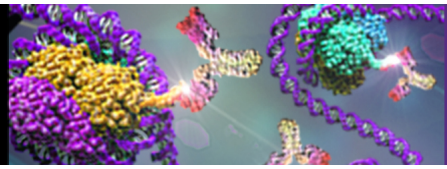

The Journal of Immunology

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Development of Asthma in Inner-City Children: Possible Roles of MAIT Cells and Variation in the Home Environment

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Humans have populations of innate-like T lymphocytes with an invariant TCR α-chain that recognize nonpeptide Ags, including invariant NKT (iNKT) cells and mucosal-associated invariant T (MAIT) cells. iNKT cell involvement in human asthma is controversial, whereas there has been little analysis of MAIT cells. Using peripheral blood cells from 110 participants from the Urban Environment and Childhood Asthma (URECA) birth cohort study, these cells were analyzed for number and function. We determined whether iNKT cell or MAIT cell frequency at 1 y is correlated with the cytokine polarization of mainstream CD4$^+$ T cells and/or the development of asthma by age 7 y. Dust samples from 300 houses were tested for iNKT cell antigenic activity. Our results show that a higher MAIT cell frequency at 1 y of age was associated with a decreased risk of asthma by age 7 y. The frequency of MAIT cells was associated with increased production of IFN-γ by activated CD4$^+$ T cells from the URECA cohort. iNKT cell antigenic activity in bedroom dust samples was associated with higher endotoxin concentration and also with reduced risk of asthma. In conclusion, MAIT cell frequency at 1 y may reflect the tendency of the immune system toward Th1 responses and is associated with protection from asthma. Additionally, iNKT cell antigenic activity may be a marker of houses with increased microbial exposures and therefore also with protection from asthma. ***The Journal of Immunology*****, 2018, 200: 1995–2003.**

Asthma is a significant health problem in industrialized countries, among children as well as adults. Some studies suggest that asthma may be more prevalent in low-income inner-city populations, accompanied by higher morbidity and mortality rates (1–3). Different lymphocyte populations may contribute to asthma. In this study, we have focused on the two prevalent subsets of innate-like T cells that recognize nonpeptide Ags found in microbes. One of these populations is invariant NKT (iNKT) cells, which respond to glycolipids, and the other is mucosal-associated invariant T (MAIT) cells, which respond to certain riboflavin metabolites (4). The cognate Ags for these cells are presented by nonclassical or nonpolymorphic class I Ag-presenting molecules: CD1d for iNKT cells and MR1 for MAIT cells (5). iNKT cells express surface proteins in common with NK cells, often including NK1.1 in mice and CD161 in humans, and surface proteins typical of T lymphocytes. The great majority of iNKT cells express an invariant TCR α-chain formed by rearranged Vα24 and Jα18 (*TRAV10–TRAJ18*) gene segments in humans (4, 6). MAIT cells are characterized by the expression of a different conserved and invariant α-chain: a Vα7.2-Jα33 (*TRAV1-2–TRAJ33*) α-chain rearrangement in humans (7, 8). These innate-like T cells share a number of properties, including

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S.C., G.W., A.A.H., and M. Kronenberg designed and analyzed the immunology experiments, which were performed by S.C., G.W., A.K., and M.R.; analyses of data were performed by J.A.G., A.M.G., and K.J.; S.C. and M. Kronenberg wrote the paper; A.-P.G. helped with sample collection and analysis; J.E.G. is the lead investigator and conceived the Urban Environment and Childhood Asthma consortium with R.W., who leads the Baltimore clinical site; L.B. leads the St. Louis clinical site, G.O. leads the Boston clinical site, and M. Kattan leads the New York clinical site; L.B., A.T., R.W., G.O., M.S., and M. Kattan acquired data from the subjects. All coauthors have contributed substantially to the analyses and interpretation of the data and have provided important intellectual input and approval of the final version of the manuscript.

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Abbreviations used in this article: HDE, house dust extract; iNKT, invariant NKT; MAIT, mucosal associated invariant T; URECA, Urban Environment and Childhood Asthma.

Table I. Demographics of URECA cohort: total samples

Annual Household Income < $15,000	Cesarean-Section	Mother Ever Breastfed	Child's Sex	Child's Race
207 (Y) 96 (N)	94 (Y) 209 (N)	170 (Y) 128 (N)	144 (Female) 159 (Male)	221 (Black) 56 (Hispanic) 20 (Mixed) 4 (Other) 2 (White)

Table represents the demographics of children who provided house dust or blood.
N, No; Y, Yes.

rapid cytokine responses, recognition of nonpeptide Ags, and preferential localization to tissues, such as the lung (4).

iNKT cells have been implicated in several mouse models of asthma. These include asthma induced either by allergens or inflammatory stimuli (9, 10). In humans, it has been shown that Th2 cytokine–secreting iNKT cells often were the dominant $CD4^+$ T cell subset in the airways of both allergic and nonallergic subjects with severe asthma, but they were almost undetectable in the airways of healthy controls (11). These results are highly controversial, however, with smaller or no iNKT cell increase observed in some other studies (12–15). These discordant results may be a reflection of the variability in iNKT cell numbers in human peripheral blood (16) or disease severity.

Sterile house dust extracts (HDEs) have stimulatory compounds for innate immune cells (17) and adjuvant activity in a widely used model of airway inflammation, in which mice were sensitized to the Ag chicken OVA. Additionally, our laboratories have shown that most HDEs also had antigenic activity for mouse and human iNKT cells (9). Furthermore, the adjuvant activity of the HDEs was partially iNKT cell–dependent; therefore, airway inflammation in mice sensitized with HDEs and cOVA was significantly reduced in mice that did not have iNKT cells (9). The amount of antigenic activity in HDEs was highly variable when obtained from different houses, however, leading us to ask whether this activity correlates with the number of iNKT cells in the blood, which is also quite variable (16), or the development of asthma. Much less is known about the role of MAIT cells in allergy and asthma. A recent study, however, found a reduction of MAIT cells in blood, sputum, and biopsy specimens from asthmatic patients, which was related to disease severity (18).

The Inner-City Asthma Consortium initiated the Urban Environment and Childhood Asthma (URECA) study in 2005. This birth cohort study was designed to assess the effect of environmental factors found in urban areas with a high poverty rate on the immune system and the development of allergy and asthma. In this study, pregnant women were enrolled from central urban areas of Baltimore, Boston, New York City, and St. Louis, and their offspring, who have at least one parent with allergy or asthma (19), are being followed from birth through age 14–16 y. In this study, we focused on the frequency of iNKT cells and MAIT cells in blood samples obtained at age 1 y from children in the URECA study and determined their correlations with cytokine production by $CD4^+$ T lymphocytes, the presence of iNKT cell Ags in house dust, and clinical outcomes of recurrent wheeze and atopy at age 3 y and asthma at age 7 y.

Materials and Methods

URECA study and sample collection

The URECA birth cohort study is an observational study with a total of 560 infants, born at term in low-income, inner-city urban neighborhoods from Baltimore, Boston, New York City, and St. Louis, four cities at high risk for allergic disease. Prenatal entry criteria included a history of allergic disease or asthma in at least one parent. Subjects are predominantly African American, and some are Latin American or mixed race. Subjects underwent serial clinical evaluations and house dust samples were collected from their homes as previously described. Informed consent was obtained from all subjects. Detailed design of the study has been previously published (19). Briefly, the clinical outcomes that were used for this study are recurrent wheezing (age 3 y), aeroallergen sensitization (age 3 y), and asthma (age 7 y). Sensitization to aeroallergens was defined as either a positive skin test or positive serum-specific IgE. For skin testing, children underwent prick skin testing with a collection of 14 common indoor and outdoor allergens such *Alternia tenuis*, Timothy grass, and others as described (19) (Multi-Test II; Lincoln Diagnostics, Decatur, IL). Total and allergen-specific IgE were measured by fluoroenzyme immunoassay (UniCAP; Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). Recurrent wheezing is defined as at least two episodes of wheezing during the first 3 y of life, with at least one episode during the third year. Asthma up to age 7 y was based on several criteria as described (19). Briefly, children were classified as having asthma at age 7 y when at least one of three conditions was met: 1) a parent-reported physician diagnosis of asthma between age 4 and 7 y, combined with asthma symptoms or the use of asthma controller medication for 6 of the past 12 mo; 2) albuterol reversibility of forced expiratory volume in 1 s (FEV1) $\geq$ 10% or methacholine concentration needed to produce 20% fall in FEV1 from baseline (PC20) $\leq$ 4 mg/ml, combined with asthma symptoms or use of asthma controller medication for 6 of the past 12 mo; or 3) a report in the past 12 mo of two or more wheezing episodes, two or more doctor visits for asthma/wheeze, one or more hospitalizations for asthma/wheeze, or the use of controller medications for 6 of the past 12 mo. Endotoxin levels in the bedroom dust samples were measured as described (20) at the age of 3 mo.

Blood cell analysis

A total of 162 URECA PBMC samples were obtained from 110 unique subjects, plus 52 duplicate samples. All the blood samples were collected

Table II. Demographics of URECA cohort: blood donors

Annual Household Income < $15,000	Cesarean-Section	Mother Ever Breastfed	Child's Sex	Child's Race
68 (Y) 36 (N)	31 (Y) 73 (N)	48 (Y) 54 (N)	52 (Female) 52 (Male)	89 (Black) 8 (Hispanic) 6 (Mixed) 1 (Other) 0 (White)

Table represents the demographics of children who provided blood.
N, No; Y, Yes.

Table III. Clinical outcome in URECA cohort: total samples

	Allergic to Aeroallergens (Age 3 y)	Recurrent Wheezing (Age 3 y)	Asthma (Age 7 y)
Negative	164	201	193
Positive	139	102	77

Table represents clinical outcomes for the children who provided house dust or blood.

at the age of 1 y. Samples of frozen PMBCs were thawed at 37˚C, washed twice with prewarmed (37˚C) RPMI 1640 medium (Invitrogen) supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin-glutamine containing 10,000 U/ml penicillin, 10,000 μg/ml streptomycin, 29.2 mg/ml L-glutamine (Invitrogen), distributed in plates, and rested overnight (14–16 h). To detect iNKT cells, we used the 6B11 mAb specific for the CDR3 region of the invariant TCR α-chain (16). As the availability of Ag-loaded MR1 tetramers was highly limited, for MAIT cells we used surrogate markers including the combination of Vα7.2 and CD161, which provide a reasonably accurate detection of this population (21). Ninety percent of the cells were then stimulated with PMA (200 ng/ml) and ionomycin (1 μg/ml) (both Sigma-Aldrich) in the presence of 0.8 μl/ml GolgiPlug and 0.55 μl/ml GolgiStop (both from BD Biosciences) for 4 h at 37˚C. As a staining control, PBMCs were prepared from buffy coats pooled from two healthy donors from the San Diego Blood Bank. Control lymphocytes were purified with Ficoll, aliquoted, and frozen. One aliquot of these cells was thawed with every experimental set, that is, one control and five URECA samples. Cells were surface stained for 30 min on ice, washed, fixed with Cytofix/Cytoperm (BD Biosciences) for 15 min at 37˚C, permeabilized, and intracellular cytokine staining was done for 30 min on ice. Flow cytometry was performed as previously described (22), cells were analyzed with an LSR II Fortessa (BD Biosciences), and data were processed with CellQuest Pro (BD Biosciences) and FlowJo (Tree Star) software. The Abs used in this study are described in Supplemental Fig. 1A. Gating strategy for various populations is described in Supplemental Fig. 1B. Details of the protocol and of the surface staining used have been published elsewhere (23). The study received Institutional Review Board approval at the clinical sites and the administrative center. An independent Data Safety and Monitoring Board run by the National Institutes of Health monitors the study. Approval for this study also was obtained from the Institutional Review Board at the La Jolla Institute for Allergy and Immunology.

