Effects of dog ownership and genotype on immune development and atopy in infancy

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Background: Exposure to furred pets might confer protection against the development of allergic sensitization through a mechanism that is incompletely understood.

Objective: The objective of this study was to determine the effects of pet exposure and genotype on immunologic development and the incidence of atopic markers and diseases in the first year of life.

Methods: Pet exposure in the home was compared with cytokine secretion patterns (mitogen-stimulated mononuclear cells at birth and age 1 year) and indicators of atopy (allergen-specific and total IgE, eosinophilia, food allergy, atopic dermatitis) in 285 infants. Interactions with genotype at the CD14 locus were also evaluated in the data analyses.

Results: Exposure to dogs was associated with reduced allergen sensitization (19% vs 33%, \( P = .020 \)) and atopic dermatitis (30% vs 51%, \( P < .001 \)). The risk for atopic dermatitis was further influenced by genotype at the CD14 locus (\( P = .006 \)), even after adjusting for exposure to dogs (\( P = .003 \)). Furthermore, infants with the genotype –159TT were less likely to develop atopic dermatitis if they were exposed to a dog (5% vs 43%, \( P = .04 \)). Last, dog exposure was associated with increased IL-10 (117 vs 79 pg/mL, \( P = .002 \)) and IL-13 (280 vs 226 pg/mL, \( P = .013 \)) responses at age 1 year.

Conclusions: Having a dog in infancy is associated with higher IL-10 and IL-13 cytokine secretion profiles and reduced allergic sensitization and atopic dermatitis. These findings suggest that postnatal exposure to dogs can influence immune development in a genotype-specific fashion and thereby attenuate the development of atopy in at-risk children. (J Allergy Clin Immunol 2004;113:307-14.)

Key words: Interleukin-10, hypersensitivity, dogs, cats, children, interleukin-13, allergy, cytokines, CD14, atopic dermatitis

Although the incidence of allergic diseases and asthma in childhood has increased during the past several decades, this trend is not evenly distributed throughout the population. For example, exposure to other children, infections, animals, and farming environments during infancy has been associated with a reduced rate of atopic diseases during childhood. These observations led to the development of the hygiene hypothesis, which proposes that exposure to infections or microbial products in infancy can favorably modify immune development to inhibit atopic diseases. The consequences of owning pets are of particular interest because exposure to cats and dogs has been reported to either promote or inhibit the risk of subsequent atopy. Although mechanisms for these associations are incompletely understood, it has been postulated that exposure to animals in infancy could inhibit atopy by either boosting \( T_H^1 \)-like cytokine responses or by modifying \( T_H^2 \)-like immune responses.

To test these proposed mechanisms, we used data from the Childhood Origins of Asthma (COAST) study. Exposure to cats and dogs in the home was prospectively ascertained and compared with clinical and serologic markers of atopy at 1 year, genetic markers, and to cytokine secretion patterns of blood mononuclear cells (MNCs) obtained at birth and age 1 year.

METHODS

Study subjects

The subjects were enrolled in the COAST study, which was approved by the University of Wisconsin Human Subjects Committee. To be eligible, either the mother or father had to have either allergic rhinitis (defined as a history of symptoms and 1 or more positive aeroallergen skin test results), asthma by history, or both. Entry criteria included term delivery, 5-minute Apgar scores of 7 or greater, and the absence of significant respiratory difficulties or congenital anomalies.
Abbreviations used
AD: Atopic dermatitis
COAST: Childhood Origins of Asthma Study
MNC: Mononuclear cell
RSV: Respiratory syncytial virus

Study design
Parents prospectively completed questionnaires at intervals to assess the home environment. The birth and 2-year questionnaires specifically asked about pet ownership. Families who answered affirmatively at one of these time points were contacted by telephone to determine when the pet either entered or left the household. For this study the variable that was used in the analysis was the presence of a cat or dog at home at the time of the child’s birth.

