Am. J. Hum. Genet. 76:696–704, 2005

Report

Gene-Environment Interaction Effects on the Development of Immune Responses in the 1st Year of Life

Sabine Hoffjan,[1,*,†] Dan Nicolae,[2,*] Irina Ostrovnaya,[2] Kathy Roberg,[4,5] Michael Evans,[6] Daniel B. Mirel,[8] Lori Steiner,[8] Karen Walker,[8] Peter Shult,[7] Ronald E. Gangnon,[6] James E. Gern,[4,5] Fernando D. Martinez,[9] Robert F. Lemanske, Jr.,[4,5] and Carole Ober[1,3]

Departments of [1]Human Genetics, [2]Statistics, and [3]Obstetrics and Gynecology, The University of Chicago, Chicago; Departments of [4]Pediatrics, [5]Medicine, and [6]Biostatistics and Medical Bioinformatics, University of Wisconsin–Madison, and [7]Wisconsin State Laboratory of Hygiene, Madison; [8]Roche Molecular Systems, Alameda, CA; and [9]Tucson Respiratory Center, University of Arizona, Tucson

Asthma is a common disease that results from both genetic and environmental risk factors. Children attending day care in the 1st year of life have lower risks for developing asthma, although the mechanism for this "day care" effect is largely unknown. We investigated the interactions between day care exposure in the 1st 6 mo of life and genotypes for 72 polymorphisms at 45 candidate loci and their effects on cytokine response profiles and on the development of atopic phenotypes in the 1st year of life in the Childhood Onset of Asthma (COAST) cohort of children. Six interactions (at four polymorphisms in three loci) with "day care" that had an effect on early-life immune phenotypes were significant at $P < .001$. The estimated false-discovery rate was 33%, indicating that an estimated four *P* values correspond to true associations. Moreover, the "day care" effect at some loci was accounted for by the increased number of viral infections among COAST children attending day care, whereas interactions at other loci were independent of the number of viral infections, indicating the presence of additional risk factors associated with day care environment. This study identified significant gene-environment interactions influencing the early patterning of the immune system and the subsequent development of asthma and highlights the importance of considering environmental risk factors in genetic analyses.

The dramatic increase in asthma and allergic diseases over the past 50 years (Burr et al. 1989; Beasley 2002) has been attributed in part to the eradication of many childhood infections, the liberal use of antibiotics, and a "cleaner" lifestyle in general during this time period (Martinez 2001; Weiss 2002; Liu and Murphy 2003). This so-called hygiene hypothesis is further supported by epidemiologic studies demonstrating that children who attend day care in infancy (Celedon et al. 1999; Kramer et al. 1999; Ball et al. 2000; Haby et al. 2000) and those with older siblings (von Mutius et al. 1994; Wickens et al. 1999; Ball et al. 2000; Koppelman et al. 2003) are less likely to develop asthma, presumably because of the increased exposure to infections among these children. These studies and others (Gereda et al. 2000; von Mutius et al. 2000; Braun-Fahrlander 2001; Braun-Fahrlander et al. 2002) suggest that exposure to "germs" in early life may facilitate the development of an immune system that is appropriately balanced with respect to T helper (Th) 1 and Th2 cytokine–producing cells. Recently, a role for interleukin (IL) 10 and transforming growth factor β (TGF-β)-secreting regulatory T (T_{reg}) cells in the development of Th1 and Th2 cells has also been proposed (Umetsu et al. 2003). In this model, secretion of these cytokines by T_{reg} cells may induce immune tolerance to commensal bacteria and allergens and may promote a balance with respect to Th1- and Th2-producing cells.

Although asthma and allergic diseases are considered to be Th2-skewed conditions—with increased IL-4, IL-13, and IL-5 cytokine secretion—the role of Th1 cytokine–producing cells in the development of atopic dis-

Received December 20, 2004; accepted for publication February 2, 2005; electronically published February 22, 2005.

Address for correspondence and reprints: Dr. Carole Ober, Department of Human Genetics, 920 East 58th Street, CLSC 507C, The University of Chicago, Chicago, IL 60637. E-mail: c-ober@genetics.uchicago.edu

* These authors contributed equally to this article.

† Present affiliation: Department of Human Genetics, Ruhr-University of Bochum, Bochum, Germany.

