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Effects of dog ownership in early childhood on immune development and atopic diseases

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Summary

Background Exposure to pets in childhood has been associated with a reduced risk of wheezing and atopy.

Objective Our objective was to determine whether the effects of pet exposure on immune development and atopy in early childhood can be explained by alterations in exposure to innate immune stimuli in settled dust.

Methods Two hundred and seventy-five children at increased risk of developing allergic diseases were evaluated to age 3 years for pet ownership, blood cell cytokine responses, and atopy. Can f 1, Fel d 1, endotoxin, ergosterol, and muramic acid were measured in settled dust from 101 homes.

Results Dog exposure at birth was associated with decreased atopic dermatitis (AD) (12% vs. 27%; $P = 0.004$) and wheezing (19% vs. 36%; $P = 0.005$) in year 3. The rates of AD (23%) and wheezing (42%) in year 3 were relatively high in children who acquired dogs after birth. The prevalence of dog sensitization (10–12%) did not vary according to dog exposure. Can f 1 levels in bedroom dust were positively associated with IL-10 ($r = 0.26$; $P = 0.01$), IL-5 ($r = 0.34$, $P < 0.001$), and IL-13 ($r = 0.28$; $P = 0.004$) responses at age 1, and IL-5 ($r = 0.24$; $P = 0.022$) and IL-13 ($r = 0.25$; $P = 0.015$) responses at age 3. In contrast, endotoxin was associated with IFN- γ ($r = 0.31$; $P = 0.002$) and IL-13 ($r = 0.27$; $P = 0.01$) responses at age 3 but not at age 1, and similar relationships were present for muramic acid. Adjustment for levels of innate immune stimuli in house dust did not significantly affect the relationships between Can f 1 and cytokine responses.

Conclusions Exposure to dogs in infancy, and especially around the time of birth, is associated with changes in immune development and reductions in wheezing and atopy. These findings are not explained by exposure to endotoxin, ergosterol, or muramic acid.

Keywords atopic dermatitis, cytokines, dogs, pets, wheezing

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Introduction

Exposure to furred pets in the home can cause exacerbations of rhinitis and asthma in sensitized individuals [1–3]. However, recent evidence suggests that exposure to pets in early childhood can reduce the risk of atopic diseases [4–7]. The timing of exposure to animals might be important in determining the effects of atopy, although

this is controversial. While some studies have demonstrated a protective effect of dog exposure in infancy on clinical outcomes later in childhood [5, 7], others have suggested that these effects are dependent on or stronger for current dog exposure [8].

The mechanisms behind the protective influence of pet exposure are now under investigation. For example, chronic exposure to cats may induce a modified T-helper

type 2 (Th2) response that down-regulates IgE synthesis and favours the production of IgG4 [9]. In addition, we reported that dog exposure is associated with reduced incidence of allergic sensitization and atopic dermatitis (AD) in the first year of life [10] and also enhanced IL-10 and IL-13 responses from blood mononuclear cells at age 1 year. These findings, coupled with the identification of a gene-by-environment interaction between dog exposure and CD14 polymorphisms on AD [10], suggested that exposure to dogs in early life is associated with a unique pattern of immune development, perhaps driven by innate immune stimuli.

We continued to follow this cohort prospectively to age 3 years and have examined whether or not the timing of dog exposure influences these associations. Furthermore, in order to test the hypothesis that dog exposure increases the exposure to microbial stimuli of the innate immune system, which, in turn, influence immune development and the development of atopic disease, we conducted environmental sampling of household dust for exposure to microbial biomarkers, including endotoxin (Gram-negative bacteria), muramic acid (Gram-positive bacteria), and ergosterol (fungi).

Methods

Study subjects

A total of 289 newborns were enrolled from November 1998 through May 2000 in the Childhood Origins of Asthma (COAST) study as described earlier [11–13]. Of these children, 285 (98.6%) were followed prospectively for at least 1 year, and 275 (95.2%) were followed for 3 years [14]. To qualify, at least one parent was required to have respiratory allergies (defined as one or more positive aeroallergen skin tests) and/or a history of physician-diagnosed asthma. The Human Subjects Committee of the University of Wisconsin approved the study.