The subjects were followed prospectively from birth to 1 year of age with historical questionnaires and physical examinations that were performed at regular intervals (2, 4, 6, 9, and 12 months of age) by their own health care providers and at 1 year of age by study personnel. Atopic dermatitis (AD) was defined as physician diagnosed either by documentation by a health care provider on the medical record or by parental report of physician-diagnosed AD on the historical questionnaires. Three groups were defined and used for this study: (1) children with no AD anytime within the first year of life, (2) children with AD documented at any point within the first year, and (3) children with active disease at 1 year of age.

Food allergy was defined by using allergen-specific IgE test results (CAP; Pharmacia, Philadelphia, Pa) and by historical reports (parental reports or physician documentation). Three groups were defined: (1) food allergy, which included a positive fluoroenzyme immunoassay (FEIA) and a convincing history, which included a typical response and timing to the food in question and reproducibility; (2) possible food allergy, which included positive FEIA with an indeterminate history or negative FEIA with a convincing history; and (3) no food allergy, which included a negative FEIA with a negative history or a positive FEIA with a history of eating the food(s) in question without having adverse reactions.

Immunologic secretion assays
Heparinized samples of umbilical cord blood and peripheral blood at age 1 year were collected, kept at room temperature, and processed on the same day or, in the case of cord blood collected after 4 pm, on the following morning. Blood MNCs (10^6 cells/mL) were stimulated with incubated (24 hours) PHA (5 µg/mL; Sigma, St Louis, Mo) or medium alone, and supernatants were frozen (–80°C) pending analysis for IFN-γ, IL-5, IL-10, and IL-13 by ELISA (Pharmingen, San Diego, Calif).17 The assay sensitivities were 4.7, 1.9, 7.8, and 3.1 pg/mL, respectively, and the coefficient of variation of the ELISA tests was generally less than 10%. The samples were run in duplicate, and mean values are reported.

Total and allergen-specific IgE were performed as previously described.17 Allergen-specific IgE values of 0.35 kU/L (class I) or greater were considered positive, and the sensitivity for detection of total IgE was 2 kU/L.

Genotyping studies
Genotypes for the –159C/T polymorphism at the CD14 locus were determined by using an immobilized linear array system designed by Roche Molecular Systems (Alameda, Calif).18

Statistical analysis
Effects of dog or cat exposure at birth on allergic sensitization (1 or more positive RAST test results) were analyzed by using a chi-square test. In addition, logistic regression analysis was performed to adjust for potential confounding variables (parental allergy and asthma, daycare attendance, respiratory syncytial virus [RSV] infection, and presence of older siblings).

The effects of pet exposure on cytokine responses, total IgE, and absolute eosinophils were analyzed by using nonparametric tests: Wilcoxon rank sum test for 2 group comparisons and Kruskal-Wallis test for 4 groups. In addition, ANOVA models were performed to adjust for potential confounding variables (see list above) after log transformation of the responses. Single-locus tests of association between CD14 genotypes and AD and atopy were carried out by using the chi-square statistic for contingency tables. The P values were calculated by Monte Carlo simulation from contingency tables with given marginals. Interactions between genotypes and qualitative covariates were tested by using logistic regression. The P values were determined by using large sample approximations to the likelihood ratio statistic. Only white children were included in the genetic studies.

RESULTS
Study participants
Of the 312 families who gave informed consent, cord blood specimens were not obtained in 14 subjects, 9 subjects did not meet the inclusion criteria, and 4 families withdrew from the study. The group included 14% ethnic minorities, and 73% of mothers and 70% of fathers attended college; these demographics are reflective of the general population of Madison, Wis. Complete information regarding pet ownership was available for each of the remaining 285 families (Table I). The babies were born between November 1998 and May 2001, and births were distributed uniformly across the calendar year.

Pet exposure
At the time of birth, 101 infants were exposed to dogs and 84 infants were exposed to cats at home. When exposure at the time of birth to both types of animals was considered, 68 children were exclusively exposed to dogs, 51 children were exclusively exposed to cats, 33 were exposed to both dog and cat, and 133 reported no home exposure to either animal. Pet ownership was relatively stable during the first year; 3 families (1.1%) obtained new pets (2 dogs, 1 cat), and 6 families (2.2%) gave away their pets (2 dogs, 4 cats).

