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# Regulatory T Cells in Prenatal Blood Samples: Variability with Pet Exposure and Sensitization

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## Abstract

Fetal exposures have come under investigation as risk factors of early life allergic disease. In this study we aimed to examine the relationships between dog or cat exposure and naturally occurring regulatory T cells (Treg cells), thought to play an important role in immune tolerance, in pregnant women. A cross-sectional analysis was conducted among 204 pregnant women who were queried regarding dog and cat exposure. Treg cells (CD4+CD25+Foxp3+ lymphocytes) and allergen-specific IgE were measured in venous blood samples. Atopy was defined as allergen-specific IgE ≥ 0.35 kU/L reactive with common allergens including dust mite, dog, cat, Timothy grass, ragweed, *Alternaria alternata*, egg white or cockroach. Nonparametric Wilcoxon rank sum tests and linear regression models of log transformed Treg cell levels were used in analyses. Among women sensitized to dog, those who had a dog or cat in the home had lower Treg cell levels compared with those who had no dog or cat. However, among women not sensitized to dog, those with a dog or cat in the home had higher Treg cell levels compared with those who did not. Among women sensitized to cat, those who had a dog or cat in the home had lower Treg cell levels compared with those who had no dog or cat. Gestational age at blood draw did not affect the associations. We conclude that Treg cell levels during pregnancy vary in association with both dog and cat exposure and atopic status.

### Keywords

Pregnancy; Immunology; Regulatory T Cells

## INTRODUCTION

A recent cross-sectional study reported that almost half (45.6%) of the children in the United States ages 6–9 years are atopic (positive skin test to at least one of ten allergens) (Arbes SJ, et al. 2005). Research on childhood allergic disease has largely focused on early childhood exposures, and causal mechanisms remain poorly understood. Over the last several years, fetal exposures, especially to pets and livestock, have come under investigation as potentially critical

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factors in the development of allergic diseases. However, studies of maternal exposure to pets and livestock during pregnancy have provided mixed evidence about whether prenatal exposure to pets or livestock might reduce the risk of allergic disease in early life (Aichbhaumik N, et al. 2008; Kerkhof M, et al. 2005; Schönberger HJ, et al. 2005; Ege MJ, et al. 2006; Ege MJ, et al. 2008). Additionally, mechanistic investigations regarding the role of prenatal pet exposure in subsequent allergic disease risk are lacking.

To investigate potential mechanisms, we studied the relationship between regulatory T cells and pet exposure in a cohort of pregnant women. Although there is no consensus, regulatory T cells are thought to play an important role in immune tolerance. The subset of CD25+ regulatory T cells may be key in allergic disease mechanisms because they may suppress Th2 and Th1 responses (Shi HZ, et al. 2005; Seroogy CM and Gern JE 2005; Bacchetta R, et al. 2007; Bellinghausen I, et al. 2005; Lee JH, et al. 2007; Maggi L, et al. 2007).

For this current investigation, we examined the associations between self-reported prenatal exposure to dogs and cats and regulatory T cell percentages (Treg cells were defined as CD4 +CD25+Foxp3+ lymphocytes) measured in the blood of 204 pregnant women in the Detroit, Michigan area.

## MATERIALS AND METHODS

### 1. Study Population

The Wayne County Health, Environment, Allergy and Asthma Longitudinal Study (WHEALS) is an ongoing birth cohort study in southeastern Michigan designed to examine the relationships between early life exposures such as pets, infections or endotoxin and allergic diseases in early childhood. We recruited 21–49 year old women from a predefined geographic area who were in their second trimester or later of pregnancy, were seeing a Henry Ford Health System (HFHS) medical group obstetrician at one of five clinics, and planned to stay in the Detroit area for at least two years after delivery. Women completed an interview about their health histories at the time of recruitment and provided a venous blood sample. Women were required to speak English well enough to provide written informed consent. This research was approved by the HFHS IRB.

Recruitment began in September 2003 and was completed in November 2007. However, maternal blood samples were analyzed for Treg cells only in women recruited after November 28, 2006 because that is when funding for Treg cell analyses in a subset of the mothers became available. We have analyzed all 204 prenatal blood samples collected from the women recruited after that date. These maternal blood samples were drawn between 1 and 102 days prior to delivery.