Study design

Blood was obtained annually for evaluation of phytohaemagglutinin (PHA)-induced peripheral blood mononuclear cell (PBMC) cytokine responses, including IFN- γ , IL-5, IL-10, and IL-13, as well as allergen-specific IgE to foods and aeroallergens as described previously [13]. The presence or absence of a dog at home was determined by questionnaires on a yearly basis. The presence of AD [13] or wheezing [14] in the past year was determined on a yearly basis as described previously.

Dust sampling and processing

House dust samples were collected from the bedrooms of a subset of 101 children aged 3 years who had not moved

since birth using a Eureka Mighty Mite Smart Vac[®] vacuum (Electrolux, Peoria IL, USA) with an attached crevice/thimble device in the wand. Collection times were as follows: 5 min on flooring directly under and to the side of the bed (18–24 × 72 in), 30 s on the main sleeping pillow, 2.5 min on all bedding layers, and 2 min on the top surface of the mattress. Dust samples were sieved and weighed and then stored in a –80 °C freezer until processing. For measurement of allergens, samples were extracted in borate-buffered saline with 5% bovine serum albumin, and the major dog allergen Can f 1 and the major cat allergen Fel d 1 were measured by sandwich ELISA [15, 16]. For measurement of endotoxin, samples were suspended in pyrogen-free phosphate-buffered saline, sonicated, and diluted in pyrogen-free water [17]. Endotoxin concentration was measured by the Limulus Amebocyte Lysate assay (Bio-Whittaker QCL-1000, Walkersville, MD, USA) under pyrogen-free conditions. Endotoxin concentration is expressed in endotoxin units per mL. Serial dilutions of each sample were run in triplicate. Ergosterol and muramic acid were measured by gas chromatography–ion trap mass spectrometry. These samples were stored dry in baked glass tubes, and detailed protocols are as described elsewhere [18, 19].

Cytokine enzyme-linked immunosorbent assay

PBMCs were stimulated with PHA, and cytokine responses were measured as described previously [13, 20]. Cytokine data were not available for three subjects at age 1 and 33 subjects at age 3. Reasons for the absence of cytokine data included not enough blood obtained at blood draw or failure to obtain a blood sample during the allotted window of time. The levels of IFN- γ , IL-5, IL-10, and IL-13 in supernatants were measured by ELISA (Pharmingen, San Diego, CA, USA) [13]. The sensitivities are as follows: IFN- γ = 4.7 pg/mL; IL-5 = 1.9 pg/mL; IL-10 = 7.7 pg/mL; and IL-13 = 3.1 pg/mL. Duplicate wells were run, and mean values are reported.

Allergen-specific immunoglobulin E

Total IgE and allergen-specific IgE levels for dust mites (Der p 1 and Der f 1), cat (Fel d 1), dog (Can f 1), *Alternaria alternata*, egg, peanut, and milk were determined as described previously [13]. Allergen-specific values of 0.35 kU/L (Class I) or greater were considered to be positive.

Statistical analysis

Categorical outcomes were compared between two groups with χ^2 tests. Continuous outcomes were compared between two groups with Wilcoxon's rank-sum tests. The effects of dog exposure on clinical outcomes were also

analysed using multivariate logistic regression adjusting for covariates (RSV infection in the first year, daycare attendance, older siblings, maternal asthma and allergy, and paternal asthma and allergy, race/ethnicity, parental education, and income). Correlations between continuous variables were assessed with Pearson's correlation coefficients on the log-transformed values.

Results

Pet exposure

Two hundred and seventy-five subjects were followed to age 3 years and were included in this analysis. At the time of birth, 97 (35%) children were exposed to dogs and 81 (29%) were exposed to cats in the home. At age 3 years, 89 (32%) subjects were exposed to a dog, and 69 (25%) were exposed to a cat in the home. While dog ownership was relatively stable over 3 years, 21 (8%) children were exposed to a dog at birth but not at age 3, and 13 (5%) children did not have a dog at birth and then obtained a dog by age 3.

Demographic variables for subjects and their families were compared in relation to the presence or absence of dog exposure in the home at birth and age 3 years (Table 1). There were relatively few differences in demographics associated with dog ownership. Children exposed to a dog at birth and throughout the first 3 years of life were less likely to be non-white and less likely to have

maternal cat allergy. Dog ownership at birth and at age 3 was independent of maternal and paternal asthma, allergy, and dog allergy. Removal of the dog present at birth by age 3 was not associated with an increased prevalence of maternal or paternal dog allergy, allergy in general, or asthma (data not shown).