Demographic variables were compared for families with and without indoor pets (Table 1). Children exposed to dogs were less likely to be minorities. Dog ownership was associated with reduced prevalence of maternal allergy to cats, and there were nonsignificant trends toward associations with reduced allergy in general and allergy to dogs. In addition, cat exposure was associated with a reduced prevalence of paternal cat allergy. Finally, parents with and without AD had similar rates of owning dogs (37% vs 33%, P = .48) and cats (27% vs 32%, P = .34).

AD and food allergy
The clinical diagnosis of AD in the first year of life was associated with other atopic markers at age 1 year, including significantly higher total IgE (17 vs 11 pg/mL, P = .02), percentage of children with detectable egg-specific
IgE (29% vs 8%, P < .001), and a trend toward increased circulating eosinophils (237 vs 192 cells/µL, P = .07). In addition, the incidence of AD varied with the pattern of pet exposure; children exposed to either dogs or both pets were less likely to have AD than children exposed only to cats or to neither animal (Table II). When exposure to individual animals was considered, exposure to dogs was associated with reduced AD (30% vs 51%, P < .001); exposure to cats was not (45% vs 43%, P = .70). Similarly, the prevalence of active AD at the 1-year examination was 12% in children exposed to dogs, compared with 34% in children without dogs (P < .001).
Food allergy was ascertained in 283 subjects; 15 subjects (5.3%) had confirmed food allergies, whereas 249 subjects (88%) had no signs or symptoms of food allergy. An additional 19 subjects (6.7%) had possible food allergy, defined as a history that was suggestive, but not diagnostic, for food allergy. There was no significant effect of cat or dog exposure on confirmed food allergy (Table II).

Markers of atopy

Serum was available at age 1 year for 279 subjects. For the group as a whole, 78 subjects (28%) had at least one positive RAST test result, including egg (n = 47), milk (n = 31), peanut (n = 28), cat (n = 6), dog (n = 15), dust mite (Dermatophagoides pteronyssinus, n = 7; D farinae, n = 6), and Alternaria (n = 7). There were nonsignificant trends for reduced sensitization in infants exposed to dogs only (18%), the combination of dogs and cats (21%) compared with exposure to cats alone (34%), or to neither animal (33%). Fewer subjects who were exposed to dog (dog alone or dog and cat) had positive RAST test results compared with children with no dog exposure (cat alone or neither) (19% vs 33%, P = .013, Table II). This relationship persisted after adjustment for covariates (minority status, maternal and paternal allergy, maternal and paternal asthma, siblings, RSV infection, and daycare). Furthermore, the percentage of children with at least one RAST test result that was class 2 or greater (0.70 kU/mL or greater) varied with exposure to dogs (13% dog-exposed vs 22% no exposure, P = .05) but not cats (19% with or without cat exposure). In contrast, cat exposure did not affect sensitization in general (29% vs 28%, Table II) and tended to be associated with increased sensitization to cat (9.6% vs 4.1%, P = .068). Dog exposure did not affect the incidence of dog sensitization (6.1% vs 5.1%, P = .72). There were no significant relationships between pet ownership and total blood IgE levels or eosinophil counts at age 1 year (Table II).
Patterns of cytokine secretion

Cytokine (IL-5, IL-10, IL-13, IFN-γ) responses from PHA-stimulated MNCs from cord blood and 1-year peripheral blood were compared with pet status. Pet exposure at birth was not associated with significant differences in cytokine secretion from cord blood cells (data not shown), except for a slight reduction in IL-5 responses associated with cat (median, 2 vs 3 pg/mL; P = .016).

At age 1 year (Table III and Fig 1), IL-10 responses were higher in infants exposed to dogs only (116 pg/mL) or dogs and cats (120 pg/mL), compared with exposure to cats alone (78 pg/mL) or neither pet (80 pg/mL). Overall, dog exposure was associated with a 48% increase in IL-10 responses and a 24% increase in IL-13 responses compared with no dog exposure. The relationships between dog exposure and cytokine responses at age 1 year were still seen in ANOVA models, which included parental allergy and asthma, daycare, the presence of siblings in the house, and RSV infection in the first year of life. There were no significant associations between exposure to cats and cytokine responses at age 1 year.