0002-9297/2005/7604-0017$15.00

ease is less clear. Many studies of unselected children have shown that low production of Th1 cytokines (usually interferon-γ [IFN-γ]) in infancy is a marker for the subsequent development of atopic disease (Holt et al. 1992; Rinas et al. 1993; Tang et al. 1993, 1994; Warner et al. 1994; Martinez et al. 1995; Liao et al. 1996; Pohl et al. 1997; Kondo et al. 1998; Prescott et al. 1999). However, this attenuated IFN-γ response during the 1st 6 mo of life may be transient in children who develop atopic disease, because Th1 cytokine hyperresponsiveness was present in these children at age 18 mo (Rowe et al. 2004) and at age 12 years (Smart and Kemp 2002). Furthermore, it has been suggested that the switch from hypo-IFN-γ responsiveness to hyper-IFN-γ responsiveness may occur earlier in children with a family history of asthma or allergy (Rowe et al. 2004).

It is likely that early-life exposures interact with a child's genotype to determine response to environmental factors and subsequent risk for disease. To explore this hypothesis, we studied 72 polymorphisms in 45 genes involved in the immune response (table 1) in a cohort of high-risk children participating in the prospective Childhood Onset of Asthma (COAST) Study (Lemanske 2002; Gern et al. 2003; Neaville et al. 2003; Hoffjan et al. 2004). Th1 (IFN-γ), Th2 (IL-5 and IL-13), and T_{reg} (IL-10) cytokine responses were measured in these children at birth and at age 1 year, and atopic phenotypes were documented in the 1st year (table 2). Day care attendance and the presence of older siblings in the household during the 1st year were determined prospectively by a questionnaire. Day care attendance was defined by >10 h of care outside of the home per wk or >10 h of care per wk in which the mother cared for one or more unrelated children in her own home. Among 208 white COAST children, 47.8% attended day care for at least 6 mo in the 1st year of life, 55.9% had older siblings living in the same household, and 80.7% either attended day care or had older siblings. Of the children in day care, 97% were in environments that included other children, and 95% of the day care environments were outside the child's home. Ninety-nine children (47.8%) experienced at least one moderate-to-severe respiratory syncytial virus (RSV) infection, and 109 children (52.7%) experienced at least one moderate-to-severe rhinovirus (RV) infection in the 1st year. The average number of moderate-to-severe viral infections was 1.4 (range 0–7). Day care attendance or the presence of older siblings at home was associated with an increased number of viral infections in the 1st year, and the number of viral infections was positively correlated with the change in IFN-γ response during the 1st year among COAST children (Copenhaver et al. 2004).

We examined the effects of day care attendance, the presence of older siblings, and viral infections on immunologic phenotypes in the 1st year by use of likelihood-ratio tests on a truncated normal model. The clinical phenotypes were investigated using logistic regression. In all analyses, we considered an additive model in which genotypes and exposures interact to influence each phenotype, and we tested the null hypothesis that the effects of each potential risk factor (the genotypes and day care exposure) are independent. Cytokine measurements were normalized using a log (for IFN-γ measurements) or square root (for IL-5, IL-10, and IL-13 measurements) transformation. The change in cytokine response was calculated as the difference of the transformed values from birth to age 1 year. Censoring was used for the quantitative phenotypes to model the sensitivity of the assay. The interaction between the covariates and genotypes was modeled using a factorial model. *P* values were determined using large-sample approximations for the likelihood-ratio statistic. Because of the large number of comparisons that were made in this study, we report only results with an interaction $P < .01$. Further, we estimated the false-discovery rate (FDR), which is the proportion of false-positive results in the set of rejected hypotheses (Benjamini and Hochberg 1995). The FDR for a fixed rejection region (e.g., $P < .001$) can be estimated as the ratio of the expected number of false-positive results to the observed number of rejections (Storey 2002). We calculated the expected number of false-positive results by repeating the analyses with simulated data sets. The simulated data sets preserve the relationship between phenotypes (the response variables) and between day care exposure and genotypes (the covariates) and were created by random permutation of the vectors of phenotypes among individuals. The average number of *P* values in the rejection region in 100 simulations gives a conservative estimate of the expected number of false-positive results.

Sixteen polymorphisms at 10 loci revealed 22 significant interactions with day care attendance on cytokine profiles and/or atopic phenotypes in the 1st year of life at the $P < .01$ level; 6 interactions were significant at $P < .001$ (interaction *P* values are given in table 3). Only one polymorphism (*IL13*_−1112) was modestly associated with the same phenotype in the baseline analysis (main-effect *P* value is given in table 3). Therefore, neither the exposure variable (day care) nor these polymorphisms were individually associated with 1st-year phenotypes. The estimated FDR was 73% for results with $P < .01$ and was 33% for results with $P < .001$, suggesting that, in this data set, we would expect

Table 1

European American COAST Children ($n = 208$) Genotyped for 73 Markers in 45 Genes