Analysis of the antigenic activity in HDEs

Three hundred house dust samples were collected at the age of 3 mo from URECA subjects, including all unique subjects who donated blood. The samples were suspended in sterile PBS at 100 mg/ml and then were placed on a rotor at room temperature for 24 h before filtration and storage at −80˚C. House dust samples were diluted in PBS to a final concentration of 1 mg/ml for the experiment. To test for antigenic activity, stimulation of an iNKT cell hybridoma on plates coated with soluble, recombinant mouse CD1d was performed as described (24). Briefly, 96-well flat-bottom MicroWell plates were coated with mouse CD1d by incubation of 100 μl of CD1d solution (1 μg/ml in PBS) for 1 h at 37˚C. Wells were then washed five times with PBS and blocked by incubation for 1 h at 37˚C with 10% (v/v) FBS (Mediatech) in PBS. After washing, HDE samples, and as a positive control the potent Ag α-galactosylceramide, were added to duplicate wells and incubated for 24 h at 37˚C. After being washed with medium (RPMI 1640 [Invitrogen] supplemented with 5% [v/v] FBS, 1% [v/v] penicillin-streptomycin-glutamine containing 10,000 U/ml penicillin, 10,000 μg/ml streptomycin, 29.2 mg/ml L-glutamine (Invitrogen), and 50 μM 2-ME [Sigma-Aldrich]), 1×10^5 DN3A4-1.2 Vα14i NKT hybridoma cells (25) were added to the plates and incubated for 16–20 h at 37˚C. Supernatants were collected and IL-2 was determined using a sandwich ELISA (BD Pharmingen).

Statistical analysis

Matrices containing cell population statistics for different samples were created. For the 52 duplicate blood samples an average value was used. The "cor" and "cor.test" functions in the R "stats" package were used to calculate R and *p* values for Pearson correlations among the log-transformed frequencies of the various cell populations. The "t.test" function was used to calculate *p* values for equivalence of clinical parameter groups. Adjusted *p* values were calculated using the Benjamini–Hochberg algorithm in the "p.adjust" function of R, where appropriate. All statistical calculations were performed with R version 3.1.0.

Results

Subject characteristics

The baseline characteristics of the URECA consortium birth cohort have previously been described (19). Summary demographics of 303 children related to this study are described in Table I and demographics for children who provided blood are described in Table II. Detailed demographic information on individual children is listed in Supplemental Table I. From these 303 children, we received 300 house dust samples. Blood samples were available from 110 of these individuals, 107 who also provided house dust, plus the 3 additional donors (Tables I, II). Both sexes were well represented; almost 50% were female. The blood samples were collected from the subjects at the age of 1 y, whereas house dust was collected at 3 mo of age. These ages were chosen to assess early influences on asthma, but they also reflect the availability of material that is widely shared among a consortium of investigators. Furthermore, we analyzed the clinical data available for these individuals. One hundred thirty-nine were found to be sensitized to aeroallergens and 102 had recurrent wheezing by the age of 3 y (Table III). By the age of 7 y, some children dropped out of the study and at that time data were available for 270 children, of whom 77 (29%) developed asthma. Similarly, when we only consider the children who provided blood, by age 3 y, 42% were sensitized to aeroallergens and 25% developed recurrent wheezing. By the age of 7 y, 18.9% of these children developed asthma (Table IV).

Frequencies of iNKT and MAIT cells

The gating strategy for detecting T cell populations is shown in Supplemental Fig. 1B and primary data on cell frequencies are in Supplemental Fig. 1C. The mean frequency of MAIT cells in the population was 0.097% (median 0.07%) of total $CD3^+$ T cells. The

Table IV. Clinical outcome in URECA cohort: blood donors

	Allergic to Aeroallergens (Age 3 y)	Recurrent Wheezing (Age 3 y)	Asthma (Age 7 y)
Negative	60	78	77
Positive	44	26	18

Table represents clinical outcomes for the children who provided blood.

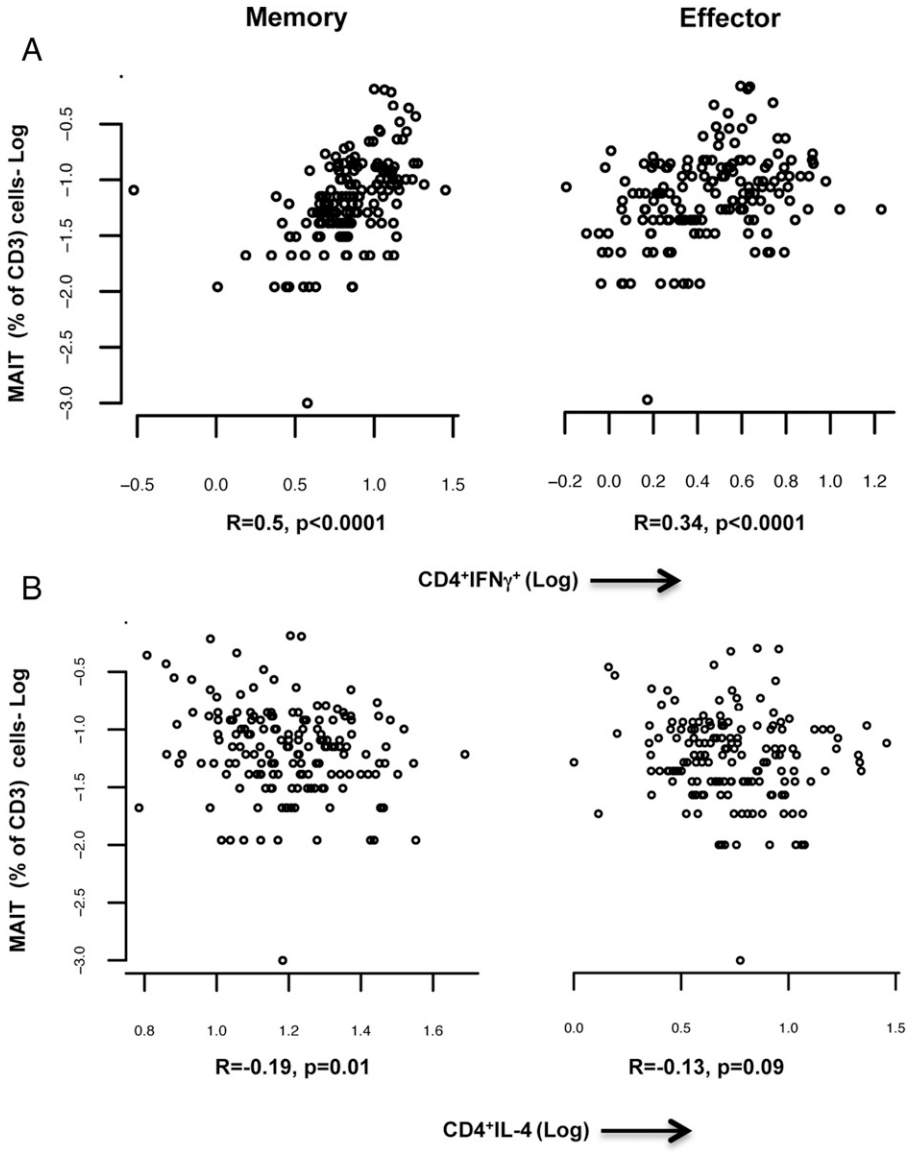

FIGURE 1. Correlation of MAIT cells with $CD4^+$ T cells producing IFN-γ (**A**) or IL-4 (**B**). Pearson correlations were determined between MAIT cells as a percentage of total $CD3^+$ cells and cytokine production in the indicated $CD4^+$ T cell populations. Correlation coefficients and *p* values are indicated below the plot ($n = 110$).

proportion of iNKT cells was lower, at 0.038% (median 0.029%). The MAIT cell frequency of URECA participants is lower than what has been reported for adults, consistent with literature indicating that this cell population expands steadily after birth (21, 26, 27).

Correlation of MAIT cell frequency with IFN-γ–producing $CD4^+$ T cells

After a brief in vitro activation, we gated on two subpopulations of $CD3^+CD4^+$ T lymphocytes: memory and effector. We observed a strong tendency for the activated $CD4^+$ T cells to produce IL-4 compared with IFN-γ, although production of both cytokines was detectable. This Th2 pattern of cytokine skewing is consistent with other work indicating that T cells before birth and early in life produce stronger Th2 responses (28–30). We found a significant positive correlation between the frequency of MAIT cells and the cytokine secretion by $CD4^+$ T cells, prominently with their capability of producing IFN-γ (Fig. 1A), and a negative correlation was observed in the case of $CD4^+$ T cells producing IL-4 (Fig. 1B). In contrast, there generally were no significant correlations between iNKT cell frequency and the $CD4^+$ T cell subpopulations capable of producing IFN-γ or IL-4 (Supplemental Fig. 2).

Association of innate-like cell populations with clinical outcomes

To test the relationship between the frequencies of iNKT and MAIT cells in the blood and clinical outcomes related with allergy and asthma, we compared the frequencies of the two innate-like T cell populations to subsequent aeroallergen sensitivity and recurrent wheezing at age 3 y, and asthma at age 7 y. iNKT cell population frequency distributions at age 1 y were not related to the later development of allergic sensitization or asthma at ages 3–7 y (Fig. 2A–C). Although MAIT cell frequency was not associated with risk of aeroallergen sensitization or wheezing at age 3 y (Fig. 2D, 2E), the frequency of MAIT cells was significantly lower ($p = 0.003$) in 1-y-old children who later developed asthma at age 7 y (Fig. 2F). Benjamini–Hochberg adjustment indicated a low likelihood of false discovery ($q = 0.015$).