Dog ownership and CD14 genotype

To determine whether genetic variation in CD14, the gene encoding the LPS receptor, influences risk for atopy in children with and without dogs in the home, we genotyped the COAST children for a functional promoter region polymorphism, −159C/T (Table IV). Overall, there was a significantly different risk of AD in the 6 groups formed by the CD14 genotypes and exposure to a dog (P = .003). Although there was a significant association between CD14 genotype and AD (P = .006), the prevalence of AD among children with the TT genotype was significantly less among children with a dog compared with children without a dog in the home (5% vs 43%, P = .04). There were no associations between CD14 genotype and allergic sensitization, total IgE, or cytokine responses at 1 year.

**DISCUSSION**

This study was conducted to determine whether having pets in the home changes the development of cytokine responses in such a way as to inhibit the development of atopy in infancy. Information regarding pet ownership, clinical findings, and immune development was gathered prospectively from birth; this study design eliminates recall bias and improves the accuracy of these observations. Furthermore, we were able to take into account the possible confounding of allergic status among family members. Collectively, our findings support the hypothesis that postnatal exposure to dogs modifies immune development by enhancing IL-10 and, to a lesser extent, IL-13 responses and reducing allergic sensitization in the first year of life. Moreover, children exposed to dogs had a reduced incidence of AD, which is often the first clinical expression of atopy in childhood.

Although this study does not establish how dog exposure leads to increased IL-10 responses, it is known that pets can increase home exposure to innate immune stimuli such as endotoxin.22 Endotoxin is a potent innate stimulus for the production of IL-10,23,24 and increased exposure to endotoxin has been linked to reduced prevalence of AD, allergic sensitization, hay fever, and asthma.25-27 Furthermore, in a murine model of allergic sensitization, IL-10–producing dendritic cells in the lung were found to stimulate the development of tolerogenic T cells that produce high levels of IL-10,28,29 These findings suggest that dogs increase levels of endotoxin or other innate immune stimuli, and that this environment in early life boosts MNC IL-10 responses.

The gene-by-environment interaction involving CD14, which is part of the endotoxin receptor complex, supports the concept that dogs in the home reduce the incidence of AD by increasing exposure to endotoxin or another Toll-like receptor ligand. Recently, Vergez,30 in response to conflicting published reports of CD14 associations, predicted that there would be no association between genotypes and atopy with very low levels of endotoxin exposure, but that the −159T allele would be protective among children with moderate exposure. Our findings of a protective effect of the CD14−159TT genotype only in children with a dog at home is consistent with this hypothesis.

Our findings raise the possibility that enhanced IL-10 and IL-13 responses are responsible for the lower risk for allergic sensitization and AD. In support of this concept, IL-10 has been linked to allergen tolerance in an epidemiologic study31 and in mouse models of allergen exposure.28,32,33 Furthermore, IL-10 can modify TH2-like antibody responses by shifting IL-4–induced isotype switching from IgE to IgG4,34 and this modified TH2 response might promote tolerance rather than allergy.13 In the COAST cohort, we have previously reported that PHA-induced IL-10 responses are inversely related to the risk of egg sensitization but were not related to the risk of AD.17 Whether IL-10 is the tolerogenic factor that reduced sensitization cannot be established by this observational study, and the mechanism for the effects of dog exposure on reduced AD remain speculative.

Several other prospective studies have evaluated effects of pet exposure on allergen sensitization and wheezing.7,8,11 For example, in Swedish children, dog or cat exposure at birth was associated with reduced allergic rhinitis at age 4 years.8 Furthermore, children in Detroit

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<td>CC</td>
<td>7/18 (0.38)</td>
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<tr>
<td></td>
<td>CT</td>
<td>15/38 (0.39)</td>
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Proportion of children in each category are shown (frequency). P value for the interaction between dog ownership and genotype on AD risk = .0071.
who were exposed to dogs in the first year had reduced total and allergen-specific IgE at age 6 to 7 years.\textsuperscript{11} Moreover, exposure during infancy to either dogs\textsuperscript{7} or cats\textsuperscript{35} has been associated with reduced wheezing later in childhood. Although there are differences in experimental design, environmental conditions, and family characteristics, these prospective studies suggest that exposure to pets in early life promote respiratory health.