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Table 2

Phenotypes and Covariates Examined in the COAST Cohort

Characteristic	Children Attending Day Care (n = 99)	Children Not Attending Day Care (n = 109)
Clinical phenotype:		
No. of children with:		
AD in the 1st year	44	48
Allergic sensitization at age 1 year[a]	31	29
Wheeze in the 1st year	31	27
Wheeze with RSV infection in the 1st year	25	19
Immunologic phenotype[b]:		
PHA-induced response from MNC at age 1 year (pg/ml)[c]:		
IL-13	308.9 ± 25.4	287.3 ± 23.4
IL-5	182.8 ± 12.8	168.1 ± 19.5
IL-10	113.9 ± 7.5	118.8 ± 7.5
IFN-γ	42.6 ± 4.4	39.2 ± 4.4
Ratio at age 1 year:		
IFN-γ:IL-13	.27 ± .052	.30 ± .042
IL-10:IL-13	.52 ± .042	.71 ± .070
IL-10:IL-5	.89 ± .081	1.56 ± .40
Change in response from birth to age 1 year (pg/ml):		
IL-13 (ΔIL-13)	−48.4 ± 35.1	−48.3 ± 30.9
IL-5 (ΔIL-5)	177.4 ± 12.8	162.4 ± 19.4
IL-10 (ΔIL-10)	−7.8 ± 10.1	−11.5 ± 10.9
IFN-γ (ΔIFN-γ)	−4.6 ± .7	−4.8 ± .6
LPS-induced response from MNC at age 1 year (pg/ml)[c]:		
IFN-γ	9.7 ± 1.8	12.0 ± 3.5
IL-10	597.7 ± 52.3	693.9 ± 68.1
Eosinophil count at age 1 year (cells/mm^3)	256.6 ± 22.9	244.3 ± 19.8
Total immunoglobulin E at age 1 year (IU/ml)	40.3 ± 13.9	46.6 ± 15.8
Covariates:		
No. of children with:		
Older siblings in the household	46	69
Day care attendance in the 1st year or older siblings	99	69
RSV infection in the 1st year	52	47
RV infection in the 1st year	57	52
No. of viral infections in the 1st year (mean ± SE)	1.73 ± .16	1.19 ± .12

[a] ⩾1 positive radio allergo sorbent test.
[b] The mean ± SE of the immunologic phenotypes (untransformed) are shown.
[c] PHA = phytohemagglutinin; MNC = mononuclear cells.

to observe 16 P values < .01 and 2 P values < .001 by chance alone. Six of the 22 results with $P < .01$ and four of the six results with $P < .001$ are expected to be true associations.

Polymorphisms at three loci—*NOS3, FCER1B,* and *IL4RA*—showed associations with multiple phenotypes and had at least one interaction $P < .001$. The *NOS3*_298 polymorphism (Glu→Asp) showed significant interaction effects on Th2 cytokine response profiles during the 1st year, as measured by the change in IL-5 (ΔIL-5), ΔIL-13, IL-5 response, and the IL-10:IL-5 ratio. The *NOS3*_−922 (A→G) polymorphism also showed an interaction effect on the IL-10:IL-5 ratio. The interaction plot of the relationship between ΔIL-13, the *NOS3*_298 genotype, and day care is shown in figure 1*A*. Children with the Asp/Asp genotype who attended day care had a large decrease in mean IL-13 responsiveness in the 1st year, whereas children with the Asp/Asp genotype who did not attend day care had a large increase in mean IL-13 responsiveness. A nearly identical pattern was seen for ΔIL-5, IL-5 response, and the IL-10:IL-5 ratio values (not shown). Overall, the children with the Asp/Asp genotype who did not attend day care had the highest levels of and greatest increases in Th2 cytokine responses, whereas the children with the Asp/Asp genotype who attended day care had the lowest levels of and smallest increases in Th2 cytokine responses.

The 237Glu→Gly polymorphism at the *FCER1B* locus also showed interaction effects on Th2 cytokine responses, as measured by IL-5 response and the IL-10:IL-5 ratio. The mean IL-5 response was 4-fold greater among children with the Glu/Gly genotype who did not attend day care than among children with the Glu/Gly

Table 3

Interactions between Genotypes and Day Care Attendance and the Effects on Cytokine-Response Levels and Atopic Phenotypes in the 1st Year