### 2. Prenatal Interviews

Details on pet exposure were collected during the prenatal interview. Women were asked whether they had owned or cared for any pets in their home for more than 1 week since they learned they were pregnant. Pet keepers were then asked to identify the type of pet, the length of time the pet had been in their home and the usual number of hours the pet was inside the home each day. For this report, we define a woman as exposed to a pet if she reported owning or caring for dogs or cats that spent 12 or more hours each day in the home for more than 1 week.

Additional covariates considered in the analyses were maternal self-reported race, medications, and pregnancy history. Women were asked to report their race based on U.S. Census 2000 categories. They were also asked to report all medications taken during pregnancy and whether they were currently taking them at the time of the blood draw. Medications were then grouped

into any medication for asthma (currently using pill or inhalant taken daily or as needed), any medication for allergies (currently using pill or inhalant taken daily or as needed) and antibiotics (any during pregnancy). Maternal report of the timing (month and year ended), duration (weeks or months) and outcome of each of their prior pregnancies also was recorded.

Details on maternal smoking and secondhand smoke exposure were also collected during the prenatal interview. Women were asked if they were currently smoking at least 1 tobacco product per day and whether they had smoked in the first three months of their pregnancy (at least 1 tobacco product per day). Women were also asked whether other residents of their home smoked. For the analyses, each woman was assigned an indicator variable for maternal smoking during pregnancy and another indicator for whether she lived with smokers. We did not collect information on smokeless tobacco use.

### 3. Laboratory Measurements

Aliquots (0.5 ml) of whole blood collected in heparinized tubes were incubated for 6 hours at 37°C. Brefeldin A was added after two hours of incubation. The samples then were incubated with BD FastImmune™ EDTA Solution (BD Biosciences, San Jose, CA) for 15 minutes followed by BD FACS Lysing Solution (BD Biosciences) for 10 minutes to lyse red blood cells and fix white blood cells.

Samples were stored at − 80°C until staining and flow cytometry analysis. At the time of staining, samples were thawed and wash buffer (phosphate buffered saline (PBS) plus 0.5% bovine serum albumin) was added. Samples then were centrifuged, the supernatant decanted and the cells resuspended in wash buffer. Each 0.5 ml blood sample was subdivided into 100 μl aliquots for staining.

Fluorochrome-conjugated antibodies against the T cell surface marker CD25 (anti-CD25 FITC, BD Biosciences) along with anti-CD4 PerCP-Cy5.5 (BD Biosciences) for detection of CD4+ T cells were added to each 100 μl staining aliquot. Following incubation with cell surface marker antibodies, cells were then permeabilized by addition of Fixation/Permeabilization buffer (eBioscience Inc., San Diego, CA) and incubated with fluorochrome-conjugated antibody against Foxp3 (anti-Foxp3 PE, eBioscience), a transcription factor used as an intracellular marker of Treg cells. Cells were incubated at room temperature in the dark and fixed with 1% paraformaldelhyde in PBS. Flow cytometry was performed within 24 hours of staining.

Flow cytometry was performed with an LSR flow cytometer (BD Biosciences). The data were collected and analyzed using CellQuest Pro software (BD Biosciences). Matched isotype controls were included in each assay to adjust for nonspecific staining. Cells were gated on lymphocytes using forward and side light scatter properties and a minimum of 10,000 lymphoctye-gated events were acquired. CD4+ T lymphocytes were analyzed with bivariate dot plots of CD25 versus Foxp3. Values were obtained for the proportion of CD4+ lymphocytes that are CD25+ and express Foxp3. Treg cells are expressed as the percentage of CD4+ lymphocytes that are CD25+Foxp3+.

We performed reproducibility tests for flow cytometry analysis of duplicate samples within the same run and of duplicate samples run on different days. The coefficients of variation (CV) for these tests were within 5% for duplicate samples within the same flow cytometry run and within 15% for duplicate samples run on different days. A single technician performed all staining and flow cytometry procedures. All Treg cells analyses were reviewed by a single immunologist (KRB).