It is possible that the type of immune responses mounted by iNKT cells and/or MAIT cells would reflect disease status more accurately than their frequencies. Therefore, we activated peripheral blood cells and measured cytokine production by iNKT and MAIT cells by intracellular cytokine staining. The cytokine production profiles of these two populations were very different. iNKT cells from the 1-y-old children were generally less responsive

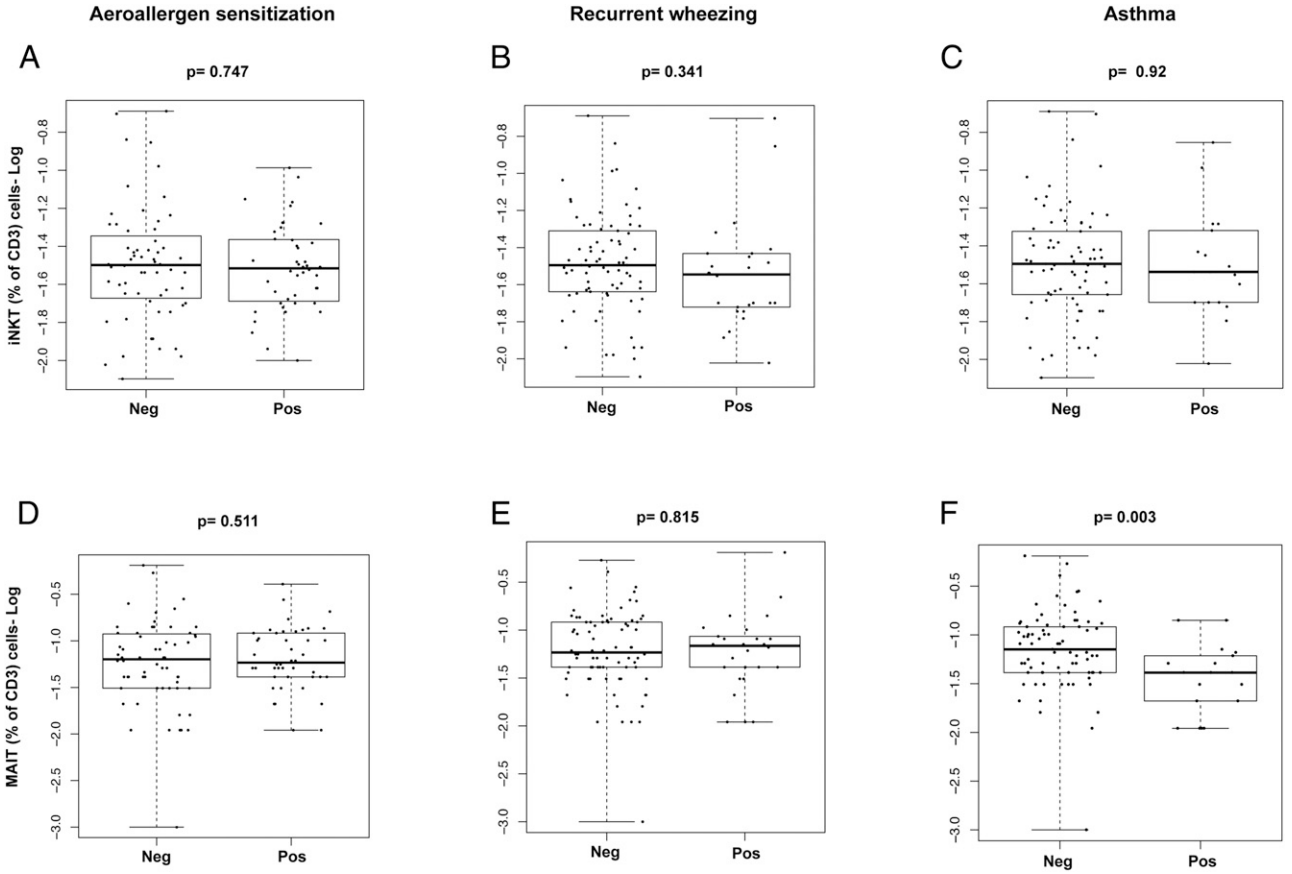

FIGURE 2. Association of iNKT and MAIT cell frequency with clinical outcomes. A comparison of the percentage of iNKT cells, of total $CD3^+$ cells, in 1-y-old children that did or did not show aeroallergen sensitization at age 3 y (Neg, $n = 60$; Pos, $n = 44$) (**A**), recurrent wheezing at age 3 y (Neg, $n = 78$; Pos, $n = 26$) (**B**), and asthma (**C**) at age 7 y (Neg, $n = 77$; Pos, $n = 18$). MAIT cell percentage in 1-y-olds were compared with subsequent development of (**D**) aeroallergen sensitization at age 3 y (Neg $n = 60$, Pos $n = 44$), (**E**) recurrent wheezing at age 3 y (Neg, $n = 78$; Pos, $n = 26$), and (**F**) asthma at age 7 y (Neg, $n = 77$; Pos, $n = 18$). The p values were calculated using a Student t test.

with relatively few cells that produced either IL-4 (Fig. 3A) (mean, 7.0%; median, 9.2%) or IFN-γ (mean, 16.7%; median, 12.6%). There was not a significant difference, however, in the percentage of iNKT cells producing one of these cytokines in children who subsequently became sensitized or who developed asthma (Supplemental Fig. 3A–F). In comparison, MAIT cells from the children exhibited a vigorous immune response potential (Fig. 3B), which closely resembled that reported for MAIT cells from mature adults. In the URECA cohort children, nearly all MAIT cells could be activated to produce IFN-γ (mean, 79.3%; median,

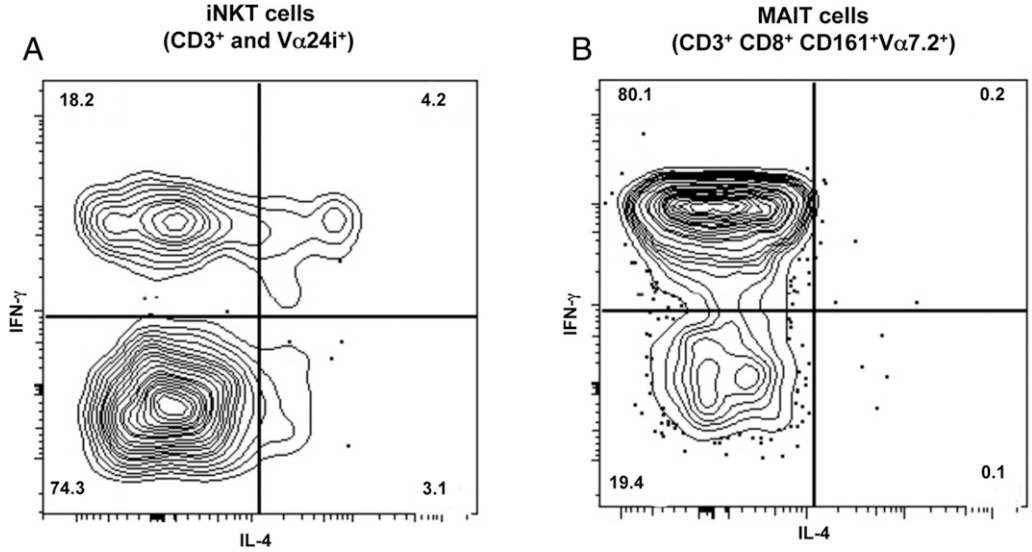

FIGURE 3. Cytokine production by iNKT and MAIT cells. Representative flow cytometry data for gated iNKT cells (**A**) and MAIT cells (**B**) activated with PMA and ionomycin and then analyzed by intracellular cytokine staining. Data from one of many similar analyses are shown; see also Supplemental Fig. 3 for compiled results for iNKT and MAIT cells.

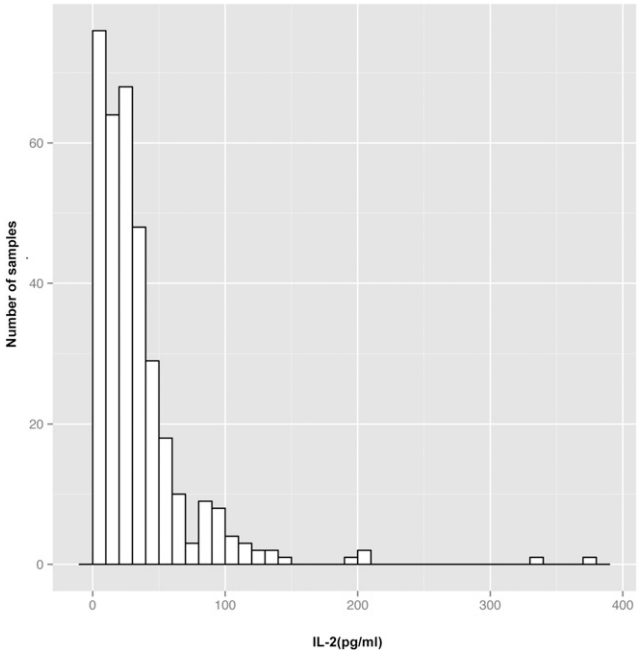

FIGURE 4. HDEs from different houses vary in their antigenic activity. iNKT cell antigenic activity (IL-2 release) of URECA HDEs (n = 300) grouped by the magnitude of cytokine release in 10-pg/ml intervals (e.g., 0–10, 11–20).

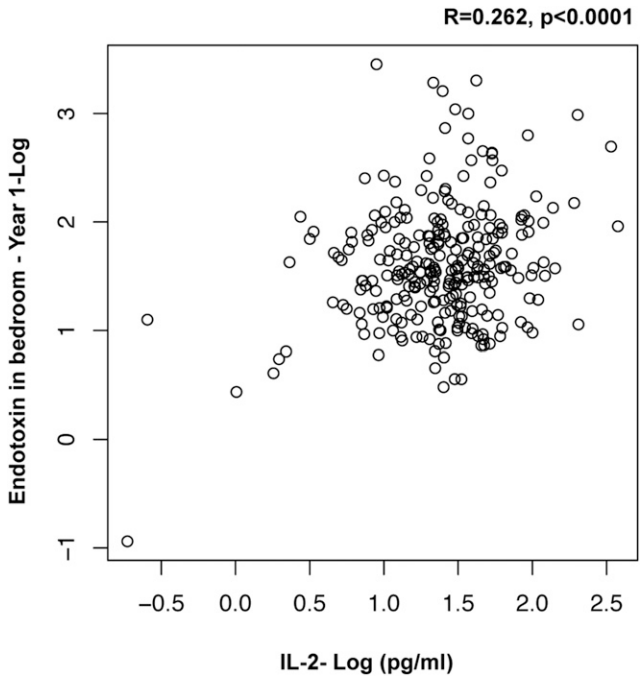

FIGURE 5. Endotoxin in bedroom is correlated with the amount of iNKT cell antigenic content in HDEs. Pearson correlation was determined between endotoxin from the bedrooms of children at age 3 mo and the iNKT cell antigenic content in HDEs.

82.8%) and very few produced IL-4 (Fig. 3B), despite a strong Th2 or IL-4 skewing of the bulk $CD4^+$ T cell response. This Th1 cytokine bias of MAIT cells from the children is similar to their cytokine output in adults (27, 31–33), but there were no significant associations with MAIT cell cytokine production and allergic sensitization, recurrent wheezing, or asthma (Supplemental Fig. 3G–I).

Variation in iNKT cell Ag in house dust

In a previous publication, we demonstrated that households from Southern California varied greatly in the antigenic activity for iNKT cells in their HDEs (9). To determine whether iNKT cell antigenic activity in the environment is related to asthma, we analyzed house dust extracts from URECA cohort households using an APC-free assay. Fig. 4 depicts the amount of stimulation of IL-2 secretion by the HDE samples. In agreement with our previous results, the antigenic content in URECA cohort households varied greatly. Interestingly, although there are outliers with high iNKT cell Ag and little endotoxin, and vice versa, antigenic content was correlated with the amount of endotoxin in the bedrooms when analyzed at 3 mo of age (Fig. 5).