Locus and Associated Phenotype	Main-Effect *P*	Interaction *P*	No Day Care ($n = 109$)		Day Care ($n = 99$)	
			Genotype Effect	*P*	Genotype Effect	*P*
*NOS3*_298:						
ΔIL-5	.84	***.00032***	Asp/Asp ↑	.011	Asp/Asp ↓	.027
ΔIL-13	.68	***.00049***	Asp/Asp ↑	.028	Asp/Asp ↓	.012
IL-5	.33	***.00090***	Asp/Asp ↑	.0019	No effect	.32
IL-10:IL-5	.22	.0070	Asp/Asp ↓	.0081	No effect	.58
*NOS3*_−922:						
IL10:IL-5	.20	.0088	AG ↑	.0065	No effect	.71
*FCER1B*_237:						
IL-5	.69	***.00085***	Glu/Gly ↑	.014	Glu/Gly↓	.024
IL-10:IL-5	.16	.0094	Glu/Gly ↓	.0097	No effect	.32
*IL4RA*_50:						
LPS-induced IFN-γ	.98	***.00029***	Val/Val ↑	.037	Val/Val ↓	.0075
*IL4RA*_142:						
AD	.85	***.00078***	CT ↓	.097	CT ↑	.0070
*IL13*_−1112:						
Wheeze	.01	.0016	No effect	.52	AA ↑	***.0005***
*IL13*_110:						
Wheeze	.28	.0053	No effect	.41	Gln/Gln ↑	.095
IL10:IL-13	.67	.0021	No effect	.20	No effect	.23
*IL13*_int3:						
Wheeze	.21	.0016	TT ↓	.089	No effect	.13
IL10:IL-13	.86	.0080	No effect	.10	No effect	.20
*IL10*_−571:						
ΔIL-5	.32	.0021	CC ↑	.0013	No effect	.29
*IL10*_−854:						
ΔIL-5	.29	.0039	GG ↑	.0073	No effect	.26
*IL1B*_−1418:						
RSV-associated wheeze	.94	.0026	TT ↑	.063	TT ↓	.11
Wheeze	.78	.0039	TT ↑	.018	TT ↓	.07
*CSF2*_117:						
IL-5	.24	.0069	Thr/Thr ↑	.041	Thr/Thr ↓	.036
*IL6*_174:						
IFN-γ	.05	.0015	GC ↑	.046	No effect	.95
*IFNG*_+5644:						
LPS-induced IFN-γ	.68	.0024	TT ↑	.073	TT ↓	.020
*LTC4S*_−444:						
Wheeze	.56	.0095	CC ↑	.013	No effect	.32

Note.—*P* values <.001 are shown in bold italics. The arrows indicate that the genotype is associated with increased (↑) or decreased (↓) amounts or prevalences of the associated phenotype. Phenotype data were available for all children.

genotype who attended day care (fig. 1*B*). The pattern of interaction was similar for the ratio of IL-10:IL-5 (not shown), with the highest ratios among children with the Glu/Gly genotype who attended day care and the lowest ratios among children with the Glu/Gly genotype who did not attend day care. Thus, consistent with the results at the *NOS3* locus, the *FCER1B* genotype that was associated with the highest IL-5 responsiveness among children not attending day care was associated with the lowest IL-5 responsiveness among children attending day care.

At the *IL4RA* locus, the 50Ile→Val polymorphism showed a significant interaction effect on lipopolysaccharide (LPS)-induced IFN-γ production and the synonymous 142C→T polymorphism on the development of atopic dermatitis (AD) during the 1st year. Among children with the *IL4RA*_50 Val/Val genotype, there was a highly significant effect of day care exposure; the mean IFN-γ response to LPS was significantly lower among those who attended day care than among those who did not attend day care (fig. 1*C*). At the 142C/T polymorphism, the proportion of AD cases was significantly higher among children with the CT genotype who attended day care than among children with the CT genotype who did not attend day care (0.67 vs. 0.33), whereas the proportion of AD cases did not differ for children with the CC genotype (0.43 among children attending day care and 0.49 among children not at-

