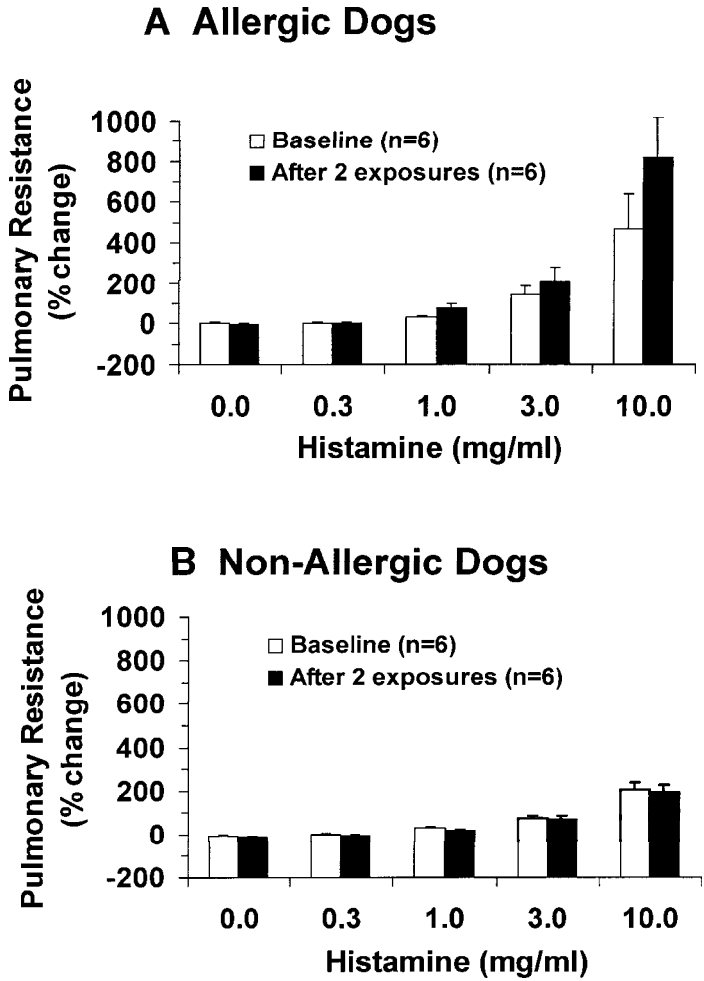


FIGURE 5 R_L in response to increasing doses of histamine in allergic (A) and nonallergic (B) dogs. Data are presented as the mean $\pm$ standard error of the mean.

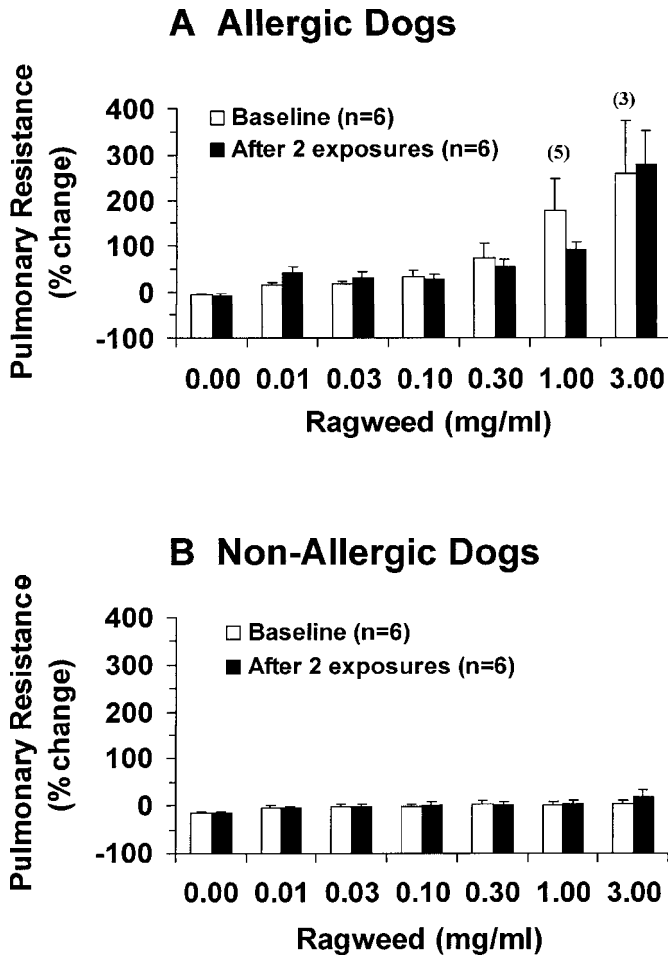


FIGURE 6 Dose response of R_L to increasing doses of ragweed before (baseline) and after two ragweed aerosol exposures in allergic (*A*) and nonallergic dogs (*B*). Data are presented as the mean ($n = 6$ unless otherwise indicated in parentheses) $\pm$ standard error of the mean.