Venous blood was also collected for assessment of allergen-specific IgE. Plasma was isolated and stored at − 80°C until assayed. Measurements of allergen-specific IgE were performed following the standard manufacturer's protocols using the Pharmacia UniCAP system (Pharmacia-Upjohn Diagnostic Division, Kalamazoo, MI). Allergen-specific IgE was analyzed for dust mite, dog, cat, Timothy grass, ragweed, *Alternaria alternata*, egg white and cockroach. One percent of all assays were repeated in a different assay run on a different day to provide estimates of inter-assay reliability. The geometric mean coefficient of inter-assay variation was 5.9% for all eight allergens. Sensitization was defined as a positive allergen-specific IgE result of ≥ 0.35 kU/L. Atopy was defined as having at least one allergen-specific sensitization.

### 4. Statistical Methods

We used robust descriptive statistics (geometric means and 95% confidence intervals) to describe Treg cell levels during pregnancy for all women and for different subgroups. Firstborn status, first pregnancy, medication use, current asthma, sensitization to any of eight allergens, sensitization to dog, sensitization to cat, tobacco smoke exposure and self-reported African-American race were evaluated as effect modifiers and then as confounders through stratified analyses and change in effects criteria (20%), respectively. These factors were chosen because they have been identified as having potential impact on allergic risk in prior studies. Linear regression models with interaction terms were also used to evaluate effect modification and confounding of associations with log transformed Treg cell levels. Using the blood draw date and the expected delivery date from the interview, and confirmed in the medical record, we calculated the gestational age at the time of blood draw. Gestational age at draw was considered as a factor potentially affecting the associations between pet exposure and Treg cell levels.

## RESULTS

The majority of the 204 women in our sample were African American (67.2%), and had a prior pregnancy (74.0%) and a prior live birth (58.8%) (Table 1). The average age was 29.4 years (standard deviation, 5.4 years), and some women smoked during pregnancy (10.8%) or had current asthma (12.3%). Almost a quarter of the women (23.0%) lived with at least one smoker during pregnancy. Most women were atopic (59.9%), and 28.4% had a dog or cat in the home 12 or more hours per day during pregnancy. All but one pet was in the home for at least 1 month prior to the interview.

The geometric mean for the percentage of Treg cells (% of CD4+ lymphocytes that were CD25+Foxp3+) for all 204 women was 0.83% (95% CI = 0.69%, 1.01%). The levels of Treg cells did not vary by pregnancy history (Table 2), race, baby sex, maternal allergic sensitization, maternal smoke exposure, dog or cat in the home, medications or current asthma status (Table 3), even after adjusting for gestational age at time of blood draw.

In order to assess potential relationships between pet exposure and Treg cell levels we stratified results by whether a pet, either a cat or dog, was present in the home during pregnancy (Table 4). The geometric means of Treg cell levels for the pet exposed and pet unexposed women are presented for different subgroups of women. No association between Treg cells and pets were found among the entire group nor among any of the subgroups evaluated including status by parity, gravidity, ethnicity, antibiotic or allergy medication use, atopy or asthma history.

We also wished to assess whether chronic exposure to pets among pet allergic women was associated with altered Treg cell levels. Among women sensitized to dog, those who had a dog or cat in the home had lower Treg cell levels compared with those who had no dog or cat (geometric mean = 0.32%, 95% CI = 0.11%, 0.93% versus 0.89%, 95%CI = 0.56%, 1.42%; p value = 0.04) (Table 4). However, among women not sensitized to dog, those with a dog or

cat in the home had higher Treg cell levels compared with those who did not (1.15%, 95% CI = 0.83%, 1.60% versus 0.75%, 95% CI = 0.57%, 0.99%; p value = 0.09) (Table 4). In a linear regression model of log transformed Treg cell levels, the interaction term between being sensitized to dog and dog or cat exposure was statistically significant (p value = 0.007). These associations were not affected by time of blood draw.