Dog ownership and markers of atopic diseases

Exposure to dogs at birth was associated with reduced cumulative prevalence of AD (ever AD) in the first 3 years of life (14% vs. 37%; $P < 0.001$), and there was a similar reduction in the prevalence of active AD at the 3-year clinic visit (12% vs. 27%; $P = 0.004$) (Table 2). Although dog exposure was not related to wheezing in the first year of life (data not shown), exposure at birth was associated with a reduced risk of wheezing in the third year of life (19% vs. 36%; $P = 0.005$). These relationships persisted after adjustment for covariates (RSV infection in the first year, daycare at 6 months, older siblings, and both maternal and paternal asthma and allergy). There were no associations between dog exposure at birth and food or aeroallergen-specific IgE results at age 3. In particular, dog exposure at birth did not influence the risk of sensitization to dog (11% vs. 12%; $P = 0.94$).

Cross-sectional relationships between dog ownership at age 3 years and contemporaneous atopic indicators were somewhat weaker (Table 2). Dog ownership at age 3 was associated with a reduction in the prevalence of active AD

Table 1. Characteristics of subjects ($N = 275$)

Characteristics	No dog at birth ($n = 178$)	Dog at birth ($n = 97$)	P -value	No dog at age 3 ($n = 186$)	Dog at age 3 ($n = 89$)	P -value
Subject						
Non-white	18%	6%	0.007	16%	10%	0.22
RSV in year 1	50%	44%	0.37	51%	43%	0.22
Daycare in first 6 months	47%	46%	0.90	47%	47%	0.95
Older siblings	60%	47%	0.053	56%	53%	0.57
Smoke exposure in year 1	27%	20%	0.17	26%	20%	0.27
Income ($> \$50\,000$)	57%	60%	0.58	58%	58%	0.98
Maternal						
Age (years)	31	31	0.93	31	31	0.92
Asthma	42%	39%	0.70	42%	38%	0.56
Allergy*	86%	77%	0.062	86%	78%	0.12
Allergy (dog)	45%	33%	0.054	42%	37%	0.44
Allergy (cat)	59%	39%	0.003	55%	45%	0.14
Education ($>$ high school)	92%	91%	0.72	93%	89%	0.25
Paternal						
Age (year)	33	33	0.56	33	33	0.99
Asthma	29%	25%	0.53	29%	25%	0.56
Allergy*	83%	76%	0.21	81%	79%	0.64
Allergy (dog)	30%	35%	0.48	31%	35%	0.55
Allergy (cat)	44%	42%	0.79	44%	43%	0.89
Education ($>$ high school)	85%	86%	0.71	85%	85%	0.96

*At least one positive skin test result.

Table 2. Dog exposure and clinical outcomes at age 3 ($N = 275$)

	No dog at birth ($n = 178$)	Dog at birth ($n = 97$)	OR	P -value	No dog at age 3 ($n = 186$)	Dog at age 3 ($n = 89$)	OR*	P -value*
AD (% ever)	37	14	0.24 (0.11, 0.53)	< 0.001	34	19	0.42 (0.20, 0.88)	0.015
AD (% active)	27	12	0.35 (0.15, 0.83)	0.016	26	13	0.39 (0.17, 0.92)	0.029
Wheezing (%)	36	19	0.49 (0.25, 0.95)	0.029	33	23	0.75 (0.38, 1.47)	0.22
Allergic sensitization (%)	45	35	0.68 (0.36, 1.30)	0.33	43	38	0.91 (0.48, 1.73)	0.67
Dog-specific IgE (%)	12	11	0.92 (0.33, 2.56)	0.89	12	11	0.99 (0.36, 2.76)	0.99

*Odds ratios (ORs) (95% CIs) and P -values were calculated using multivariate logistic regression adjusting for covariates (non-white, RSV infection in first year, daycare attendance, older siblings, smoke exposure in year 1, income, maternal asthma, allergy, and education, and paternal asthma, allergy, and education).
AD, atopic dermatitis.

at age 3 (13% vs. 26%; $P = 0.016$) and in ever AD (19% vs. 34%; $P = 0.011$) in the univariate analysis, and these findings remained significant in the multivariate logistic regression model. However, there were no significant associations between dog exposure at age 3 and wheezing or allergic sensitization to foods or aeroallergens, including dog. Cat ownership, either at birth or at age 3, was not associated with the prevalence of AD, wheezing, or atopy at age 3 (data not shown).