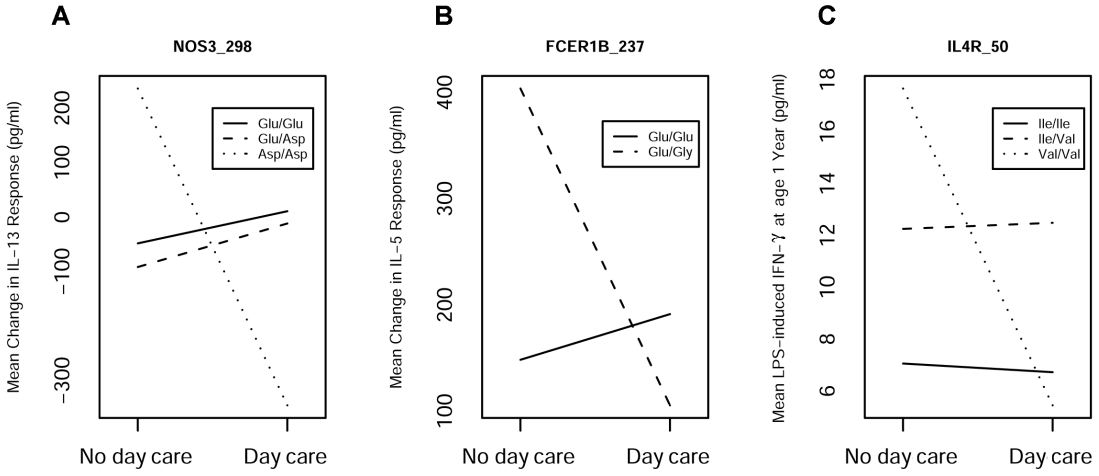

Figure 1 Interaction plots of representative associations between immunologic phenotypes, genotypes, and day care. Untransformed cytokine values are shown.

tending day care). (There were no TT homozygotes in the sample.) Other interactions and the direction of the genotype-associated risks are shown in table 3.

To determine whether the "day care" effect on cytokine-response profiles and atopic phenotypes was due to viral infections, which are increased among COAST children attending day care (Copenhaver et al. 2004), we next repeated the analyses of the three most significant loci, using the number of viral infections, the occurrence of one or more RSV infections, and the occurrence of one or more RV infections as covariates. We also considered "day care or older siblings" as a covariate, to try to tease apart the effects of being around other children from the effects specific to day care. The results of these analyses are shown in table 4.

At the *NOS3* locus, the highly significant interactive effects with day care attendance on Th2 cytokine responses were not mirrored in the analyses with the number of viral infections, RSV infections, or RV infections as covariates, whereas the covariate "day care or older siblings" showed a similar pattern of association to that of "day care" alone. Thus, the effects of day care attendance on Th2 cytokine profiles in children with specific genotypes at the *NOS3* locus are not due to increased symptomatic viral infections among children attending day care. Rather, these effects are due to risk factors that are independent of viral illnesses but correlated with the presence of other children. This is consistent with results of a study of the Hutterites, a population isolate that lives communally and has large family sizes. In that study, the Asp allele was increased in nonasthmatic Hutterites (Bourgain et al. 2003), indicating that the Asp allele has a protective effect in children exposed to a day care–like environment, perhaps as a result of smaller increases in Th2 cytokine responses during infancy in these children. In contrast, the interactive effects of "day care" and the *FCER1B* Glu237Gly genotype on IL-5 response profiles and the *IL4RA* Ile50Val genotype on IFN-γ responses are likely due to the increased number of viral infections in the children attending day care. The effects of the interactions between day care attendance and genotypes at the 142C/T polymorphism on the development of AD in the 1st year of life were not correlated with effects of either viral infections or older siblings, which indicates the presence of additional protective (or risk) factors that may differ between children who attend and those who do not attend day care in the 1st year, such as exposure to endotoxin and allergens, socioeconomic status, or breast-feeding patterns. Thus, although we considered day care attendance as the environmental risk factor, day care attendance is likely correlated with many other potential risk factors, any one of which could be influencing our results.

The results of our study are consistent with studies in model organisms, in which the effects of QTLs are often dependent on environmental exposures (Reifsnyder et al. 2000; Dilda and Mackay 2002; Coulter et al. 2003; Ungerer et al. 2003; Barr et al. 2004). Moreover, genotype-environment interactions have been reported in both linkage (Martin et al. 2002; Colilla et al. 2003; Nicolae et al. 2005; Weiss et al. 2005) and association (Werner et al. 2003; Berman et al. 2004; Leng et al. 2004; McIntire et al. 2004; Padyukov et al. 2004) studies of human diseases. Thus, this phenomenon is not limited to immune phenotypes or to the specific environmental exposures examined in this study but is likely a common feature of many—if not all—complex traits (Merikangas and Risch 2003).

The identification in this study of significant genotype-

Table 4

Interactions between Exposures (Day Care Attendance, Siblings, and Viral Infections) and Genotypes at the *NOS3*_298, *FCERB1*_237, and *IL4RA*_50 Loci and the Effects on Cytokine-Response Levels and Atopic Phenotypes

Locus and Associated Phenotype	*P* Value for Interaction				
	Day Care	Day Care or Older Siblings	RSV Infection	RV Infection	Viral Infections
*NOS3*_298:					
ΔIL-5	.00032	.0009	NS	NS	NS
ΔIL-13	.00049	.000078	NS	NS	NS
IL-5	.00090	NS	NS	NS	NS
IL-10:IL-5	.0070	NS	NS	NS	NS
*FCERB1*_237:					
IL-5	.00085	NS	NS	.015	.00083
IL-10:IL-5	.0094	NS	NS	.021	.0011
*IL4RA*_50:					
LPS-induced IFN-γ	.00029	NS	NS	NS	.00037
*IL4RA*_142:					
AD	.00078	NS	NS	NS	NS