It was reported that the frequency of iNKT cells in peripheral blood also shows a large individual variation, with this frequency being stable over time (34). To identify a possible environmental influence on the iNKT cell frequency, we analyzed whether there was a relationship between iNKT cell frequency and HDE antigenic activity. These measures were not correlated (Fig. 6).

We also analyzed the association of iNKT cell antigenic activity in the HDEs collected at the age of 3 mo with clinical outcomes. Dust-induced IL-2 secretion was similar in children grouped according to allergic sensitization (Fig. 7A) or recurrent wheezing (Fig. 7B) at age 3 y. Interestingly, however, iNKT cell antigenic activity was increased in the households of the children who did not develop asthma at age 7 y (Fig. 7C).

Discussion

MAIT cells and iNKT cells are two expanded populations of T cells that express αβ Ag receptors with invariant α-chains that recognize nonpeptide Ags, and that make rapid effector responses. In a cohort of children from asthma-prone families and environments, we tested whether the frequency and function of iNKT cells and MAIT cells early in life correlated with the subsequent development of clinical outcomes, including aeroallergen sensitization and recurrent wheeze at 3 y, and asthma at age 7 y. We also determined whether variation in antigenic activity for iNKT cells in the living environment was associated with allergic sensitization or asthma. We found that the average frequencies of iNKT cells and MAIT cells in the young children were not highly divergent, with MAIT cells only slightly >2-fold more frequent than iNKT cells. In contrast, in adults MAIT cells have been found to be much more frequent than iNKT cells (35, 36). Two lines of evidence indicate that we identified MAIT cells using surrogate markers such as Vα7.2 Ab, CD8α, and CD161, rather than Ag-loaded MR1 tetramers, which were not available when the analysis was done. First, the population identified as MAIT cells had a uniquely Th1-skewed capability of cytokine production compared with the other populations from the children, but similar to adult MAIT cells. Second, when we performed a subsequent staining of cells from young children, the great majority of cells positive for the surrogate markers stains with MR1 tetramers loaded with 5-OP-RU (data not shown), in agreement with work from a previous study of samples from young children (37). Despite their relatively low frequency compared with adults, our main findings are that a higher MAIT cell frequency in the blood of 1-y-old children is associated with reduced risk of asthma. Additionally, although iNKT cell frequencies in blood and cytokine production were not associated with asthma, increased iNKT cell antigenic activity in HDEs was associated with a lower probability of developing asthma.

Several factors distinguish this report from several previously published studies (11–15, 18) that addressed similar questions for iNKT cells. These include the larger size of our study population, the very young age of the subjects, the close match of subjects and controls for their age, environment, and background, the monitoring of the environment that was undertaken in parallel, the

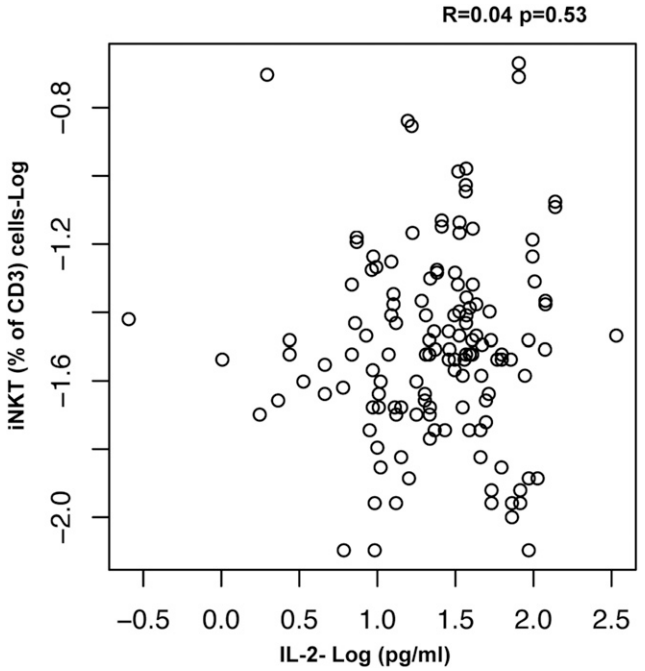

FIGURE 6. The frequency of iNKT cells is not correlated with the amount of iNKT cell Ag in HDEs. Pearson correlation was determined between the frequency of iNKT cells in peripheral blood, measured as a percentage of total $CD3^+$ cells, and the antigenic content in HDEs.

inclusion of an analysis of MAIT cell frequency and function, and the prospective design in which the subjects were followed for 7 y.

We measured the production of IFN-γ and IL-4 by mainstream $CD4^+$ T cells to determine the extent to which their immune response is related to the frequency or activity of innate-like T cells in peripheral blood. A higher percentage of the $CD4^+$ T cells from the children were capable of producing IL-4 than IFN-γ, but there was a strong positive correlation between increased IFN-γ production by $CD4^+$ T cells and an increased MAIT cell frequency. Compared to iNKT cells, MAIT cells are more dedicated to producing Th1 cytokines (21), and therefore our data suggest that the frequency of MAIT cells might reflect the presence of factors in the children's immune system driving a Th1-skewed response generally.

We also analyzed the association of iNKT cell and MAIT cell frequency, as well as cytokine production by these cells, with sensitization and wheezing up to age 3 y and asthma by age 7 y. Our data did not provide strong evidence for a protective or pathogenic role for iNKT cells, in agreement with several investigations that did not find differences in the frequency of iNKT cells in peripheral blood in asthma patients (38–42). The caveat for these studies is that events in the blood might not reflect the state of the tissue. We note that, unlike in our results, an increased Th2 cytokine response by iNKT cells was correlated with asthma in two previous studies (42, 43); this correlation is more consistent with most data from mouse asthma models (11, 44). The discordant outcomes regarding iNKT cell frequencies and asthma could reflect study design, as nearly all of the previous reports analyzed adult patients with long-term disease, rather than pediatric patients, and none looked at the earliest stages of disease or had the prospective design and highly matched control group that was part of our study. We note, however, that in a pediatric asthma study using somewhat older children, between ages 6 and 12 y, iNKT cells produced less IFN-γ and more IL-4 in children with asthma (43).

When we analyzed MAIT cell populations, we also could not find an association with allergen sensitization or wheezing up to age 3 y. Despite this, MAIT cell frequency was increased in the blood of those children at age 1 y who did not develop asthma by age 7 y. This result is consistent with the notion that the immune system can be programmed early in life (45). Although it is uncertain why there was not a correlation between MAIT cell frequency and wheezing at age 3 y, the data suggest that the early in life MAIT cell frequency may be one marker for imprinting of the immune system in young children that is relevant to subsequent risk for asthma. Blood samples from older children were not available for this study, but we note that in agreement with our results, a recent study found a decreased MAIT cell frequency in blood and lung of adult asthma patients (18).

One factor that might drive iNKT cell and MAIT cell frequencies could be exposure to certain microbes or their products, both the species that are present in the environment and the magnitude of the exposure. To analyze this parameter for iNKT cells, we measured the Ag content of HDEs in URECA cohort households. We found that there was a correlation in children who were protected from asthma and HDEs who contained a higher antigenic activity for iNKT cells, even though this was measured years earlier. We also found a positive correlation of antigenic content in HDEs and endotoxin in the bedrooms of children. These data suggest that environments with a higher load of microbial products may also contain a higher concentration of substances that stimulate

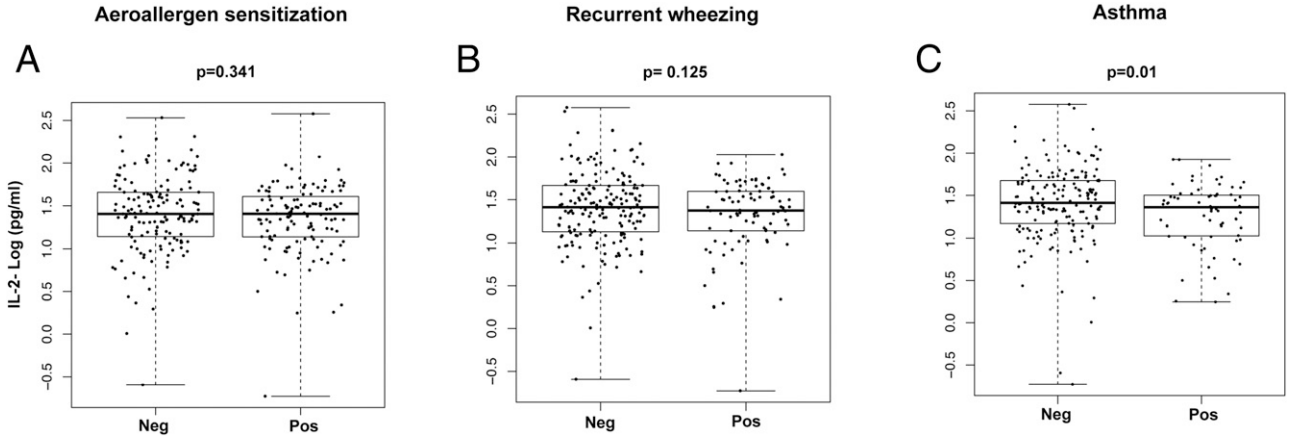

FIGURE 7. Association of HDE antigenic activity with clinical outcomes. iNKT cell antigenic activity (IL-2 release) in URECA HDEs at 3 mo of age was compared with aeroallergen sensitization at age 3 y (Neg, $n = 152$; Pos, $n = 125$) (**A**), recurrent wheezing at age 3 y (Neg, $n = 184$; Pos, $n = 93$) (**B**), and asthma (**C**) at age 7 y (Neg, $n = 181$; Pos, $n = 67$). The p values were calculated using a Student t test.

iNKT cells. Overall, the association of NKT cell Ag in house dust with endotoxin and protection from asthma is consistent with the idea that increased microbial exposure in the first years of life is protective for asthma (the "hygiene hypothesis"). The iNKT cell antigenic content in house dust was not correlated, however, with the percentage of iNKT cells in peripheral blood. This result is perhaps not too surprising, considering that there likely are other routes of iNKT cell microbial Ag exposure (46), and that alterations in the population size and phenotype of iNKT cells were fairly subtle in germ free mice (47).

In conclusion, our study demonstrates that in 1-y-old children from asthma-prone households in inner-city environments a higher MAIT cell frequency in blood was correlated with increased $CD4^+$ T cells producing IFN-γ and protection from asthma at age 7 y. These findings suggest that an early in life tendency to increased MAIT cells could be asthma protective in many children as they grow older, and could reflect early immune programming or imprinting phenomena promoting Th1 responses. MAIT cells become highly abundant in adult humans, and although MAIT cell frequency may only be a biomarker of a broader Th1 cytokine skewing early in life that suppresses Th2 responses, the strongly Th1-skewed cytokine profile of MAIT cells might actually provide protective function.

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Disclosures

The authors have no financial conflicts of interest.