Similar to those sensitized to dog, among women sensitized to cat, those with a pet in the home had lower Treg cell levels compared with those who had no dog or cat (geometric mean = 0.42%, 95% CI = 0.12%, 1.39% versus 1.10%, 95% CI = 0.73%, 1.66%; p value = 0.04) (Table 4). Among women not sensitized to cat, those with a dog or cat in the home had similar Treg cell levels compared with those who did not (1.03%, 95% CI = 0.72%, 1.47% versus 0.71%, 95% CI = 0.54%, 0.94%; p value = 0.15) (Table 4). In a linear regression model of log transformed Treg cell levels, the interaction term between being sensitized to cat and dog/cat exposure was statistically significant (p value = 0.02). Again, these associations were not affected by time of blood draw.

To further describe these observed associations and to evaluate potential confounders, we built final models, including an interaction term between dog or cat in the home, whether the mother was sensitized to dog, and whether the mother was sensitized to cat. There were no confounding covariates (Table 5). The final model indicates a pattern in which Treg cell levels were lowest in those women who were sensitized to either a dog or cat and kept at least one dog or cat in their home during pregnancy.

Of the 42 women with dog sensitization, 9 had at least 1 dog, 2 had at least 1 cat with no dogs and 31 had no dogs or cats. Among the 37 women with cat sensitization, 4 had at least 1 cat, 5 had at least 1 dog with no cats and 28 had no dogs or cats. Limited sample size did not allow us to investigate whether sensitization and keeping of that same type of animal affected levels of Treg cell levels differently than our “unmatched” analyses.

## DISCUSSION

Overall, there was a pattern of lower Treg cell levels in pregnant women who were sensitized to dog or cat and had a dog or cat in their home. Since the data are cross-sectional, we could not assess whether a cause and effect relationship exists between sensitization and lower Treg cell levels among indoor dog or cat owners. Theoretically, low Treg cell levels may increase the risk of sensitization. Alternatively, chronic exposure to allergen among pet-sensitized women may result in lower Treg cell levels. Further speculation based on our data would be premature. The data call to mind the statement from Maggi et al. (2007): “[C]aution should be advised before considering allergic disorders as the simple consequence of defective T-cell regulatory mechanisms in the response to common environmental allergens.” There is complexity in the relationships between exposures and Treg cells that we have only begun to explore.

Our data are unique and not directly comparable to other studies. We used Foxp3 to identify Treg cells in a non-experimental setting and in unstimulated blood samples from women who were exposed or not exposed to dogs or cats in their usual environment. This approach may better reflect actual environmental exposure compared with experimental analyses in which ex-vivo cell stimulation with specific antigens may not directly translate to usual human exposure. Also, we did not directly measure the suppressive function of the Treg cells following allergen-specific stimulation, a methodologic approach that could have been informative.

Though our study population included a large proportion of African American women (67.2%), the data do not indicate that there are racial differences in the associations between pets and Treg cells. Thus we do not think that our results limit comparison to other study populations.

As with any study, this work had limitations. A larger sample size would have improved precision of these estimated associations of Treg cell levels to the likelihood of sensitization among those who lived with dogs or cats. For example, if non-sensitized women with chronic exposure to pets had high Treg cell levels, this could indicate that the presence of a pet may provoke a functional immune response to increase Treg cell levels in the presence of chronic exposure to a potential allergen. In contrast, those who were sensitized may fail to increase Treg cell levels resulting in progression to the production of allergen-specific IgE and immediate hypersensitivity.

An additional limitation is that we did not validate maternal report of pet exposure. However, it is unlikely that accuracy of pet reporting varied by Treg cell levels, which would have been unknown to the women. Thus, biased results due to inaccurate pet reporting are unlikely.

Although the mechanisms are not known, the role of prenatal exposure to pets on the risk of allergic diseases in children has come under study. Since the role of Treg cells on allergic disease risk has been the recent focus of much discussion (Shi HZ, et al. 2005; Seroogy CM and Gern JE 2005; Bacchetta R, et al. 2007; Bellinghausen I, et al. 2005; Lee JH, et al. 2007; Maggi L, et al;. 2007), we chose to study Treg cells in pregnant women to further investigate the potential mechanisms underlying the role of prenatal exposures in early life allergic disease risk. We view this presented work as an early step in further understanding the relationship between maternal environmental exposures, maternal immune function and the immune function and allergic disease risk of her newborn. The results seen in these pregnant women may facilitate the study of risk factors in their children's health, but may not be the same in women who are not pregnant. Future analyses will further study the relationship between maternal immunity during pregnancy and the child's early life health and immune parameters, including cytokines and IgE levels.