To further assess the influence of the timing of dog ownership on associations with atopy and atopic markers, subjects were characterized into four groups based on dog exposure at birth and at age 3 years: 76 (28%) had a dog throughout the first 3 years (Group D_{B+3}), while 165 (60%) of children were never exposed to a dog at home (Group D_0). There were some changes in dog ownership over time: 13 (5%) without a dog at birth obtained a dog by age 3 (Group D_3) and 21 (8%) with a dog at birth no longer had a dog by age 3 (Group D_B).

The groups exhibited different patterns of atopy in the first 3 years (Fig. 1). Compared with Group D_0 , Group D_{B+3} had a lower incidence of AD (11% vs. 27%; $P = 0.006$) and wheezing (20% vs. 35%; $P = 0.021$). These relationships were similar after multivariate analysis (AD, $P = 0.015$; wheezing, $P = 0.072$). Group D_B had low rates of AD (14%) and wheezing (15%) that were comparable to Group D_{B+3} . In contrast, Group D_3 had higher rates of AD (23%) and wheezing (42%) that were similar to those of Group D_0 . The timing of dog ownership was not associated with rates of allergic sensitization (Fig. 1c), dog-specific IgE (Fig. 1d), or total IgE levels (data not shown) at age 3 years.

Pet proteins and microbial markers in settled dust

Settled dust samples from the bedrooms of 101 children at age 3 who had not moved since birth were analysed for

Can f 1, Fel d 1, as well as markers of microbial exposure: endotoxin, muramic acid, and ergosterol. Compared with the rest of the cohort, families who had dust sampling were less likely to be non-white (7% vs. 18%; $P = 0.012$) and more likely to have attended daycare in the first 6 months (55% vs. 42%; $P = 0.31$ (see online supplementary data)). In addition, the parents were slightly older (maternal age 33 vs. 31 years, paternal age 34 vs. 32 years,

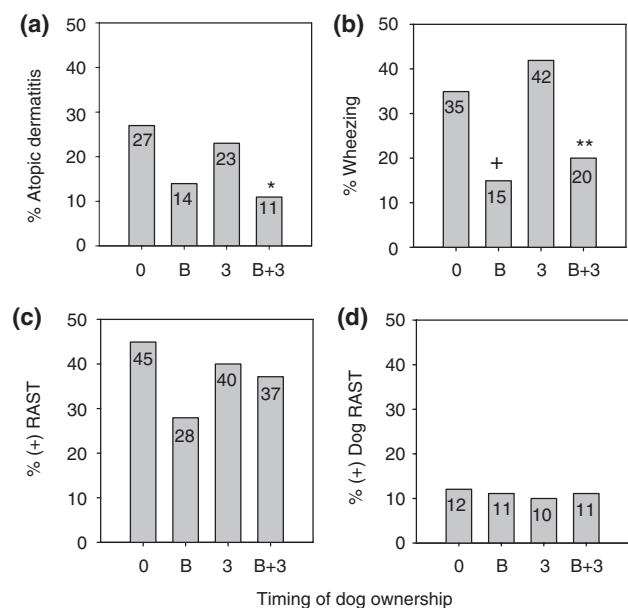


Fig. 1. Timing of dog exposure and clinical outcomes at the age of 3 years, including active atopic dermatitis (a), wheezing (b), any allergic sensitization (c), and dog-specific IgE at age 3 (d). Dog ownership categories: 0, no dog ownership from birth to age 3; B, dog ownership at birth but not age 3; 3, dog ownership by age 3 but not at birth; B+3, dog ownership from birth to age 3. * $P = 0.006$ ($P = 0.015$), ** $P = 0.021$ ($P = 0.072$), + $P = 0.07$ ($P = 0.13$); all comparisons are in relation to Group D_0 . P -values in parentheses are adjusted for covariates.