resistance was sustained in allergic dogs and was significantly different from nonallergic dogs after the second aerosol ragweed exposure, 8 days and as long as 5 months following the third ragweed exposure (Figure 7). Dogs sensitized from birth and found to have increased airway reactivity to ragweed do not appear to lose their increased sensitivity to ragweed or histamine. Based on preliminary observations, sensitivity to ragweed or histamine in allergic dogs tends to increase with age.

DISCUSSION

The aim of this study was to determine the effects of ragweed exposure by inhalation on pulmonary immunity in allergic dogs. About half of the

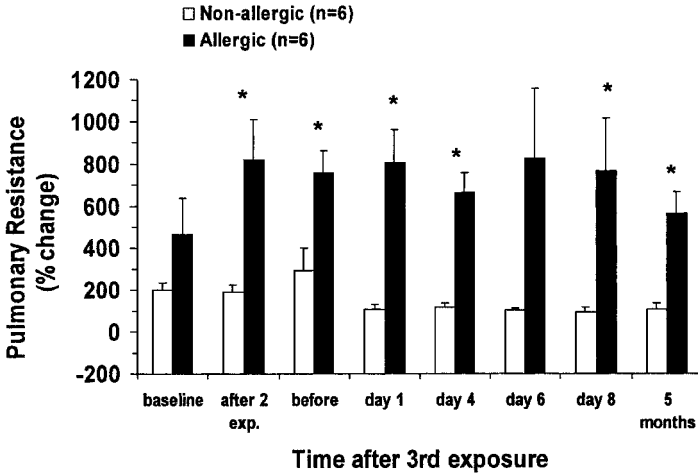


FIGURE 7 Change in R_L in response to histamine (10 mg/mL) over time in allergic and nonallergic dogs. Data are presented as the mean $\pm$ standard error of the mean. A significant difference ($P < .05$) in R_L in allergic compared to nonallergic dogs is indicated by *.

dogs born to allergic males and females develop allergic airway responses similar to humans when sensitized and exposed to antigen. These allergic dogs develop pulmonary eosinophilia, elevated levels of IgE, and airway hyperresponsiveness when challenged with antigen. They, therefore, are appropriate for modeling human allergic asthma.

Although in the present study sensitized dogs were immunized systemically (IP and SC), they developed measurable pulmonary immunity in response to antigen challenge delivered by aerosol inhalation to their lungs. Immunized dogs developed antigen-specific antibody in serum, pulmonary immunity characterized by inflammatory cell infiltration, as well as antigen-specific IgE and IgG antibody production. In contrast, nonsensitized dogs challenged by aerosol exposure to antigen did not develop antigen-specific antibody. These data indicate that antibody responses in the lungs of sensitized dogs are derived from cells that were sensitized in the periphery following IP injection and subsequently migrated to the lungs after antigen challenge. Following antigen challenge, these memory cells in the lungs secrete mediators that interact with other cells and lead to the cascade of events ultimately responsible for the symptoms of allergic asthma, including pulmonary eosinophilia, elevated levels of serum and bronchoalveolar IgE, and excessive mucus production. Sensitized dogs had greater antibody levels and more pulmonary inflammation than nonsensitized dogs, as indicated by the significantly increased number of lung eosinophils following antigen challenge.

Several studies have used the dog to model components of human allergic asthma, including airway hyperresponsiveness [31, 37–39], airway

eosinophilia [40, 41], and increased serum IgE concentration [31, 42]. In addition, similar to humans, dogs sensitized with allergen in their airways develop long-term antibody-producing cells in the interstitium of the lungs, which may play an important role in asthma pathogenesis [43]. The present study demonstrates that allergic dogs sensitized to ragweed provide an alternate model in which to study the features and characteristics of allergic asthma. Like humans, dogs develop allergic disease, particularly atopic dermatitis [44], food allergy [30], and lower respiratory tract disease characterized by eosinophilic infiltrates [45, 46]. Allergic dogs do not wheeze, probably because of lung anatomic factors such as large airways relative to lung volume and excellent collateral ventilation, not because they do not develop allergic responses in their lungs.