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## Abbreviations

**Treg cell**

Regulatory T Cells

## RERERENCES

Aicbhaumik N, Zoratti EM, Strickler R, et al. Prenatal expsoure to household pets influences fetal immunoglobulin E production. Clin Exp Allergy 2008;38:1787–1794. [PubMed: 18702655]

Arbes SJ, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: Results from the Third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2005;116:377–383. [PubMed: 16083793]

Bacchetta R, Gambineri E, Roncarlo M. Role of regulatory T cells and FOXP3 in human diseases. J Allergy Clin Immunol 2007;120:227–235. [PubMed: 17666212]

Bellinghausen I, König B, Böttcher I, Knop J, Saloga J. Regulatory activity of human CD4+CD25+ T cells depends on allergen concentration, type of allergen and atopy status of the donor. Immunol 2005;116:103–111.

Bønnelykke K, Pipper CB, Bisgaard H. Sensitization does not develop in utero. J Allergy Clin Immunol 2008;121:646–651. [PubMed: 18328892]

Cook DG, Strachan DP. Health effects of passive smoking-10: Summary of effects of parental smoking on the respiratory health of children and implications for research. Thorax 1999;54:357–66. [PubMed: 10092699]

Devereux G, Seaton A, Barker RN. In utero priming of allergic-specific helper T cells. Clin Exp Allergy 2001;31:1686–1695. [PubMed: 11696044]

Ege MJ, Bieli C, Frei R, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunuity and to atopic sensitization in school-age children. J Allergy Clin Immunol 2006;117:817–823. [PubMed: 16630939]

Ege MJ, Herzum I, Büchele G, et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. J Allergy Clin Immunol 2008;122:407–412. [PubMed: 18678343]

Kerkhof M, Wijga A, Smit HA, et al. The effect of prenatal exposure on total IgE at birth and sensitization at twelve months and four years of age: The PIAMA study. Pediatr Allergy Immunol 2005;16:10–18. [PubMed: 15693906]

Kulig M, Luck W, Lau S, et al. Effect of pre- and postnatal tobacco smoke exposure on specific sensitization to food and inhalant allergens during the first 3 years of life. Allergy 1999;54:220–228. [PubMed: 10321557]

Kuyucu S, Saraçlar Y, Tuncer A, et al. Determinants of atopic sensitization in Turkish school children: effects of pre- and post-natal events and maternal atopy. Pediatr Allergy Immunol 2004;15:62–71. [PubMed: 14998384]

Lee JH, Yu HH, Wanh LC, Yang YH, Lin YT, Chiang BL. The levels of CD4+CD25+ regulatory T cells in paediatric patients with allergic rhinitis and bronchial asthma. Clin Exp Immunol 2007;148:53–63. [PubMed: 17349011]

Maggi L, Santarlasci V, Liotta F, et al. Demonstration of circulating allergen-specific CD4+CD25+Foxp3+ T-regulatory cells in both nonatopic and atopic individuals. J Allergy Clin Immunol 2007;120:429–436. [PubMed: 17604089]

Noakes PS, Holt PG, Prescott SL. Maternal smoking in pregnancy alters neonatal cytokine responses. Allergy 2003;58:1053–1058. [PubMed: 14510725]

Prescott SL, Jones CA. Cord blood memory responses: are we being naïve? Clin Exp Allergy 2001;31:1653–1656. [PubMed: 11696038]

Prescott SL. Allergy: when does it begin and where will it end? Allergy 2003a;58:864–867. [PubMed: 12911413]

Prescott SL. 2003 (b) Early origins of allergic disease: a review of processes and influences during early immune development. Curr Opinion Allergy Clin Immunol 3:125–132.

Schonberger HJ, Dompeling E, Knotternus JA, et al. Prenatal exposure to mite and pet allergens and total serum IgE at birth in high-risk children. Pediatr Allergy Immunl 2005;16:27–31.