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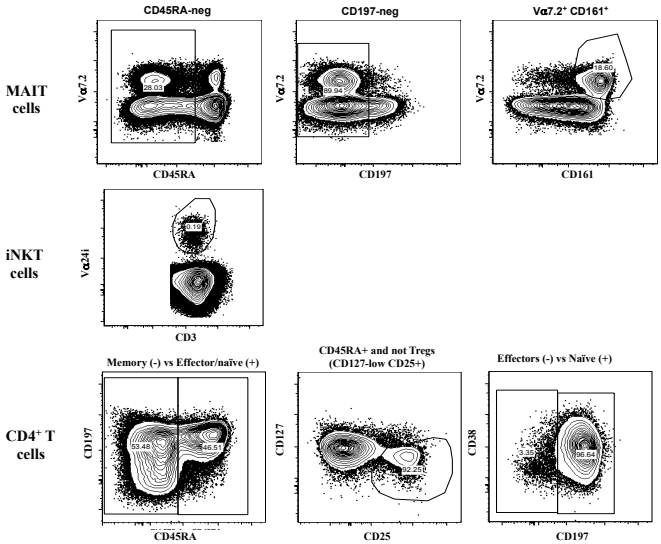
Supplemental Figure 1: Antibodies, gating strategy and cell number

a

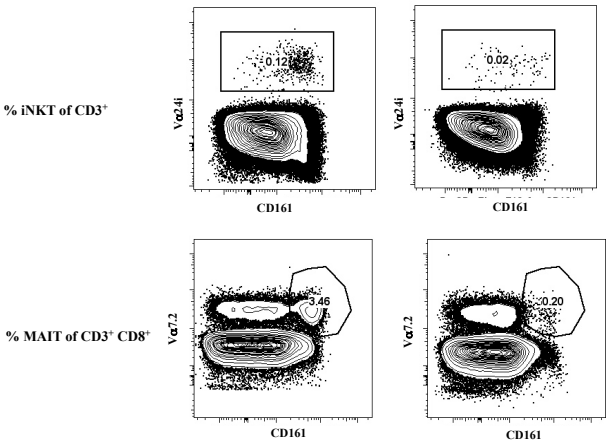
Antigens	Reagents used	Surface stain	2nd/ICS stain	Clones used	Vendors	Amount (per test)
L/D-Blue	L/D-Blue	X		n/a	Invitrogen	0.4ul
CD4	BV510		X	SK3	BD	1ul
CD45RA	BV570		X	HI100	BioLegend	1ul
CD3	QD605	X		UCHT1	Invitrogen	0.7ul
CD8	eF650	X		RPA-T8	eBioscience	2ul
CD56	BV711	X		HCD56	BioLegend	1.5ul
IFNγ	BV785		X	4S.B3	BioLegend	1.5ul
Vα7.2	FITC	X		3C10	BioLegend	1ul
+anti-FITC -Ab	AF488		X	polyclonal	Invitrogen	0.2ul
CD161	PerCP-Cy5.5	X		HP-3G10	BioLegend	2.5ul
CD197 (CCR7)	PE-CF594	X		150503	BD	1ul
CD20	PE-Cy5.5		X	2H7	eBioscience	2ul
IL-4	PE-Cy7		X	8D4-8	eBioscience	0.15ul
Vα14*i*	APC	X		6B11	BioLegend	3ul

L/D = Live/Dead
ICS = Intracellular staining

b

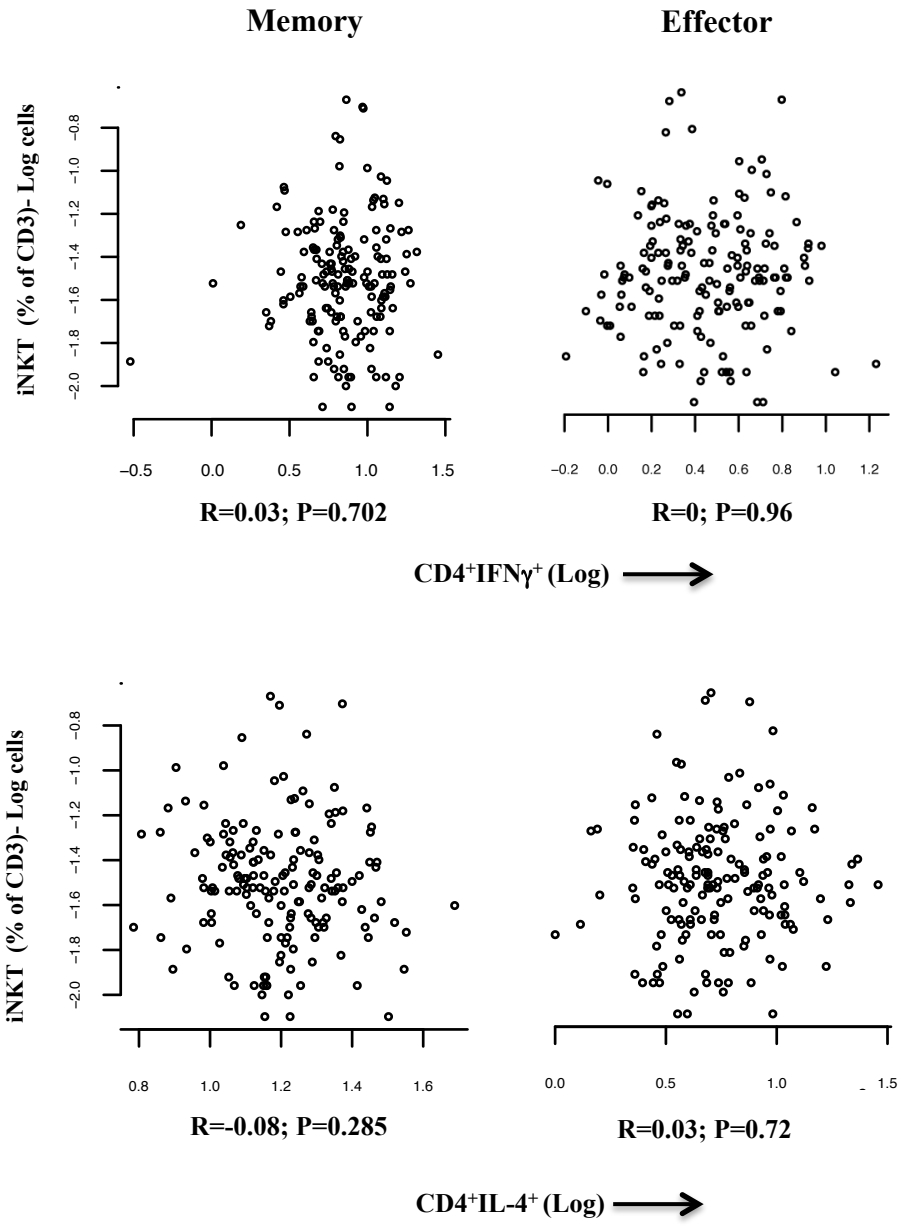


c



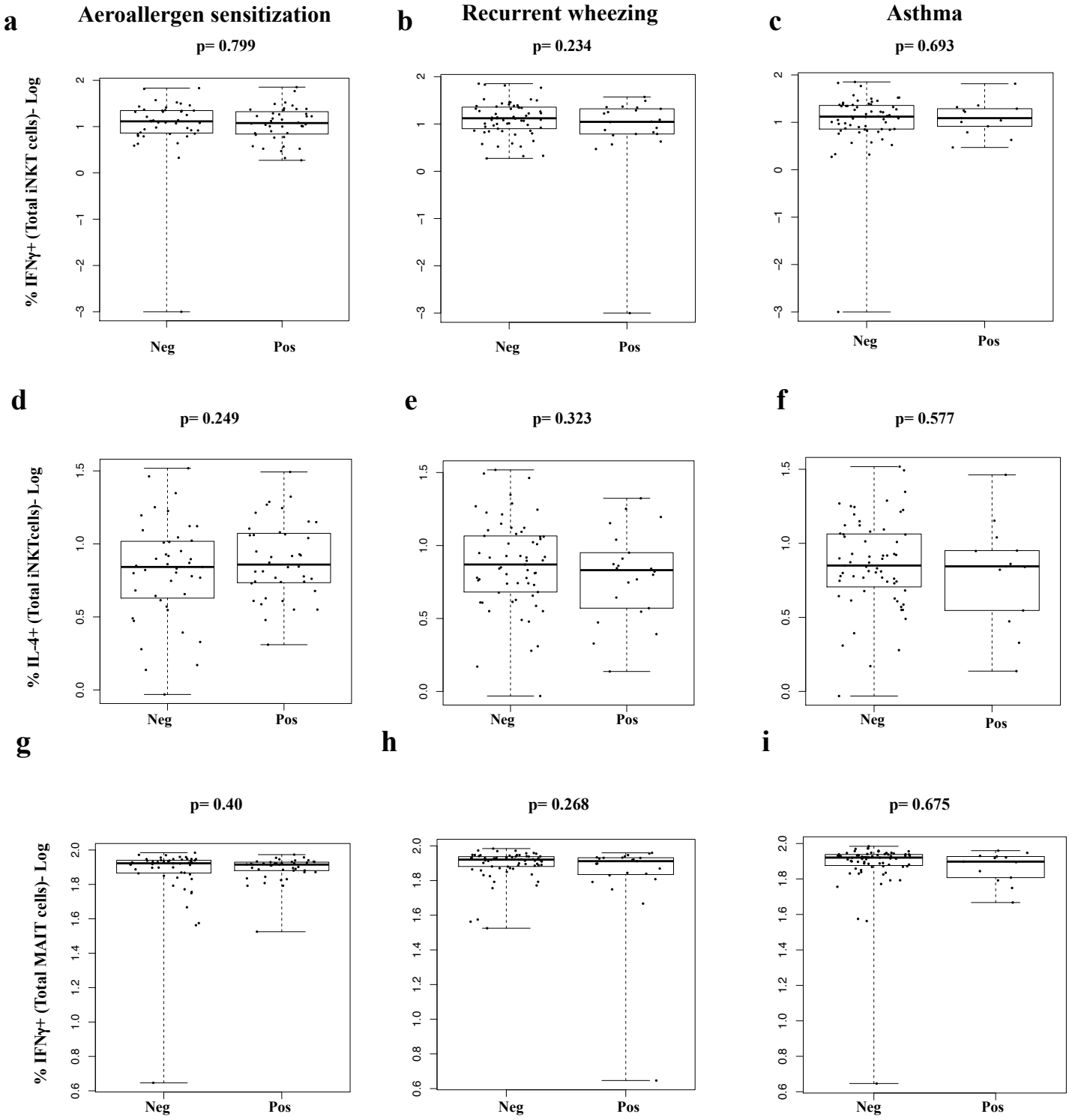
(a) Description of antibody clones and amount used.(b) Gating strategies for MAIT, iNKT and CD4$^+$ T cells. MAIT cells were identified as live cells that were CD20$^-$, CD4$^-$, CD45RA$^-$, CD197$^-$, Vα24i$^-$ (to exclude iNKT cells) and CD3$^+$, CD8α$^+$, CD161$^+$ and Vα7.2$^+$. iNKT cells were identified as live cells that were CD20$^-$, Vα7.2$^-$, and CD3$^+$ and Vα24i$^+$and CD4$^+$ T lymphocytes were identified by flow cytometry as CD20$^-$ Vα24i$^-$ CD56$^-$ CD8$^-$ CD3$^+$ CD4$^+$ cells, which included naïve (CD45RA$^+$ CD197$^+$), memory (CD45RA$^-$) and effector (CD45RA$^+$ CD197$^-$) subsets. Percentages of positive cells in a representative sample are indicated. (c) iNKT and MAIT cell percentages. Representative high and low percentages of iNKT cells in peripheral blood of URECA subjects. Similarly, representative high and low percentages of MAIT cells in peripheral blood of URECA subjects.