The airway reactivity data support the use of allergic dogs sensitized to ragweed as a model of allergic asthma. Following aerosol antigen challenge, sensitized dogs, whose immune cells were previously exposed to antigen in the periphery exhibited significantly greater increases in R_L in response to ragweed than nonsensitized dogs. Furthermore, the increased change in R_L in immunized dogs was sustained and persisted as long as 5 months following the third aerosol ragweed exposure. Perhaps persistent immune cells are responsible for mediating this sustained change in R_L in response to ragweed challenge in immunized dogs because antigen-specific antibody can be detected in BALF as long as 3 years following antigen exposure [22, 43, 47]. Sensitized dogs also had significantly greater changes in R_L than nonsensitized dogs when challenged with allergen (ragweed). In contrast, nonsensitized dogs had significantly reduced changes in R_L when challenged with increasing doses of ragweed than sensitized dogs. This was not surprising because nonsensitized dogs did not contain antigen-specific immune cells as indicated by ragweed-specific serum and BALF antibody data. We cannot explain why no change in R_L was detected in allergic dogs after 2 aerosol exposures to ragweed. However, preliminary data indicate that R_L to ragweed is higher 1 year after aerosol exposure to ragweed than at baseline.

Murine models of asthma, as well as other animal species, have been useful for investigating the numerous components important in the pathophysiology of allergic asthma [48]. Data from murine models emphasize the importance of T cells [49], mast cells, basophils, and adhesion molecules, as well as soluble mediators including immunoglobulins and cytokines in allergic asthma. For example, murine models facilitated the identification of interleukin-4 (IL-4) as an essential regulator of terminal differentiation of B cells to IgE-secreting plasma cells [50]. Similarly, IL-5 was found to be critical in the production of eosinophils by bone marrow [51]. Analysis of lymphocytes isolated from patients confirmed the association of IL-4 and IL-5 expression with allergic disease [52]. Although T cells clearly play an

important role, antibody production to allergens localized to the lungs of asthmatics is also important in asthma pathogenesis.

Clearly, murine models have been powerful tools for understanding allergic asthma. However, not all manifestations of the human disease can be reproduced in mice (reviewed in [48]). Airway responses and inflammation in mice are transient with little or no late-phase airway reactivity [53] and are not chronic as seen in human asthmatics [54]. With only 1 exception [55], airway reactivity in mice has been measured in response to the nonspecific agonist methacholine and not in response to aerosol antigen provocation. Another important difference between mouse and human pulmonary immunity is that mice, but not humans, develop bronchial-associated lymphoid tissue (BALT) consisting of bronchial lymphoid aggregates covered by a lymphoid epithelium [56, 57]. Antibody produced in the BALT enters the circulation via the lymphatics and is not released directly into the lungs. In contrast, human, primate, and dog lungs contain cells that actively produce and secrete antibody directly into the lung tissues and airways [26–29, 47]. It is not clear that the induced responses in mice reflect the events that mediate allergic disease in humans.

The data presented here demonstrate that the similarity to humans with allergic disease makes the allergic Beagle dog an excellent model for studying the role of pulmonary immunity in allergic disease. Unlike mice but similar to humans, sensitized allergic dogs challenged with aerosolized antigen develop sustained allergic immunity. These allergic dogs demonstrate persistent airway reactivity not only to agonists (histamine, methacholine, etc.) but also to allergen. Furthermore, dogs do develop interstitial inflammation and chronic pulmonary eosinophilia similar to human asthmatics rather than increased accumulation of BALT as seen in mice. The allergic dog has potential not only for investigating the basic mechanisms of pulmonary immunity but also for demonstrating efficacy of therapeutic agents targeting allergic asthma. Although immunological and molecular reagents are not as readily available for dogs as for mice, the advent of molecular technology is increasing the number of important immunological reagents available to study allergic disease in dogs.

REFERENCES

1. Haley KJ, Sunday ME, Wiggs BR, Kozakewich HP, Reilly JJ, Mentzer SJ, Sugarbaker DJ, Doerschuk CM, Drazen JM: Inflammatory cell distribution within and along asthmatic airways. *Am J Respir Crit Care Med.* 1998;158:565–572.
2. Howarth PH: Small airways and asthma. An important therapeutic target? *Am J Respir Crit Care Med.* 1998;157:S173.
3. Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G, Assoufi B, Collins JV, Durham S, Kay AB: Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis.* 1990;142:1407–1413.