There might be a tendency for families with parents or children with allergy to avoid owning pets, a variable that can be an important confounding factor in studies of pets and allergies.\textsuperscript{8,36} Our study has several features that help to minimize this potential bias. First, although every family in this cohort has at least 1 parent with allergies or asthma, rates of pet ownership (158 of 285, 55\%) were comparable with the range reported for unselected populations (45\% to 61\%).\textsuperscript{7,11,37} These data indicate that many children were exposed to pets in the first year of life, and that there was no evidence of a strong tendency to avoid pet ownership in our cohort. There were indications of interactions between pet ownership and pet allergy: families who owned cats were less likely to have a father with cat allergy, and a similar nonsignificant trend was noted for families with dogs and maternal dog allergy (Table I). Although the causality of this relationship is unknown, it should be emphasized that the protective effects of dog on childhood atopy were observed after controlling for the parental history of dog and cat allergies in multivariable regression models. Furthermore, the clinical and immunologic effects that were observed were specific for dog exposure. If there were a systematic bias related to pet ownership and allergies, it would be expected to affect relationships between the outcome measures and exposure to cats as well as dogs. Finally, although high dropout rates can introduce a strong bias into cohort studies,\textsuperscript{36} only 4 subjects (1.4\%) withdrew during the first year of the COAST study, thus providing excellent data for longitudinal analysis.

In this study, in contrast to several others,\textsuperscript{9,12,15} home exposure to cats did not inhibit allergic sensitization. Platt's-Mills et al\textsuperscript{13} have suggested that high levels of cat allergen exposure might be required for protection to occur. Because we did not measure allergen levels in house dust, we were unable to test this hypothesis. Alternatively, cats might have less of an effect on personal exposure to airborne endotoxin,\textsuperscript{22} perhaps because of their relatively small size (less endotoxin), or because direct animal-human contacts are less intense as a result of the unique social habits of cats compared with dogs.

This study should be interpreted with an understanding of its limitations. First, because effects of pet exposure on allergen tolerance might be stronger for children with a family history of allergy,\textsuperscript{15} our results might overestimate effects of pet exposure in an unselected population. In addition, it should be noted that dog exposure was associated with less allergic sensitization but not confirmed food allergy, although the numbers of affected children were low. Although these children at age 1 year were too young to manifest symptoms of respiratory allergy, sensitization to foods in infancy has proved to be a strong risk factor for the subsequent development of respiratory allergies and asthma.\textsuperscript{38} Finally, the duration of the study was relatively short (1 year), and it is possible that pet exposure later in life or continued pet exposure for several years might have distinct effects. We are continuing to monitor these children and intend to address some of these remaining questions.

In conclusion, our study supports the growing body of evidence suggesting that exposure to pets in early life might reduce the incidence of atopic disorders and provides new information regarding potential immunologic and genetic mechanisms. Indeed, the data raise the possibility that tolerance induced by exposure to dog is mediated by enhanced production of IL-10 or modified Th2 responses, and that the protective effects of dog exposure might be genotype specific. Greater understanding of mechanisms that modify immune development to promote tolerance in infancy might lead to new preventive strategies for allergic diseases.

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Wacholz, David Weber, Bonny Whalen, Margaret Wilcots, Gary Williams, W. Michael Wilson, Robin Wright, Michael Yaffe, and Kok-Peng Yu. The support and participation of the following hospitals and clinics have been key to the success of the project: the obstetrical nursing staff at Meriter Hospital, St Mary’s Medical Center, Fort Atkinson Memorial Health Services, Inc, St Clare Hospital, Reedsburg Area Medical Center, Sauk Prairie Memorial Hospital, and, in addition, the clinic staff from Physicians Plus, Associated Physicians, Dean Medical Center, Group Health Cooperative, and other clinics in southwestern Wisconsin who care for individual COAST families.