Supplemental Figure 2: Correlation of iNKT cells with CD4$^+$ T cells producing IFNγ or IL-4



Pearson correlations were determined between iNKT cells as a percentage of total CD3$^+$ cells and cytokine production in the indicated CD4$^+$ T cell populations. Correlation coefficients and p-values are indicated below the plot (n = 110).

Supplemental Figure 3: Association of iNKT and MAIT cell cytokines with clinical outcomes



(a-f) A comparison in one year-old children of the percentage of iNKT cells that produced IFNγ or IL-4 after a brief in vitro activation with aeroallergen sensitization at age 3 years (Neg, n = 43; Pos, n = 40) (a, d), recurrent wheezing at age 3 years (Neg, n = 61, Pos, n = 22) (b, e) and asthma (c, f) at age 7 years (Neg, n = 63, Pos, n = 13). (g-i) A comparison of the percentage of MAIT cells producing IFNγ to several clinical outcomes, including, aeroallergen sensitization at age 3 years (Neg n = 51, Pos n = 41) (g), recurrent wheezing at age 3 years (Neg n = 69, Pos n = 23) (h) and asthma (i) at age 7 years (Neg n = 71, Pos n = 13). p-values were calculated using a Student's t- test. Samples with less than 50 cell counts for the population analyzed were excluded from the analysis.

Supplemental Table: Individual demographics of URECA cohort- Donors that provided blood are indicated in blue font

Accession Number	URECA Study ID	Annual Household Income	Annual Household Income < $15K?	Child's Race	Child's Gestational Age (weeks)	Child's Birthweight (grams)	C-Section?	Mother Ever Breastfed? 0=No, 1=Yes	Child's Gender
n0431	07-01-001-1	Less Than 5,000	Yes	Hispanic	40	2888	No	0	Female
n0444	07-01-014-6	10,000 to 14,999	Yes	Black	37	2102	Yes	0	Female
n0475	07-08-002-8	Less Than 5,000	Yes	Black	41	3195	Yes	0	Female
n0538	07-01-006-9	10,000 to 14,999	Yes	Black	39	2907	No	0	Female
n0540	07-01-010-5	15,000 to 19,999	No	Black	41	2753	Yes	0	Female
n0543	07-01-011-4	5,000 to 9,999	Yes	Mixed (>1 race)	40	3498	No	1	Female
n0549	07-01-007-6	30,000 to 34,999	No	Black	36	2412	No	0	Male
n0692	07-02-002-5	15,000 to 19,999	No	Black	38	3220	No	1	Female
n0701	07-07-001-5	5,000 to 9,999	Yes	Black	41	2780	No	1	Female
n0720	07-02-003-9	10,000 to 14,999	Yes	Black	39	2695	No	1	Male
n0798	07-08-001-6	5,000 to 9,999	Yes	Black	38	3065	No	1	Male
n0800	07-08-004-4	Less Than 5,000	Yes	Black	39	4625	Yes	1	Male
n0803	07-08-005-9	30,000 to 34,999	No	Black	39	3355	No	1	Male
n0848	07-07-004-3	5,000 to 9,999	Yes	Mixed (>1 race)	37	2570	No	1	Female
n0868	07-08-003-7	10,000 to 14,999	Yes	Black	37	3415	No	1	Female
n0871	07-08-008-5	Less Than 5,000	Yes	Black	39	2940	No	1	Male
n0877	07-08-010-2	Less Than 5,000	Yes	Black	39	3960	No	0	Female
n0880	07-08-014-1	Less Than 5,000	Yes	Black	37	3240	No	0	Female
n0887	07-02-005-6	Less Than 5,000	Yes	Black	37	3375	No	1	Male
n0951	07-07-008-9	10,000 to 14,999	Yes	Hispanic	36	2805	Yes	1	Male
n0965	07-07-007-0	30,000 to 34,999	No	Black	41	3170	No	1	Female
n0998	07-07-011-7	15,000 to 19,999	No	Hispanic	40	4120	No	1	Male
n1129	07-08-012-5	Less Than 5,000	Yes	Black	39	3280	No	1	Male
n1135	07-08-024-8	15,000 to 19,999	No	Mixed (>1 race)	39	2970	No	1	Female
n1138	07-08-026-9	5,000 to 9,999	Yes	Black	41	3625	Yes	0	Male
n1156	07-02-006-0	10,000 to 14,999	Yes	Black	38	2740	Yes	0	Female
n1159	07-02-009-4	5,000 to 9,999	Yes	Hispanic	41	4320	No	1	Male
n1189	07-07-014-0	10,000 to 14,999	Yes	Hispanic	36	2620	No	0	Male
n1284	07-08-015-6	Less Than 5,000	Yes	Black	39	3990	No	1	Male
n1290	07-08-022-4	5,000 to 9,999	Yes	Black	39	3145	No	1	Male
n1302	07-01-009-5	25,000 to 29,999	No	Black	39	2778	No	0	Male
n1305	07-01-013-3	Less Than 5,000	Yes	Black	40	3220	No	0	Male
n1313	07-01-020-0	Less Than 5,000	Yes	Black	40	3332	No	1	Female
n1318	07-01-022-8	10,000 to 14,999	Yes	Black	39	4462	Yes	1	Male
n1321	07-01-023-7	25,000 to 29,999	No	Mixed (>1 race)	40	3419	No	0	Male
n1327	07-01-025-9	10,000 to 14,999	Yes	Black	40	3858	No	0	Female
n1330	07-01-026-3	20,000 to 24,999	No	Black	41	4194	Yes	1	Female
n1333	07-01-029-2	15,000 to 19,999	No	Black	42	3681	No	1	Female
n1356	07-02-013-6	Less Than 5,000	Yes	Black	38	3235	Yes	1	Male
n1396	07-07-013-8	Less Than 5,000	Yes	Hispanic	35	3030	No	1	Male
n1407	07-08-021-1	5,000 to 9,999	Yes	Mixed (>1 race)	40	3295	No	1	Female
n1414	07-08-032-2	5,000 to 9,999	Yes	Black	40	3995	No	0	Male
n1416	07-07-016-4	Less Than 5,000	Yes	Hispanic	37	2710	No	1	Male
n1467	07-01-027-1	5,000 to 9,999	Yes	Black	38	2866	No	0	Male
n1476	07-01-031-8	Less Than 5,000	Yes	Black	41	3371	Yes	0	Male
n1482	07-01-034-1	5,000 to 9,999	Yes	Black	38	3201	No	0	Female
n1491	07-01-039-4	5,000 to 9,999	Yes	Black	40	3232	No	1	Female
n1494	07-01-042-1	20,000 to 24,999	No	Black	40	3631	No	0	Female
n1497	07-01-056-8	Less Than 5,000	Yes	Black	35	2385	No	0	Male
n1504	07-08-033-3	Less Than 5,000	Yes	Black	38	3375	Yes	0	Male
n1540	07-02-016-2	30,000 to 34,999	No	Black	40	3625	Yes	1	Male
n1547	07-02-019-1	15,000 to 19,999	No	Black	39	3305	Yes	1	Male
n1553	07-07-017-2	Less Than 5,000	Yes	Black	38	2805	No	0	Male
n1588	07-02-018-9	10,000 to 14,999	Yes	Hispanic	40	2820	Yes	1	Female
n1591	07-02-021-3	Less Than 5,000	Yes	Black	38	3080	No	1	Female
n1594	07-02-022-1	Less Than 5,000	Yes	Mixed (>1 race)	37	3355	No	1	Male
n1608	07-08-027-6	10,000 to 14,999	Yes	Black	35	2825	Yes	1	Female
n1611	07-08-034-6	5,000 to 9,999	Yes	Black	39	3125	Yes	0	Male
n1614	07-08-035-1	5,000 to 9,999	Yes	Black	38	3440	No	1	Female
n1620	07-08-037-9	5,000 to 9,999	Yes	Black	39	4150	No	1	Male
n1626	07-08-041-9	35,000 to 39,999	No	Black	38	3780	No	1	Female
o0005	07-01-038-7	5,000 to 9,999	Yes	Black	38	4298	No	0	Female
o0008	07-01-040-9	5,000 to 9,999	Yes	Black	41	4400	Yes	0	Male
o0014	07-01-043-2	20,000 to 24,999	No	Black	35	2379	Yes	1	Male
o0017	07-01-044-5	10,000 to 14,999	Yes	Black	39	3538	No	1	Male
o0022	07-01-048-4	20,000 to 24,999	No	Black	41	3391	Yes	0	Male
o0025	07-01-050-6	5,000 to 9,999	Yes	Black	40	3757	Yes	1	Female
o0028	07-01-052-3	25,000 to 29,999	No	Black	41	3586	No	1	Male
o0030	07-01-055-2	15,000 to 19,999	No	Black	40	3560	No	1	Female
o0033	07-01-059-9	Less Than 5,000	Yes	Black	41	3396	No	1	Male
o0044	07-01-064-9	5,000 to 9,999	Yes	Black	39	3059	Yes	0	Male
o0124	07-08-046-1	Less Than 5,000	Yes	Black	41	3275	Yes	1	Male
o0129	07-07-019-3	5,000 to 9,999	Yes	Hispanic	34	3210	Yes	1	Male
o0132	07-07-024-7	Less Than 5,000	Yes	Black	40	3480	No	1	Female
o0141	07-07-020-6	10,000 to 14,999	Yes	Hispanic	40	3295	No	1	Male
o0154	07-08-044-2	20,000 to 24,999	No	Black	38	3155	No	0	Male
o0158	07-08-047-4	20,000 to 24,999	No	Black	38	3065	No	0	Male
o0160	07-08-050-1	10,000 to 14,999	Yes	Black	38	3435	Yes	1	Male
o0166	07-02-027-8	5,000 to 9,999	Yes	Black	36	2565	Yes	1	Female
o0169	07-02-028-4	5,000 to 9,999	Yes	Mixed (>1 race)	37	2510	Yes	0	Female
o0194	07-08-029-5	25,000 to 29,999	No	Black	36	3520	No		Male
o0200	07-08-051-7	Less Than 5,000	Yes	Mixed (>1 race)	37	3460	Yes	0	Male
o0203	07-08-053-8	5,000 to 9,999	Yes	Black	37	2480	No	0	Female
o0209	07-01-047-8	25,000 to 29,999	No	Black	40	3657	No	0	Male