4. Bentley AM, Menz G, Storz C, Robinson DS, Bradley B, Jeffery PK, Durham SR, Kay AB: Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Relationship to symptoms and bronchial responsiveness. *Am. Rev. Respir Dis.* 1992;146:500–506.
5. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR: Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol.* 1993;92:313–324.
6. Sur S, Crotty TB, Kephart GM, Hyma BA, Colby TV, Reed CE, Hunt LW, Gleich GJ: Sudden-onset fatal asthma. A distinct entity with few eosinophils and relatively more neutrophils in the airway submucosa? *Am Rev Resp Dis.* 1993;148:713–719.
7. Djukanovic R, Wilson SJ, Howarth PH: Pathology of rhinitis and bronchial asthma. *Clin Exp Allergy.* 1996;26(Suppl 3):44–51.
8. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE: Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy.* 1977;7:503–513.
9. O'Byrne PM: Allergen-induced airway hyperresponsiveness. *J Allergy Clin Immunol.* 1988;81:119–127.
10. Carter PM, Heinly TL, Yates SW, Lieberman PL: Asthma: the irreversible airways disease. *J Invest Allergy Clin Immunol.* 1997;7:566–571.
11. National Center for Health Statistics: Report of Final Mortality Statistics: 1970–1997. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention 2000.
12. Michel FB, Neukirch F, Bousquet J: Asthma: a world problem of public health. *Bull Acad Natl Med.* 1995;179:279–293.
13. Anderson HR: Air pollution and trends in asthma. *Ciba Found Symp.* 1997;206:190–202.
14. Charpin D, Vervloet D: Role of atmospheric pollutants in asthma. *Rev Pneumol Clin.* 1996;52:70–78.
15. Forastiere F, Corbo GM, Pistelli R, Michelozzi P, Agabiti N, Brancato G, Ciappi G, Perucci CA: Bronchial responsiveness in children living in areas with different air pollution levels. *Arch Environ Health.* 1994;49:111–118.
16. Glovsky MM, Miguel AG, Cass GR: Particulate air pollution: possible relevance in asthma. *Allergy Asthma Proc.* 1997;18:163–166.
17. Wardlaw AJ: Air pollution and allergic disease. Report of a Working Party of the British Society for Allergy and Clinical Immunology. *Clin Exp Allergy* 1995;25:6–8.
18. Custovic A, Simpson A, Woodcock A: Importance of indoor allergens in the induction of allergy and elicitation of allergic disease. *Allergy.* 1998;53:115–120.
19. Braback L, Breborowicz A, Dreborg S, Knutsson A, Pieklik H, Bjorksten B: Atopic sensitization and respiratory symptoms among Polish and Swedish school children. *Clin Exp Allergy.* 1994;24:826–835.
20. Cookson WO, Moffatt MF: Asthma: an epidemic in the absence of infection? *Science.* 1997;275:41–42.
21. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM: The inverse association between tuberculin responses and atopic disorder. *Science.* 1997;275:77–79.
22. Bice DE, Williams AJ, Muggenburg BA: Long-term antibody production in canine lung allografts: Implications in pulmonary immunity and asthma. *Am J Respir Cell Mol Biol.* 1996;14:341–347.
23. Corris PA, Dark JH: Aetiology of asthma: lessons from lung transplantation. *Lancet.* 1993;341:1369–1371.
24. Higenbottam T: Lung transplantation and asthma. *Lancet.* 1993;342:249.
25. Sears MR: Transplantation asthma. *Lancet.* 1993;341:1377–1378.
26. Bice DE, Harris DL, Muggenburg BA, Bowen JA: The evaluation of lung immunity in chimpanzees. *Am Rev Respir Dis.* 1982;126:358–359.
27. Bice DE, Shopp GM: Antibody responses after lung immunization. *Exp Lung Res.* 1988;14:133–155.
28. Mason MJ, Gillett NA, Bice DE: Comparisons of systemic and local immune responses after multiple pulmonary antigen exposures. *Regen Immunol.* 1989;2:149–157.
29. Weissman DN, Bice DE, Crowell RE, Schuyler MR: Intrapulmonary antigen deposition in the human lung: local responses. *Am J Respir Cell Mol Biol.* 1994;11:607–614.
30. Ermel RW, Kock M, Griffey SM, Reinhart GA, Frick OL: The atopic dog: a model for food allergy. *Lab Anim Sci.* 1997;47:40–49.
31. Bice DE, Collie DDS, Muggenburg BA, Basbaum CB: Effects of anti-ragweed immunity in the lung on airway mucus production. *Am J Respir Crit Care Med.* 1998;157:A832.