Seroogy CM, Gern JE. The role of T regulatory cells in asthma. J Allergy Clin Immunol 2005;116:996–999. [PubMed: 16275366]

Shi HZ, Qin XJ. CD4+CD25+ regulatory T lymphocytes in allergy and asthma. Allergy 2005;60:986–995. [PubMed: 15969678]

Søyseth V, Kongerud J, Boe J. Postnatal maternal smoking increases the prevalence of asthma but not of bronchial hyperresponsiveness or atopy in their children. Chest 1995;107:389–94. [PubMed: 7842766]

Strachan DP, Hartkins LS, Johnston IDA, Anderson HR. Childhood antecedents of allergic sensitization in young British adults. J Allergy Clin Immunol 1997;99:6–12. [PubMed: 9003205]

**Table 1**

Demographic characteristics of women in the study (all women, N=204)

| | N | % |
|---|---|---|
| African American | 137 | 67.2 |
| White | 38 | 18.6 |
| Hispanic | 13 | 6.4 |
| Middle eastern | 10 | 4.9 |
| Other | 6 | 2.9 |
| Current Asthma | 25 | 12.3 |
| Mother is atopic* | 115 | 59.9 |
| Had prior live birth | 120 | 58.8 |
| Had prior pregnancy | 151 | 74.0 |
| Maternal Age (Mean (SD)) | 29.4 (5.4) | |
| Female baby | 103 | 50.5 |
| Woman smoked during pregnancy | 22 | 10.8 |
| Woman lived with a smoker during pregnancy | 47 | 23.0 |
| Woman smoked during pregnancy and/or lived with a smoker during pregnancy | 58 | 28.4 |
| Woman had dog/cat in home (12 or more hours indoors) during pregnancy | 58 | 28.4 |

*Atopy is defined as ≥ 1 allergen-specific IgE ≥ 0.35 for any of the following allergens: *Dermatophagoides farinae (Der f)*, dog, cat, grass, ragweed, *Alternaria alternate*, egg white, and cockroach. 12 women had missing allergen-specific IgE data.

**Table 2**

Geometric means (95% confidence intervals) for the percentage of Treg cells (% of CD4+ lymphocytes that were CD25+Foxp3+) according to pregnancy history (all women, N=204)

| | No Prior Pregnancies (N=53) | ≥ 1 Prior pregnancy, No Prior Live Birth (N=31) | 1 Prior Live Birth (N=62) | 2 Prior Live Births (N=32) | 3+ Prior Live Births (N=26) |
|---|---|---|---|---|---|
| CD4+CD25+Foxp3+ (as a % of CD4+ lymphocytes) | 0.85 (0.57, 1.27) | 1.02 (0.63, 1.64) | 0.67 (0.45, 1.00) | 0.98 (0.68, 1.41) | 0.86 (0.51, 1.45) |
| Geo mean (95% CI) | | | | | |
| β (s.e.)* | -ref- | 0.18 (0.31) | −0.24(0.26) | 0.14(0.31) | 0.01(0.33) |
| | | p=0.57 | p=0.36 | p=0.64 | p=0.98 |

*Overall p-value for comparing Treg cells across the five groups: p=0.62.

**Table 3**

Geometric means (95% confidence intervals) for percentages of CD4+ lymphocytes that are CD25+Foxp3+ according to potential modifying characteristics (all women, N=204)