Note.—The number of children in each exposure group is given in table 2. NS = not significant.

environment interaction effects on the development of the immune system has implications for the design and interpretation of association studies. In particular, the differential responses of specific genotypes to day care attendance, older siblings, and viral infections and the differential associations with 1st-year phenotypes could account for the inconsistent results of association studies of these variants with asthma and related phenotypes, for which there are numerous examples of nonreplication and of opposite alleles associated in different studies (reviewed by Hoffjan et al. [2003]). For example, in our study, the risk-associated genotype differed between exposure groups (e.g., *NOS3* and *FCER1B*), or the association was only evident in one exposure group (e.g., IL-10 and IL-13). Thus, one might not expect to find associations with these polymorphisms or with the same genotypes in all populations studied, as a result of differences in early-life exposures. However, because replications are important and are still a gold standard, they should be carefully designed to match the relevant factors in the original study, whenever possible. Moreover, because in many instances the environmental and genetic components studied are only correlated with the true risk factors, the power of the replication study will depend on how well these correlations are preserved in the replication sample. At the very least, possible differences in environmental exposures should be considered in the interpretation of association studies, especially when there is a lack of replication of an association reported elsewhere.

Because of the relatively large number of environmental factors that are known to influence susceptibility to asthma and atopy, these phenotypes provide outstanding models for dissection of the genetic complexities of common diseases. We hypothesized that early-life exposures would interact with a child's genotype to influence the developing immune system in a way that would predispose to (or protect from) asthma and allergic diseases. The COAST Study was designed to test this hypothesis. In this report, we examined the interaction effects, in this cohort of children, of day care attendance and polymorphisms in genes that are involved in immune pathways. The aim of this study was to identify specific genes that influence cytokine-response profiles and atopic phenotypes in an exposure-specific manner and to determine whether the day care effect could be explained by an increased exposure to viral infections among children attending day care. However, because we sampled only one or a few polymorphisms in each gene, we cannot exclude the possibility that additional variation in any one of these genes interacts with day care exposure to influence 1st-year immune phenotypes. Furthermore, the COAST children were selected on the basis of having a parent with asthma or allergy, so these results may not be directly applicable to unselected children. For example, despite the well-established protection effect of day care among unselected children (Celedon et al. 1999; Kramer et al. 1999; Ball et al. 2000; Haby et al. 2000), day care attendance in the 1st year was not protective against atopic phenotypes in the COAST children or against asthma at age 6 years in another cohort of children whose mothers had asthma (Celedon et al. 2003). Thus, parental affection status is another important risk factor that interacts with environment (day care, in this example) to determine risk. Together, these results underscore the importance of environmental factors on the developing immune system and the genotype-specific responses to these exposures. Furthermore, although the focus of this study was phenotypes related to asthma and allergy, it is likely that

these results have general implications for immune-mediated diseases and have particular implications for autoimmune diseases, which have also increased in prevalence over the past 50 years (Bach 2002) and cluster with allergic diseases in families (Sheikh et al. 2003).

Acknowledgments

We would like to thank Nancy Cox for helpful discussions; Deborah Greene, from Roche Molecular Systems, for providing genotyping reagents; and the COAST personnel, for their dedicated work: Kirstin Carlson-Dakes, Beth Anderson, Christopher Tisler, Douglas DaSilva, Claudia Rock, Heidi Hiemke, Tressa Pappas, Carol Kirk, Mary Wedig, Abhik Bhattacharya, Zhanhai Li, Debbie Yoshihara, Marzena Krawiek, Gloria Akan, Christopher Copenhaver, Dan Jackson, William Neaville, Dan Brooks, Lance Mikus, Louis Rosenthal, Mark Moss, Sarah Sund, Addie Barth, Hoda Ahmadi, Kelly Anklam, Kiva Adler, Rebekah Hamilton, Stephanie Gilbertson-White, Patricia Green, Andrea Williams, Partricia Meyer, Kunling Shen, Karen Moucha, Lori Meinguth, Nan Peterson, Matthew Martin, Andrew Chang, Jennifer Tran, Kevin Gilmartin, and Matthew Sund. This project would not have been possible without the support and cooperation of the following south-central Wisconsin health care organizations: Associated Physicians LLP, Dean Medical Center, Group Health Cooperative, Physicians Plus Group Practices, UW Health, Prairie Clinic SC, Reedsburg Physician Group, St. Mary's Hospital Medical Center, Meriter Hospital, St. Clare Hospital and Health Care Services, Sauk Prairie Memorial Hospital, Reedsburg Area Medical Center, and Fort Atkinson Memorial Health Care Services. This research was supported by National Institutes of Health grants M01 RR03186, R01 HL61879, R01 DK55889, and P01 HL70831. S.H. was supported in part by the Deutsche Forschungsgesellschaft.