32. Collie DD, DeBoer DJ, Muggenburg BA, Bice DE: Evaluation of association of blood and bronchoalveolar eosinophil numbers and serum total immunoglobulin E concentration with the expression of nonspecific airway reactivity in dogs. *Am J Vet Res.* 1997;58:34–39.
33. Rebar AH, DeNicola DB, Muggenburg BA: Bronchopulmonary lavage cytology in the dog: normal findings. *Vet Pathol.* 1980;17:294–304.
34. Uhl RR, Lewis FJ: Digital computer calculation of human pulmonary mechanics using a least squares fit technique. *Comput Biomed Res.* 1974;7:489–495.
35. Wald A, Jason D, Murphy TW, Mazzia VD: A computer system for respiratory parameters. *Comput Biomed Res.* 1969;2:411–429.
36. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL: Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl.* 1993;16:53–83.
37. Becker AB, Hershkovich J, Simons FE, Simons KJ, Lilley MK, Kepron MW: Development of chronic airway hyperresponsiveness in ragweed-sensitized dogs. *J Appl Physiol.* 1989;66:2691–2697.
38. Chung KF, Becker AB, Lazarus SC, Frick OL, Nadel JA, Gold WM: Antigen-induced airway hyperresponsiveness and pulmonary inflammation in allergic dogs. *J Appl Physiol.* 1985;58:1347–1353.
39. Mitchell RW, Antonissen LA, Kepron W, Kroeger EA, Stephens NL: Effect of atropine on the hyperresponsiveness of ragweed-sensitized canine tracheal smooth muscle. *J Pharmacol Exp Ther.* 1986;236:803–809.
40. Baldwin F, Becker AB: Bronchoalveolar eosinophilic cells in a canine model of asthma: two distinctive populations. *Vet Pathol.* 1993;30:97–103.
41. Woolley MJ, Lane CG, Ellis R, Stevens WH, Woolley KL, O'Byrne PM: Role of airway eosinophils in the development of allergen-induced airway hyperresponsiveness in dogs. *Am J Respir Crit Care Med.* 1995;152:1508–1512.
42. Frick OL, Brooks DL: Immunoglobulin E antibodies to pollens augmented in dogs by virus vaccines. *Am J Vet Res.* 1983;44:440–445.
43. Bice DE, Jones SE, Muggenburg BA: Long-term antibody production after lung immunization and challenge: role of lung and lymphoid tissues. *Am J Respir Cell Mol Biol.* 1993;8:662–667.
44. Nesbitt GH: Canine allergic inhalant dermatitis: a review of 230 cases. *J Am Vet Med Assoc.* 1978;172:55–60.
45. Brownlie SE: A retrospective study of diagnosis in 109 cases of canine lower respiratory disease. *J Small Anim Pract.* 1990;31:371–376.
46. Corcoran BM, Thoday KL, Henfrey JI, Simpson JW, Burnie AG, Mooney CT: Pulmonary infiltration with eosinophils in 14 dogs. *J Small Anim Pract.* 1991;32:494–502.
47. Bice DE, Weissman DN, Muggenburg BA: Long-term maintenance of localized antibody responses in the lung. *Immunology.* 1991;74:215–222.
48. Bice DE, Seagrave J, Green FHY: Animal models of asthma. *Inhal Toxicol.* 2000;12:829–862.
49. Gavett SH, Chen X, Finkelman F, Wills-Karp M: Depletion of murine CD4 T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol.* 1994;10:587–593.
50. Brenner R, Savelkoul HF: Regulation of IgE production in mice. *Eur Respir J Suppl.* 1991;97s–104s.
51. Yamaguchi Y, Suda T, Suda J, Eguchi M, Miura Y, Harada N, Tominaga A, Takatsu K: Purified interleukin 5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. *J Exp Med.* 1988;167:43–56.
52. Corrigan CJ, Kay AB: T cells and eosinophils in the pathogenesis of asthma. *Immunol Today.* 1992;13:501–507.
53. Hessel EM, Van Oosterhout AJ, Hofstra CL, De Bie JJ, Garssen J, Van Loveren H, Verheyen AK, Savelkoul HF, Nijkamp FP: Bronchoconstriction and airway hyperresponsiveness after ovalbumin inhalation in sensitized mice. *Eur J Pharmacol.* 1995;293:401–412.
54. McFadden ER Jr, Gilbert IA: Asthma. *N Engl J Med.* 1992;327:1928–1937.
55. Cieslewicz G, Tomkinson A, Adler A, Duez C, Schwarze J, Takeda K, Larson KA, Lee JJ, Irvin CG, Gelfand EW: The late, but not early, asthmatic response is dependent on IL-5 and correlates with eosinophil infiltration. *J Clin Invest.* 1999;104:301–308.

56. Evans MJ, Guha SC, Cox RA, Moller PC: Attenuated fibroblast sheath around the basement membrane zone in the trachea. *Am J Respir Cell Mol Biol.* 1993;8:188–192.
57. Macklem PT: The physiology of small airways. *Am J Respir Crit Care Med.* 1998;157:S181–S183.