o0215	07-01-054-7	15,000 to 19,999	No	Black	41	2705	No	0	Female
o0218	07-01-057-5	Less Than 5,000	Yes	Black	41	3618	Yes	0	Female
o0222	07-01-060-8	40,000 to 44,999	No	Black	39	3715	No	0	Male
o0236	07-01-074-3	20,000 to 24,999	No	Other	40	3492	No	1	Female
o0239	07-02-036-9	10,000 to 14,999	Yes	Hispanic	40	3165	No	1	Female
o0253	07-02-038-2	Less Than 5,000	Yes	Black	40	3435	Yes	1	Male
o0256	07-07-029-9	10,000 to 14,999	Yes	Black	39	3490	No	0	Female
o0338	07-08-061-5	10,000 to 14,999	Yes	Black	40	3210	No	0	Female
o0341	07-08-064-3	5,000 to 9,999	Yes	Mixed (>1 race)	36	2085	Yes	0	Male
o0344	07-08-067-0	10,000 to 14,999	Yes	Hispanic	37	2935	No	1	Male
o0347	07-08-068-9	10,000 to 14,999	Yes	Black	36	2520	No	1	Male
o0357	07-01-075-8	Less Than 5,000	Yes	Black	39	3549	No	0	Female
o0363	07-01-077-0	5,000 to 9,999	Yes	Black	39	3468	Yes	0	Male
o0365	07-01-078-9	5,000 to 9,999	Yes	Black	40	2829	No	1	Male
o0368	07-01-079-1	45,000 to 49,999	No	Other	41	4021	No	0	Male
o0380	07-01-084-0	25,000 to 29,999	No	Black	39	3727	Yes	1	Male
o0404	07-08-069-1	Less Than 5,000	Yes	Black	38	2720	Yes	0	Female
o0410	07-02-023-2	5,000 to 9,999	Yes	Black	40	3525	No	1	Female
o0413	07-02-033-0	40,000 to 44,999	No	Black	40	3935	No	1	Female
o0419	07-02-046-3	More than 50,000	No	Black	39	3935	No	1	Male
o0425	07-07-027-5	Less Than 5,000	Yes	Hispanic	39	2840	No	0	Male
o0431	07-02-040-0	25,000 to 29,999	No	Black	36	2635	No	1	Male
o0476	07-08-054-0	15,000 to 19,999	No	Black	37	2950	Yes	0	Female
o0482	07-08-065-8	Less Than 5,000	Yes	Black	37	1845	No	1	Male
o0485	07-08-074-9	10,000 to 14,999	Yes	Black	36.6	2525	No	1	Female
o0488	07-08-076-5	Less Than 5,000	Yes	Black	38	3100	No	0	Female
o0511	07-02-042-8	25,000 to 29,999	No	Black	39	3535	No	1	Female
o0527	07-08-071-2	Less Than 5,000	Yes	Black	39	3310	No	1	Male
o0530	07-08-077-7	Less Than 5,000	Yes	Black	36	2170	No	0	Female
o0536	07-08-080-6	Less Than 5,000	Yes	Black	36	2460	No	0	Female
o0539	07-07-032-1	5,000 to 9,999	Yes	Hispanic	40	3595	No	1	Male
o0544	07-07-035-0	20,000 to 24,999	No	Hispanic	40	3810	No	0	Male
o0551	07-07-042-5	15,000 to 19,999	No	Hispanic	39	2410	No	0	Male
o0575	07-08-075-4	Less Than 5,000	Yes	Black	38.6	3335	No	1	Female
o0578	07-08-081-0	Less Than 5,000	Yes	Black	41	3770	Yes	1	Male
o0581	07-08-082-3	15,000 to 19,999	No	Black	39	3300	No	0	Female
o0584	07-08-085-2	5,000 to 9,999	Yes	Black	38	2495	No	1	Female
o0594	07-01-070-4	15,000 to 19,999	No	Mixed (>1 race)	39	3394	No	0	Male
o0597	07-01-082-9	5,000 to 9,999	Yes	Black	39	2784	Yes	1	Male
o0600	07-01-085-5	5,000 to 9,999	Yes	Black	39	2813	No	0	Female
o0603	07-01-086-4	10,000 to 14,999	Yes	Black	39	3886	No	0	Male
o0606	07-01-087-2	Less Than 5,000	Yes	Black	39	2864	Yes	0	Male
o0609	07-01-089-3	5,000 to 9,999	Yes	Black	40	3673	No	1	Male
o0614	07-01-092-6	15,000 to 19,999	No	Black	35	2324	Yes	0	Male
o0617	07-01-093-5	5,000 to 9,999	Yes	Black	39	3739	No	1	Male
o0619	07-01-094-2	More than 50,000	No	Black	40	3944	No	0	Male
o0622	07-01-095-7	15,000 to 19,999	No	Black	41	3378	No	0	Female
o0626	07-01-096-1	15,000 to 19,999	No	Black	39	3164	No	0	Female
o0631	07-01-098-8	10,000 to 14,999	Yes	Black	40	3224	Yes	0	Female
o0640	07-01-103-4	Less Than 5,000	Yes	Black	39	3050	Yes	0	Male
o0641	07-01-110-9	Less Than 5,000	Yes	Black	39	3250	Yes	0	Female
o0652	07-02-047-1	15,000 to 19,999	No	Hispanic	34	2260	No	1	Female
o0655	07-02-054-2	5,000 to 9,999	Yes	Hispanic	37	3015	No	1	Male
o0667	07-07-038-4	35,000 to 39,999	No	Hispanic	40	3220	No	1	Female
o0682	07-08-084-7	5,000 to 9,999	Yes	Black	40	2980	No	1	Female
o0685	07-08-086-8	5,000 to 9,999	Yes	Mixed (>1 race)	40	3620	No	0	Female
o0691	07-08-092-1	5,000 to 9,999	Yes	Black	37	2810	No	0	Female
o0790	07-02-068-6	5,000 to 9,999	Yes	Black	40	2920	No	0	Male
o0793	07-02-075-1	10,000 to 14,999	Yes	Hispanic	38	3345	No	1	Female
o0814	07-07-052-8	45,000 to 49,999	No	Mixed (>1 race)	40	3050	No	1	Female
o0884	07-07-055-9	5,000 to 9,999	Yes	Hispanic	38	2610	No	1	Female
o0893	07-02-061-7	5,000 to 9,999	Yes	Black	40	3260	Yes	1	Male
o0896	07-02-069-3	35,000 to 39,999	No	Hispanic	40	3060	Yes	1	Male
o0902	07-02-073-3	5,000 to 9,999	Yes	Hispanic	38	4020	Yes	1	Male
o0921	07-08-102-9	5,000 to 9,999	Yes	Black	39	3655	Yes	0	Male
o0927	07-08-105-5	Less Than 5,000	Yes	Black	38	3295	No	0	Female
o0930	07-08-109-3	Less Than 5,000	Yes	Black	39	3135	No	1	Female
o0945	07-01-104-7	40,000 to 44,999	No	Black	38	2583	No	0	Female
o0948	07-01-105-2	5,000 to 9,999	Yes	Black	35	2185	Yes	0	Male
o0950	07-01-107-5	15,000 to 19,999	No	Black	41	4060	Yes	0	Male
o0953	07-01-108-1	20,000 to 24,999	No	Black	40	3108	Yes	0	Male
o0958	07-01-114-5	5,000 to 9,999	Yes	Black	38	3029	No	0	Female
o0960	07-01-117-8	5,000 to 9,999	Yes	Black	39	3179	No	0	Male
o0962	07-01-118-4	30,000 to 34,999	No	Black	39	3909	Yes	1	Male
o0997	07-02-056-1	10,000 to 14,999	Yes	Hispanic	39	4070	No		Male
o1000	07-02-059-0	10,000 to 14,999	Yes	Black	36	3165	No	1	Male
o1006	07-02-062-9	10,000 to 14,999	Yes	Black	38	3714	No	1	Male
o1009	07-02-066-4	5,000 to 9,999	Yes	Black	39	3660	No	1	Male
o1018	07-02-077-9	30,000 to 34,999	No	Black	38	2930	No	1	Male
o1021	07-02-080-8	20,000 to 24,999	No	Black	39	3315	Yes	1	Female
o1031	07-01-113-2	Less Than 5,000	Yes	Black	40	3268	No	0	Female
o1033	07-01-115-0	25,000 to 29,999	No	Hispanic	35	1842	No	0	Male
o1035	07-01-116-6	5,000 to 9,999	Yes	Black	41	3631	No	0	Male
o1038	07-01-119-7	15,000 to 19,999	No	Black	38	3224	No	0	Male
o1044	07-01-122-7	Less Than 5,000	Yes	Black	41	3672	No	1	Female
o1050	07-01-128-9	Less Than 5,000	Yes	Mixed (>1 race)	40	3519	No	0	Male
o1130	07-07-069-0	10,000 to 14,999	Yes	Hispanic	39	3310	No	1	Male
o1136	07-08-089-9	5,000 to 9,999	Yes	Black	38	3570	No	0	Female
o1142	07-08-114-2	Less Than 5,000	Yes	Black	37	3035	No	0	Female