References

Bach JF (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 347:911–920

Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL (2000) Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med 343:538–543

Barr CS, Newman TK, Lindell S, Shannon C, Champoux M, Lesch KP, Suomi SJ, Goldman D, Higley JD (2004) Interaction between serotonin transporter gene variation and rearing condition in alcohol preference and consumption in female primates. Arch Gen Psychiatry 61:1146–1152

Beasley R (2002) The burden of asthma with specific reference to the United States. J Allergy Clin Immunol 109:S482–S489

Benjamini Y, Hochberg M (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Stat Soc B 57:289–300

Berman DM, Wang Y, Liu Z, Dong Q, Burke LA, Liotta LA, Fisher R, Wu X (2004) A functional polymorphism in *RGS6* modulates the risk of bladder cancer. Cancer Res 64:6820–6826

Bourgain C, Hoffjan S, Nicolae R, Newman D, Steiner L, Walker K, Reynolds R, Ober C, McPeek MS (2003) Novel case-control test in a founder population identifies p-selectin as an atopy-susceptibility locus. Am J Hum Genet 73:612–626

Braun-Fahrlander C (2001) The role of the farm environment and animal contact for the development of asthma and allergies. Clin Exp Allergy 31:1799–1803

Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D, von Mutius E (2002) Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 347:869–877

Burr ML, Butland BK, King S, Vaughan-Williams E (1989) Changes in asthma prevalence: two surveys 15 years apart. Arch Dis Child 64:1452–1456

Celedon JC, Litonjua AA, Weiss ST, Gold DR (1999) Day care attendance in the first year of life and illnesses of the upper and lower respiratory tract in children with a familial history of atopy. Pediatrics 104:495–500

Celedon JC, Wright RJ, Litonjua AA, Sredl D, Ryan L, Weiss ST, Gold DR (2003) Day care attendance in early life, maternal history of asthma, and asthma at the age of 6 years. Am J Respir Crit Care Med 167:1239–1243

Colilla S, Nicolae D, Pluzhnikov A, Blumenthal MN, Beaty TH, Bleecker ER, Lange EM, Rich SS, Meyers DA, Ober C, Cox NJ (2003) Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure. J Allergy Clin Immunol 111:840–846

Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, Kirk CJ, Roberg KA, Anderson EL, Tisler CJ, DaSilva DF, Hiemke HJ, Gentile K, Gangnon RE, Lemanske RF Jr (2004) Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. Am J Respir Crit Care Med 170:175–180

Coulter AA, Bearden CM, Liu X, Koza RA, Kozak LP (2003) Dietary fat interacts with QTLs controlling induction of *Pgc-1α* and *Ucp1* during conversion of white to brown fat. Physiol Genomics 14:139–147

Dilda CL, Mackay TF (2002) The genetic architecture of Drosophila sensory bristle number. Genetics 162:1655–1674

Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, Liu AH (2000) Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 355: 1680–1683

Gern JE, Reardon CL, Hoffjan S, Nicolae D, Li Z, Roberg KA, Neaville WA, Carlson-Dakes K, Adler K, Hamilton R, Anderson E, Gilbertson-White S, Tisler C, Dasilva D, Anklam K, Mikus LD, Rosenthal LA, Ober C, Gangnon R, Lemanske RF Jr (2003) Effect of dog ownership and genotype on immune development and atopy in infancy. J Allergy Clin Immunol 113:307–314

Haby MM, Marks GB, Peat JK, Leeder SR (2000) Daycare attendance before the age of two protects against atopy in preschool age children. Pediatr Pulmonol 30:377–384

Hoffjan S, Nicolae D, Ober C (2003) Association studies for

asthma and atopic diseases: a comprehensive review of the literature. Respir Res 4:14–28

Hoffjan S, Ostrovnaja I, Nicolae D, Newman DL, Nicolae R, Gangnon R, Steiner L, Walker K, Reynolds R, Greene D, Mirel D, Gern JE, Lemanske RF Jr, Ober C (2004) Genetic variation in immunoregulatory pathways and atopic phenotypes in infancy. J Allergy Clin Immunol 113:511–518