$P < 0.001$). As expected, there were significantly higher levels of Can f 1 and Fel d 1 in the bedrooms of children who owned dogs and cats, respectively, at age 3 years (Table 3). There were no significant differences in the levels of endotoxin, muramic acid, or ergosterol in relation to dog ownership at age 3 (Table 3). There was a significant inverse correlation between Can f 1 and ergosterol ($r = -0.23$; $P = 0.023$) and indications of a positive association between Can f 1 and endotoxin ($r = 0.18$; $P = 0.08$). Cat owners had higher dust levels of muramic acid (101 vs. 67 ng/mg dust, $P = 0.002$), and there was a positive correlation between Fel d 1 and muramic acid ($r = 0.38$; $P < 0.001$). Finally, there were positive correlations between endotoxin and both muramic acid ($r = 0.41$; $P < 0.001$) and ergosterol ($r = 0.22$; $P = 0.032$). These relationships were unchanged after repeating the analysis after excluding 11 families who had either gained ($n = 1$) or lost ($n = 10$) their dogs over the interval from birth to age 3 years (data not shown).

Dust analyses, cytokine response profiles, and atopy

We have reported previously that PHA-induced IL-10 and IL-13 responses at age 1 were increased in children exposed to a dog beginning at the time of birth [10]. Analysis of house dust revealed positive correlations between Can f 1 levels and IL-5 ($r = 0.34$; $P < 0.001$), IL-10 ($r = 0.26$; $P = 0.01$), and IL-13 ($r = 0.28$; $P = 0.004$) responses at age 1 (Fig. 2). The strength of the association between Can f 1 levels and year 3 IL-5 ($r = 0.22$; $P = 0.022$) and IL-13 ($r = 0.25$; $P = 0.15$) responses was weaker, and there was no correlation with year 3 IL-10 responses ($r = 0.06$; $P = 0.59$). Furthermore, exposure to dog at birth was not related to IL-10 or IL-13 responses at age 3 years, and Can f 1 levels were not associated with IFN- γ responses at either age (data not shown). Finally, there were no associations between Fel d 1 levels and cytokine responses at either age (data not shown).

Concentrations of selected innate immune stimuli were then compared with the development of PHA-induced cytokine responses (Table 4). In contrast to findings with Can f 1,

there were no significant correlations between endotoxin, muramic acid, or ergosterol levels and PHA-induced cytokine responses at age 1. At age 3 years, endotoxin was positively related to IFN- γ ($r = 0.31$; $P = 0.002$) and IL-13 ($r = 0.27$; $P = 0.01$) responses, with a similar trend for IL-10 ($r = 0.19$; $P = 0.066$). Muramic acid levels were also positively related to age 3 IFN- γ ($r = 0.29$; $P = 0.007$) and IL-13 ($r = 0.26$; $P = 0.015$) responses, with a positive trend for IL-5 ($r = 0.21$; $P = 0.055$). There was a trend towards a negative correlation between ergosterol levels and IL-13 responses at age 3 ($r = -0.18$; $P < 0.089$). There were no significant associations between Can f 1, Fel d 1, endotoxin, ergosterol, or muramic acid levels and the clinical outcomes of atopy including wheezing, AD, and allergic sensitization. We next performed a post hoc analysis to determine whether the association between Can f 1 and changes in cytokine responses would be altered by adjustment for innate immune stimuli in house dust. Adjustment for endotoxin or muramic acid did not affect the association between Can f 1 and cytokine responses at age 1 year, and had little effect on cytokine responses at age 3 years (Table 4).

Discussion

The findings from this prospective birth cohort study demonstrate that exposure to dogs, and not cats, is associated with a unique pattern of immune development and a reduced risk of AD and wheezing. This concept was further supported by the presence of a dose-related association between Can f 1 levels in settled dust and cytokine response profiles. Interestingly, the immunologic relationships with dog exposure were strongest at age 1 year, while the clinical relationships were strongest at age 3 years. Furthermore, dog exposure at the time of birth appeared to have the most influence on the clinical outcomes. Finally, although we hypothesized that the effects of dog exposure on immune development would be driven by increased exposure to microbial stimuli, the alterations in immune development and clinical outcomes were not related to levels of endotoxin, ergosterol, or muramic acid in settled dust. The children in the COAST

Table 3. Median (25th, 75th) environmental exposure levels in settled bedroom dust according to pet ownership at age 3