o1167	07-01-124-3	5,000 to 9,999	Yes	Black	38	3000	No	1	Male
o1169	07-01-130-1	5,000 to 9,999	Yes	Black	41	3975	No	1	Female
o1175	07-01-132-9	Less Than 5,000	Yes	Black	40	3666	Yes	1	Male
o1178	07-01-135-5	Less Than 5,000	Yes	Black	40	2768	No	0	Female
o1190	07-07-081-4	20,000 to 24,999	No	Hispanic	40.3	3490	Yes	1	Female
o1193	07-02-048-5	15,000 to 19,999	No	Black	39	3810	Yes	0	Male
o1196	07-02-053-5	45,000 to 49,999	No	Black	40	4190	Yes	1	Male
o1202	07-02-079-8	35,000 to 39,999	No	Black	38	2995	No	1	Female
o1205	07-02-084-9	Less Than 5,000	Yes	Mixed (>1 race)	41	3880	No	1	Female
o1208	07-02-085-4	5,000 to 9,999	Yes	Hispanic	39	3630	No	1	Male
o1214	07-02-088-3	Less Than 5,000	Yes	Black	37	3075	No	1	Male
o1220	07-02-090-6	10,000 to 14,999	Yes	Black	40	3275	No	1	Female
o1237	07-07-075-3	Less Than 5,000	Yes	Black	39	3360	No	1	Male
o1240	07-07-083-3	5,000 to 9,999	Yes	Black	40.3	3340	No	0	Female
o1256	07-02-045-9	15,000 to 19,999	No	Black	38	2595	No	1	Female
o1259	07-02-082-0	5,000 to 9,999	Yes	Hispanic	40	3380	No	1	Male
o1262	07-02-091-0	35,000 to 39,999	No	Hispanic	38	4065	Yes	1	Female
o1265	07-02-094-7	5,000 to 9,999	Yes	Black	40	3280	No	1	Male
o1295	07-08-101-7	Less Than 5,000	Yes	Black	38	2910	Yes	0	Female
o1299	07-08-112-6	15,000 to 19,999	No	Black	39	4380	Yes	0	Female
o1301	07-08-117-4	5,000 to 9,999	Yes	Hispanic	38	3830	No	1	Male
o1307	07-08-121-2	5,000 to 9,999	Yes	Black	38	2905	No	1	Female
o1310	07-08-122-0	5,000 to 9,999	Yes	Black	41	3410	Yes	1	Female
o1313	07-08-123-1	Less Than 5,000	Yes	Black	40	3625	No	0	Female
o1316	07-08-124-9	15,000 to 19,999	No	Black	40	4085	No	0	Male
o1319	07-08-127-7	Less Than 5,000	Yes	Black	39	3715	Yes	1	Female
o1346	07-07-074-8	5,000 to 9,999	Yes	Hispanic	40.6	2955	No	1	Male
o1365	07-07-080-5	5,000 to 9,999	Yes	Hispanic	37.2	2820	Yes	0	Female
o1394	07-02-072-2	5,000 to 9,999	Yes	Hispanic	40	3465	No	1	Male
o1397	07-02-083-1	5,000 to 9,999	Yes	Hispanic	40	3475	Yes	1	Male
o1403	07-02-096-8	35,000 to 39,999	No	Black	38	3320	Yes	1	Female
o1406	07-02-098-1	10,000 to 14,999	Yes	Hispanic	40	3695	No	1	Female
o1409	07-02-102-6	15,000 to 19,999	No	Black	39	3655	No	1	Female
o1473	07-01-136-4	5,000 to 9,999	Yes	Black	40	4020	No	0	Male
o1479	07-01-140-8	5,000 to 9,999	Yes	Black	37	2497	Yes	0	Male
o1482	07-01-145-4	15,000 to 19,999	No	Black	39	2918	No	1	Female
o1484	07-01-146-5	Less Than 5,000	Yes	Black	41	3223	Yes	1	Female
o1487	07-01-147-7	5,000 to 9,999	Yes	Black	40	3568	No	0	Female
o1490	07-01-148-3	5,000 to 9,999	Yes	Black	40	2869	No	0	Female
o1495	07-01-153-3	5,000 to 9,999	Yes	Black	39	2818	No	0	Female
o1524	07-07-086-7	20,000 to 24,999	No	Black	37	2500	Yes	1	Male
o1527	07-07-094-9	More than 50,000	No	Hispanic	39	3790	Yes	1	Male
o1536	07-08-088-1	Less Than 5,000	Yes	Black	37	3105	No	1	Female
o1539	07-08-125-4	Less Than 5,000	Yes	Black	40	3835	No	1	Female
o1542	07-08-126-5	5,000 to 9,999	Yes	Black	41	3370	No	0	Male
o1548	07-08-133-4	Less Than 5,000	Yes	Black	39	3535	No	0	Male
o1551	07-08-141-5	Less Than 5,000	Yes	Black	37	3060	No	1	Male
o1554	07-08-144-3	10,000 to 14,999	Yes	Black	36	2885	Yes	0	Male
o1613	07-02-076-7	15,000 to 19,999	No	Black	38	2975	No	0	Male
o1616	07-02-105-7	10,000 to 14,999	Yes	Black	37	2755	Yes	0	Female
o1622	07-02-107-4	5,000 to 9,999	Yes	Hispanic	41	3200	Yes	1	Male
o1625	07-02-108-8	15,000 to 19,999	No	Hispanic	40	3350	Yes	1	Female
o1627	07-02-110-0	5,000 to 9,999	Yes	Black	38	2920	No	0	Female
o1633	07-02-113-7	5,000 to 9,999	Yes	Hispanic	37	2475	No	1	Female
p0018	07-08-132-3	5,000 to 9,999	Yes	Black	35	3695	No	1	Male
p0020	07-08-134-7	Less Than 5,000	Yes	Black	40	3480	No	1	Female
p0026	07-08-136-8	Less Than 5,000	Yes	Mixed (>1 race)	39	2855	Yes	1	Female
p0035	07-08-146-2	10,000 to 14,999	Yes	Black	39	3770	No	1	Male
p0041	07-08-150-2	Less Than 5,000	Yes	Black	38	3190	Yes	0	Male
p0050	07-02-109-0	More than 50,000	No	Black	40	3195	No	1	Female
p0053	07-02-114-4	Less Than 5,000	Yes	Hispanic	38	3360	Yes	1	Male
p0056	07-02-116-3	More than 50,000	No	Hispanic	39	3340	No	1	Male
p0061	07-02-120-5	Less Than 5,000	Yes	Hispanic	39	2750	No	1	Female
p0063	07-02-123-3	45,000 to 49,999	No	White	38	4850	Yes	0	Male
p0066	07-02-126-7	40,000 to 44,999	No	Hispanic	37	3220	No	1	Male
p0094	07-01-138-6	5,000 to 9,999	Yes	Black	39	3051	Yes	1	Male
p0099	07-01-142-0	5,000 to 9,999	Yes	Black	39	4164	No	1	Female
p0103	07-01-144-9	5,000 to 9,999	Yes	Black	39	3064	Yes	0	Female
p0111	07-01-152-2	5,000 to 9,999	Yes	Black	41	3497	No	1	Female
p0112	07-01-154-6	5,000 to 9,999	Yes	Other	39	3131	No	1	Female
p0115	07-01-156-7	15,000 to 19,999	No	Black	39	2857	Yes	0	Female
p0118	07-01-157-9	10,000 to 14,999	Yes	Black	41	2818	No	1	Female
p0121	07-01-158-0	10,000 to 14,999	Yes	Black	41	2695	Yes	0	Female
p0124	07-01-160-7	20,000 to 24,999	No	Black	42	3510	No	1	Male
p0127	07-01-162-4	10,000 to 14,999	Yes	Black	39	3093	Yes	0	Male
p0130	07-01-163-0	Less Than 5,000	Yes	Black	38	3072	No	0	Male
p0290	07-08-097-8	5,000 to 9,999	Yes	Black	37	3090	Yes		Female
p0305	07-08-151-8	Less Than 5,000	Yes	Black	38	2890	No	1	Female
p0311	07-08-153-9	5,000 to 9,999	Yes	Black	39	3015	No	0	Female
p0382	07-02-051-9	10,000 to 14,999	Yes	Black	40	3190	Yes	1	Male
p0388	07-02-121-4	10,000 to 14,999	Yes	Black	38	2810	No	1	Male
p0390	07-02-122-2	5,000 to 9,999	Yes	Mixed (>1 race)	40	3050	No	1	Female
p0399	07-02-129-8	Less Than 5,000	Yes	Hispanic	39	3410	Yes	1	Male
p0401	07-02-131-2	Less Than 5,000	Yes	Hispanic	40	3365	Yes	1	Female
p0404	07-02-133-1	5,000 to 9,999	Yes	Black	39	2855	No	1	Male
p0407	07-02-136-5	20,000 to 24,999	No	Mixed (>1 race)	40	3465	No	0	Male
p0411	07-02-138-3	30,000 to 34,999	No	Mixed (>1 race)	36	2875	No	0	Male
p0413	07-02-139-6	More than 50,000	No	White	41	3745	No	1	Male
p0416	07-02-142-9	10,000 to 14,999	Yes	Hispanic	41	3270	No	1	Female

p0429	07-07-076-9	15,000 to 19,999	No	Hispanic	37.3	3150	No	0	Male
p0479	07-07-111-8	More than 50,000	No	Black	39	3640	No	1	Male
p0482	07-07-120-7	20,000 to 24,999	No	Hispanic	39	2443	No	1	Male
p0512	07-01-126-2	10,000 to 14,999	Yes	Black	38	3463	No	0	Male
p0515	07-01-155-1	20,000 to 24,999	No	Black	38	3513	No	1	Female
p0521	07-01-167-6	5,000 to 9,999	Yes	Black	38	2999	No	0	Female
p0574	07-07-108-5	15,000 to 19,999	No	Hispanic	41	3845	No	1	Male
p0671	07-08-120-8	Less Than 5,000	Yes	Black	40	3970	No	0	Female
p0684	07-08-158-7	15,000 to 19,999	No	Black	37	3240	No	0	Male
p0686	07-08-159-4	15,000 to 19,999	No	Black	37	2485	Yes	0	Male
p0692	07-08-161-6	Less Than 5,000	Yes	Black	37	2970	No	0	Female
p0777	07-02-067-2	5,000 to 9,999	Yes	Black	38	3400	No	1	Female
p0780	07-02-100-3	25,000 to 29,999	No	Mixed (>1 race)	39	3865	Yes	1	Male
p0786	07-02-128-0	Less Than 5,000	Yes	Black	39	2410	No	1	Female
p0789	07-02-130-8	15,000 to 19,999	No	Other	39	3020	No	1	Male
p0792	07-02-140-1	25,000 to 29,999	No	Black	39	3195	Yes	1	Female
p0795	07-02-141-7	Less Than 5,000	Yes	Black	39	3140	No	1	Male
p0813	07-07-100-0	30,000 to 34,999	No	Black	39	3595	No		Female
p0816	07-07-110-2	Less Than 5,000	Yes	Hispanic	37	3310	Yes	0	Male
p0882	07-01-112-1	30,000 to 34,999	No	Black	37	2687	No	0	Female
p0884	07-01-165-3	35,000 to 39,999	No	Black	38	3529	No	0	Male
p0887	07-01-166-9	5,000 to 9,999	Yes	Black	39	3015	Yes	0	Male
p0912	07-07-092-0	40,000 to 44,999	No	Hispanic	39	4475	No	1	Female
p0927	07-02-143-8	15,000 to 19,999	No	Black	40	3345	No	1	Female
p0930	07-02-144-0	5,000 to 9,999	Yes	Hispanic	41	3860	No	1	Female
p0948	07-08-142-7	Less Than 5,000	Yes	Black	39	3065	No	1	Male
p0952	07-08-162-8	Less Than 5,000	Yes	Black	40	2775	Yes	0	Female
p0957	07-08-171-3	Less Than 5,000	Yes	Black	36	2000	No	1	Female
p1120	07-02-146-4	25,000 to 29,999	No	Hispanic	41	3180	No	1	Female
p1230	07-07-114-1	10,000 to 14,999	Yes	Hispanic	41	4010	No	1	Female
p1263	07-02-147-2	40,000 to 44,999	No	Hispanic	39	4355	Yes	1	Female
p1398	07-08-128-3	15,000 to 19,999	No	Black	41	3005	No		Male
p1404	07-08-174-5	Less Than 5,000	Yes	Black	39	3750	No	0	Male
p1407	07-08-175-0	20,000 to 24,999	No	Black	40	4055	No	0	Female
p1410	07-08-176-6	Less Than 5,000	Yes	Black	39	3490	Yes	0	Male
p1729	07-02-134-9	Less Than 5,000	Yes	Black	37	3000	Yes	0	Female
p1826	07-07-118-7	5,000 to 9,999	Yes	Black	39	2500	No	1	Male
p2398	07-08-157-3	10,000 to 14,999	Yes	Mixed (>1 race)	39	3180	Yes	0	Female
p2404	07-08-168-5	5,000 to 9,999	Yes	Black	38	3495	No	1	Male
p2407	07-08-173-2	Less Than 5,000	Yes	Black	37	2655	No	0	Female
p3632	07-08-163-7	Less Than 5,000	Yes	Black	40	3675	No	1	Male