| | Has the characteristic | Does not have the characteristic | β (s.e.)* p-value |
|---|---|---|---|
| Prior Pregnancy | 0.83 (0.66, 1.03) | 0.85 (0.57, 1.27) | −0.03 (0.22) |
| N | 151 | 53 | p=0.90 |
| Prior Live Birth | 0.78 (0.61, 1.01) | 0.91 (0.67, 1.23) | −0.15 (0.20) |
| N | 120 | 84 | p=0.45 |
| African American | 0.86 (0.68, 1.09) | 0.79 (0.56, 1.10) | 0.09 (0.21) |
| N | 137 | 67 | p=0.67 |
| Maternal atopy† | 0.79 (0.61, 1.01) | 0.80 (0.59, 1.10) | −0.02(0.20) |
| N | 115 | 77 | p=0.91 |
| Female baby | 0.98 (0.76, 1.27) | 0.71 (0.53, 0.94) | 0.33 (0.19) |
| N | 103 | 101 | p=0.09 |
| Maternal smoking during pregnancy | 0.69 (0.40, 1.20) | 0.85 (0.69, 1.05) | −0.21 (0.31) |
| N | 22 | 182 | p=0.51 |
| Mom lived with a smoker during pregnancy | 0.80 (0.52, 1.22) | 0.84 (0.68, 1.05) | −0.06 (0.23) |
| N | 47 | 157 | p=0.81 |
| Dog or cat in home during pregnancy | 0.88 (0.62, 1.26) | 0.82 (0.64, 1.03) | 0.08 (0.22) |
| N | 58 | 136 | p=0.71 |
| Took antibiotics anytime during pregnancy | 0.79 (0.44, 1.43) | 0.84 (0.69, 1.03) | −0.06 (0.29) |
| N | 26 | 178 | p=0.83 |
| Currently taking allergy medications‡ | 0.76 (0.40, 1.45) | 0.84 (0.69, 1.03) | −0.10 (0.34) |
| N | 18 | 186 | p=0.77 |
| Currently taking asthma medications^ | 0.82 (0.34, 1.95) | 0.84 (0.69, 1.02) | −0.02 (0.36) |
| N | 16 | 188 | p=0.95 |
| Current asthma** | 0.59 (0.31, 1.14) | 0.87 (0.72, 1.07) | −0.39 (0.30) |
| N | 25 | 179 | p=0.19 |

*Parameter estimates and p-values are from a linear regression model of the log-transformed Treg cell level comparing the groups.

†Atopy is defined as ≥ 1 allergen-specific IgE ≥ 0.35 kU/L for any of the following allergens: *Dermatophagoides farinae (Der f)*, dog, cat, Timothy grass, ragweed, *Alternaria alternate*, egg white, and cockroach.

‡Allergy medications include all inhalers, inhalants, nasal sprays and pills taken daily or as needed.

^Asthma medications include all inhalers, inhalants and pills taken daily or as needed.

**Current asthma is defined as ever had a doctor diagnosis of asthma and either taken asthma medications or had symptoms of asthma in the last year

**Table 4**

Geometric means (and 95% confidence intervals) for percentages of CD4+ lymphocytes that are CD25+Foxp3+ stratified by whether a pet, either a cat or dog, was present in the home during pregnancy (all women, N=204)