	No dog ($n = 61$)	Dog ($n = 33$)	Ratio (95% CI)	P -value	No cat ($n = 68$)	Cat ($n = 25$)	Ratio (95% CI)	P -value
Can f 1 (ng/g)	724 (335, 1879)	42 845 (16 665, 86 489)	45 (17.0, 122.5)	< 0.001				
Fel d 1 (ng/g)					930 (453, 2059)	176 009 (56 060, 543 310)	112 (35, 363)	< 0.001
Endotoxin (EU/mg)	83 (39, 125)	102 (50, 126)	1.2 (0.9, 1.7)	0.34	80 (37, 119)	94 (58, 137)	1.5 (1.1, 2.2)	0.08
Muramic acid (ng/mg)	79 (55, 106)	70 (54, 102)	0.9 (0.7, 1.2)	0.61	67 (50, 95)	101 (68, 133)	1.5 (1.2, 1.9)	0.002
Ergosterol (ng/mg)	1.9 (0.9, 2.8)	1.4 (0.81, 2.0)	0.7 (0.5, 1.1)	0.07	1.5 (0.9, 2.4)	1.9 (1.2, 2.7)	1.3 (0.8, 2.1)	0.28

EU, endotoxin units.

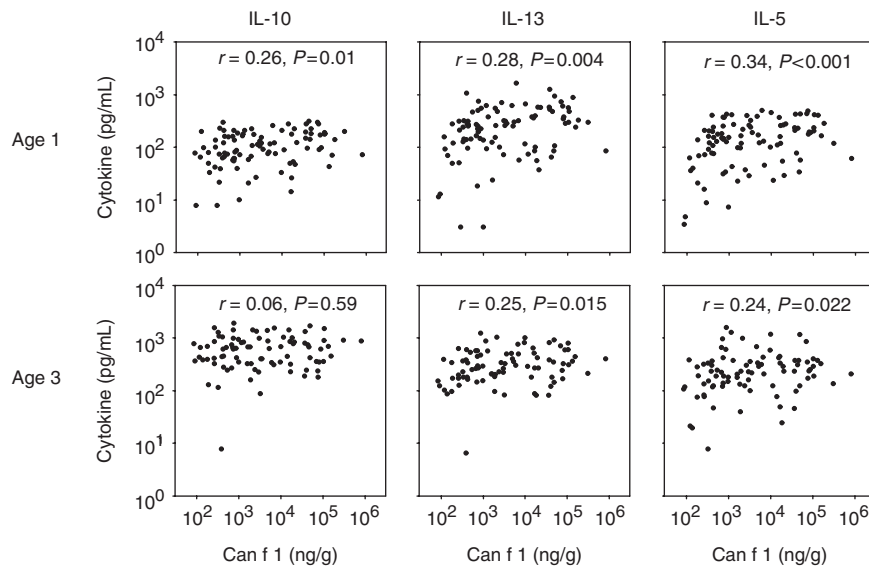


Fig. 2. Correlations between Can f 1 levels in settled dust and phytohaemagglutinin-induced peripheral blood mononuclear cell cytokine responses at 1 and 3 years of age. Can f 1 levels and cytokine response data were log transformed to approximate a normal distribution.

study are at an increased risk, based on parental histories, for the development of allergic diseases, and associations between exposure to pet proteins and immune development could be different in a non-selected population.

In our study, dog ownership was negatively associated with wheezing at age 3 but not at age 1. While the mechanism for this is speculative, it could be related to the shift in the pathogenesis of wheezing in early childhood. Wheezing in the first year of life is predominantly caused by viral infections together with individual susceptibility factors related to lung size, anti-viral responses, and genetics [21]. After infancy, wheezing with viral infections may increasingly be an indicator of atopic diathesis. In contrast to wheezing, AD in infancy is often, although not always, an indicator of atopy. Our findings therefore support previous reports that infantile wheezing and AD likely have a distinct pathogenesis based on differential effects of numerous risk factors ranging from sex and breastfeeding to pet exposure and parental asthma [22]. In contrast to wheezing, dog exposure at birth was consistently associated with reduced AD from age 1–3 years. These findings suggest that dog ownership can modify immune development to reduce the incidence of atopic disorders, but may not affect non-atopic processes such as infectious bronchiolitis.