| | Dog or cat in home during pregnancy (until blood draw) | No dog or cat in home during pregnancy (until blood draw) | Wilcoxon rank sum test p value | Interaction term p-value* |
|---|---|---|---|---|
| N | 58 | 136 | | |
| Geo mean (95% CI) | 0.88 (0.62, 1.26) | 0.82 (0.64, 1.03) | 0.71 | --- |
| Child first born | | | | 0.97 |
| Yes (n=84) | 0.94 (0.56, 1.57) n=30 | 0.89 (0.61, 1.32) n=50 | 0.88 | |
| No (n=120) | 0.83 (0.49, 1.39) n=28 | 0.77 (0.58, 1.03) n=92 | 0.82 | |
| First pregnancy | | | | 0.95 |
| Yes (n=53) | 0.91 (0.45, 1.87) n=18 | 0.82 (0.49, 1.37) n=35 | 0.81 | |
| No (n=151) | 0.87 (0.57, 1.33) n=40 | 0.81 (0.63, 1.06) n=111 | 0.78 | |
| Maternal atopy† | | | | 0.17 |
| Yes (n=115) | 0.72 (0.43, 1.19) n=29 | 0.81 (0.60, 1.09) n=86 | 0.68 | |
| No (n=77) | 1.12 (0.68, 1.87) n=25 | 0.68 (0.46, 1.02) n=52 | 0.14 | |
| Mother sensitized to dog ‡ | | | | 0.007 |
| Yes (n=42) | 0.32 (0.11, 0.93) n=11 | 0.89 (0.56, 1.42) n=31 | 0.04 | |
| No (n=151) | 1.15 (0.83, 1.60) n=43 | 0.75 (0.57, 0.99) n=108 | 0.09 | |
| Mother sensitized to cat‡ | | | | 0.02 |
| Yes (n=37) | 0.42 (0.12, 1.39) n=9 | 1.10 (0.73, 1.66) n=28 | 0.04 | |
| No (n=157) | 1.03 (0.72, 1.47) n=45 | 0.71 (0.54, 0.94) n=112 | 0.15 | |
| Mother is African American | | | | 0.75 |
| Yes (n=137) | 0.97 (0.63, 1.51) n=31 | 0.83 (0.63, 1.10) n=106 | 0.58 | |
| No (n=67) | 0.79 (0.43, 1.45) n=27 | 0.78 (0.52, 1.18) n=40 | 0.97 | |
| Took antibiotics anytime during pregnancy | | | | 0.25 |
| Yes (n=26) | 1.25 (0.50, 3.14) n=9 | 0.62 (0.28, 1.39) n=17 | 0.26 | |
| No (n=178) | 0.83 (0.56, 1.23) n=49 | 0.85 (0.66, 1.08) n=129 | 0.94 | |
| Currently taking allergy medications^ | | | | 0.16 |
| Yes (n=18) | 0.46 (0.12, 1.72) n=7 | 1.05 (0.47, 2.31) n=11 | 0.20 | |
| No (n=186) | 0.97 (0.66, 1.40) n=51 | 0.80 (0.63, 1.02) n=135 | 0.41 | |
| Currently taking asthma medications^ | | | | 0.50 |
| Yes (n=16) | 1.20 (0.39, 3.70) n=5 | 0.68 (0.19, 2.45) n=11 | 0.54 | |
| No (n=188) | 0.86 (0.59, 1.26) n=53 | 0.83 (0.65, 1.04) n=135 | 0.87 | |
| Current asthma†† | | | | 0.30 |
| Yes (n=25) | 1.01 (0.39, 2.63) n=6 | 0.50 (0.22, 1.15) n=19 | 0.35 | |
| No (n=179) | 0.87 (0.59, 1.28) n=52 | 0.88 (0.22, 1.15) n=127 | 0.97 | |

[*] The interaction term is from a linear regression model of log transformed Treg cell levels which includes and interaction term between the row factor and doc/cat exposure.

[†] Atopy is defined as ≥ 1 allergen-specific IgE ≥ 0.35 kU/L for any of the following allergens: *Dermatophagoides farinae (Der f)*, dog, cat, Timothy grass, ragweed, *Alternaria alternate*, egg white, and cockroach.

[‡] Sensitization defined as an allergen-specific IgE ≥ 0.35 kU/L

[^] Allergy medications include all inhalers, inhalants, nasal sprays and pills taken daily or as needed.

[**] Asthma medications include all inhalers, inhalants and pills taken daily or as needed.

[††] Current asthma is defined as ever had a doctor diagnosis of asthma and either taken asthma medications or had symptoms of asthma in the last year.

**Table 5**

Association between dog/cat exposure and percentages of CD4+ lymphocytes that are CD25+Foxp3+ by dog or cat sensitization status of pregnant woman as defined by a linear regression models of log transformed Treg cell levels* (all women, N=204)

| | N | β (SE) for log transformed percentage† | p-value |
|---|---|---|---|
| Dog Model | | | |
| Mother sensitized to dog and had dog/cat in home | 11 | − 0.87 (0.43) | 0.04 |
| Mother sensitized to dog and had neither dog nor cat in home | 31 | 0.17 (0.28) | 0.54 |
| Mother not sensitized to dog and had dog/cat in home | 43 | 0.42 (0.24) | 0.09 |
| Mother NOT sensitized to dog and had neither dog nor cat in home | 108 | Reference | |
| Cat Model | | | |
| Mother sensitized to cat and had dog/cat in home | 9 | − 0.54 (0.47) | 0.25 |
| Mother sensitized to cat and had neither dog nor cat in home | 28 | 0.43 (0.29) | 0.14 |
| Mother not sensitized to cat and had dog/cat in home | 45 | 0.36 (0.24) | 0.14 |
| Mother NOT sensitized to cat and had neither dog nor cat in home | 112 | Reference | |

* Dog/cat sensitization defined as allergen-specific IgE ≥ 0.35 kU/L for either dog allergen or cat allergen.