Dog exposure at the time of birth was most closely associated with immunologic effects and reduction in AD and wheezing. These findings suggest that dog exposure in the neonatal period, perhaps during a critical step in immune development, may be especially important to atopic outcomes. The duration of dog exposure could also be an important factor, and this concept is supported by other studies that have demonstrated a protective effect of

current dog exposure on various manifestations of atopic disease [23]. Remes et al. [7] have shown that children who lose their dog by age 3 or 6 years have an increased risk of wheezing compared with children who are consistently exposed to dogs from birth through age 6. The prospective nature of the COAST study will allow continued follow-up to address the effects of duration vs. timing of exposure.

How does dog exposure affect immune development in infancy? In our study, there was a dose-dependent relationship between Can f 1 levels in dust and IL-5, IL-10, and IL-13 responses that was most pronounced at 1 year of age. Although we had hypothesized that protection from atopy would be associated with enhanced IL-10 responses, the positive relationship with Th2 responses (IL-5 and IL-13) seems paradoxical. These findings should be considered in light of the rapid changes in immunologic development that occur during infancy. In fact, normal immune development is associated with increases in PHA-induced Th1 and Th2-like cytokine responses [24], and it has been suggested that atopic children lag behind their peers in immunologic development [25]. Collectively, these findings suggest the possibility that dog exposure contributes to the development of the immune system, and the enhanced cytokine responses may be a reflection of this activity.

Previously, we reported a gene-by-environment interaction between dog exposure and CD14 genotype on AD [10]. In addition, Simpson et al. [26] demonstrated interactions between environmental endotoxin exposure and CD14 (–159) genotype on atopic outcomes and wheezing. These findings suggest that dog exposure could influence immune development through mechanisms

Table 4. Pearson's correlation coefficients (95% confidence intervals) between environmental exposure levels and cytokine responses at the age of 3 years

	Age 3									
	IL-10	IFN- γ	IL-13	IL-5	IL-10	IFN- γ	IL-13	IL-5	IL-13	IL-5
Endotoxin	0.04 (-0.16, 0.24)	0.07 (-0.13, 0.26)	-0.02 (-0.22, 0.18)	-0.03 (-0.23, 0.17)	0.19 [†] (-0.01, 0.38)	0.31* (0.12, 0.48)	0.27* (0.07, 0.45)	0.08 (-0.12, 0.28)	0.26* (0.05, 0.45)	0.21 [†] (-0.01, 0.40)
Muramic acid	-0.02 (-0.22, 0.18)	-0.09 (-0.28, 0.12)	-0.14 (-0.33, 0.06)	-0.07 (-0.27, 0.13)	0.09 (-0.13, 0.29)	0.29* (0.08, 0.47)	0.26* (0.05, 0.45)	0.21 [†] (-0.01, 0.40)	0.26* (0.05, 0.45)	0.21 [†] (-0.01, 0.40)
Ergosterol	0.11 (-0.09, 0.31)	-0.11 (-0.30, 0.10)	-0.01 (-0.21, 0.19)	0.05 (-0.15, 0.25)	-0.18 [†] (-0.38, 0.03)	-0.08 (-0.28, 0.14)	-0.15 (-0.35, 0.07)	-0.05 (-0.26, 0.16)	0.26* (0.05, 0.45)	0.21 [†] (-0.01, 0.40)
Can f 1	0.26* (0.06, 0.43)	0.11 (-0.09, 0.30)	0.28** (0.09, 0.45)	0.34** (0.16, 0.51)	0.06 (-0.15, 0.26)	0.16 (-0.04, 0.35)	0.25* (0.05, 0.43)	0.24* (0.03, 0.42)	0.26* (0.05, 0.45)	0.21 [†] (-0.01, 0.40)
Can f 1 adjusted for endotoxin	0.24* (0.03, 0.43)	0.11 (-0.11, 0.31)	0.30** (0.10, 0.40)	0.39** (0.19, 0.55)	0.05 (-0.16, 0.26)	0.09 (-0.12, 0.29)	0.17 (-0.04, 0.36)	0.20 [†] (-0.01, 0.39)	0.26* (0.05, 0.45)	0.21 [†] (-0.01, 0.40)
Can f 1 adjusted for muramic acid	0.24* (0.03, 0.43)	0.14 (-0.07, 0.34)	0.30* (0.09, 0.48)	0.37** (0.17, 0.54)	0.10 (-0.12, 0.31)	0.12 (-0.09, 0.33)	0.21 [†] (-0.01, 0.40)	0.21 [†] (-0.01, 0.40)	0.26* (0.05, 0.45)	0.21 [†] (-0.01, 0.40)
Can f 1 adjusted for ergosterol	0.25* (0.04, 0.44)	0.14 (-0.08, 0.34)	0.29* (0.08, 0.47)	0.37** (0.17, 0.54)	0.10 (-0.11, 0.31)	0.13 (-0.08, 0.34)	0.22* (0.00, 0.41)	0.21 [†] (0.00, 0.41)	0.26* (0.05, 0.45)	0.21 [†] (-0.01, 0.40)

[†] $P < 0.1$.* $P < 0.05$.** $P < 0.005$.

involving endotoxin or perhaps other innate immune stimuli. Having indoor pets, including dogs, can increase exposure to endotoxin [27–29], which has been linked to a reduced prevalence of allergic sensitization [30], allergic rhinitis [31], atopic asthma [32], and AD [33, 34]. An inverse association between muramic acid levels and wheezing has been reported in European children [35], while positive associations have been reported between ergosterol and both fungal sensitization and wheezing in adults [36].

Based on these findings, we considered the possibility that dog-related increases in endotoxin [37] or other markers of microbial exposure were responsible for effects on immune development and clinical outcomes. As expected, dog exposure was associated with significantly higher levels of Can f 1, although there was measurable exposure even in homes without dogs. Although dog ownership per se did not significantly influence the levels of microbial products, there was a trend towards a positive correlation between Can f 1 and endotoxin levels in settled dust. Patterns of immune development were also associated with innate immune stimuli at age 3 years; both endotoxin and muramic acid were positively associated with PHA-induced IFN- γ and IL-13, and associations between endotoxin exposure and IFN- γ responses have been observed in other studies [38, 39]. This pattern was distinct from relationships observed with Can f 1, both in terms of timing (age 1 vs. age 3) and the specificity of cytokine responses. These findings, along with the multivariate analysis, indicate that neither endotoxin, muramic acid, nor ergosterol account for the protective effects of dog exposure on atopic disease. Our findings are in agreement with a recent study by Litonjua et al. [40] in which the negative effect of dog exposure on wheezing was independent of endotoxin exposure.

There are several potential explanations for the lack of association between microbial markers and atopic outcomes. It is possible that other microbial factors, such as bacterial DNA [41], mediate the protective benefit of dog exposure on the development of atopic disease. In addition, perhaps airborne dust sampling or other technologies could provide more accurate indices of personal exposure to allergens or innate immune stimuli, and studies involving these technologies could yield additional information related to effects on immune development.

The strengths of this study include the prospective design, high retention rate (95.2%) through age 3 years, and prospective measurement of cytokine responses. As a result, we were able to evaluate differential effects on immune development and based on the timing of dog exposure. One limitation of the COAST study is that the study participants all have a parental history of atopy or asthma. It has been suggested that dog exposure is associated with stronger protective effects in children with

atopic parents [42], although this is controversial [7]. It is also important to consider that studies of pet exposure and atopy can be confounded by selection bias, because pet ownership can be influenced by personal or parental atopic status [4, 43, 44]. This concern was not substantiated in our study population, because dog ownership and changes in this status in the first 3 years were independent of parental allergy, asthma, and dog allergy. Furthermore, the immunologic and clinical outcomes were specifically related to dogs, and not cats, in the home, arguing against a general selection bias related to pets. Effects were strongest if the pet was present at the time of birth, when there is the least potential for reverse causation. Finally, there was a dose-related relationship between Can f 1 in house dust and cytokine responses, and this would not be predicted by a selection bias.

In summary, early childhood is a period of rapid immunologic development, and these findings indicate that exposure to dogs can influence this process in a manner that is associated with reduced AD and wheezing in pre-disposed individuals. Although the effects of dog exposure on clinical and immunologic outcomes were not explained by the levels of several microbial products in household dust, there is genetic and epidemiologic evidence implicating innate immune stimulation. Identifying the mechanism underlying the protective effects of dog exposure is a worthwhile goal, and this information could provide the basis for new strategies to prevent atopic disorders in childhood.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Characteristics of subjects with and without dust sampling.

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