Holt PG, Clough JB, Holt BJ, Baron-Hay MJ, Rose AH, Robinson BW, Thomas WR (1992) Genetic "risk" for atopy is associated with delayed postnatal maturation of T-cell competence. Clin Exp Allergy 22:1093–1099

Kondo N, Kobayashi Y, Shinoda S, Takenaka R, Teramoto T, Kaneko H, Fukao T, Matsui E, Kasahara K, Yokoyama Y (1998) Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders—6-year follow-up study. Clin Exp Allergy 28:1340–1344

Koppelman GH, Jansen DF, Schouten JP, van der Heide S, Bleecker ER, Meyers DA, Postma DS (2003) Sibling effect on atopy in children of patients with asthma. Clin Exp Allergy 33:170–175

Kramer U, Heinrich J, Wjst M, Wichmann HE (1999) Age of entry to day nursery and allergy in later childhood. Lancet 353:450–454

Lemanske RF Jr (2002) The childhood origins of asthma (COAST) study. Pediatr Allergy Immunol 13 Suppl 15:38–43

Leng S, Dai Y, Niu Y, Pan Z, Li X, Cheng J, He F, Zheng Y (2004) Effects of genetic polymorphisms of metabolic enzymes on cytokinesis-block micronucleus in peripheral blood lymphocyte among coke-oven workers. Cancer Epidemiol Biomarkers Prev 13:1631–1639

Liao SY, Liao TN, Chiang BL, Huang MS, Chen CC, Chou CC, Hsieh KH (1996) Decreased production of IFN gamma and increased production of IL-6 by cord blood mononuclear cells of newborns with a high risk of allergy. Clin Exp Allergy 26:397–405

Liu AH, Murphy JR (2003) Hygiene hypothesis: fact or fiction? J Allergy Clin Immunol 111:471–478

Martin LJ, Cole SA, Hixson JE, Mahaney MC, Czerwinski SA, Almasy L, Blangero J, Comuzzie AG (2002) Genotype by smoking interaction for leptin levels in the San Antonio Family Heart Study. Genet Epidemiol 22:105–115

Martinez FD (2001) The coming-of-age of the hygiene hypothesis. Respir Res 2:129–132

Martinez FD, Stern DA, Wright AL, Holberg CJ, Taussig LM, Halonen M (1995) Association of interleukin-2 and interferon-γ production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. J Allergy Clin Immunol 96:652–660

McIntire JJ, Umetsu DT, DeKruyff RH (2004) TIM-1, a novel allergy and asthma susceptibility gene. Springer Semin Immunopathol 25:335–348

Merikangas KR, Risch N (2003) Genomic priorities and public health. Science 302:599–601

Mirel DB, Valdes AM, Lazzeroni LC, Reynolds RL, Erlich HA, Noble JA (2002) Association of *IL4R* haplotypes with type 1 diabetes. Diabetes 51:3336–3341

Neaville WA, Tisler C, Bhattacharya A, Anklam K, Gilbertson-White S, Hamilton R, Adler K, Dasilva DF, Roberg KA, Carlson-Dakes KT, Anderson E, Yoshihara D, Gangnon R, Mikus LD, Rosenthal LA, Gern JE, Lemanske RF Jr (2003) Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. J Allergy Clin Immunol 112:740–746

Nicolae D, Cox NJ, Lester LA, Schneider D, Tan Z, Billstrand C, Kuldanek S, Donfack J, Kogut P, Patel NM, Goodenbour J, Howard T, Wolf R, Koppelman GH, White SR, Parry R, Postma DS, Meyers D, Bleecker ER, Hunt JS, Solway J, Ober C (2005) Fine mapping and positional candidate studies identify *HLA-G* as an asthma susceptibility gene on chromosome 6p21. Am J Hum Genet 76:349–357

Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L (2004) A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum 50:3085–3092

Pohl D, Bockelmann C, Forster K, Rieger CH, Schauer U (1997) Neonates at risk of atopy show impaired production of interferon-γ after stimulation with bacterial products (LPS and SEE). Allergy 52:732–738

Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG (1999) Development of allergen-specific T-cell memory in atopic and normal children. Lancet 353:196–200

Reifsnyder PC, Churchill G, Leiter EH (2000) Maternal environment and genotype interact to establish diabesity in mice. Genome Res 10:1568–1578

Rinas U, Horneff G, Wahn V (1993) Interferon-γ production by cord-blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. Pediatr Allergy Immunol 4:60–64

Rowe J, Heaton T, Kusel M, Suriyaarachchi D, Serralha M, Holt BJ, de Klerk N, Sly PD, Holt PG (2004) High IFN-γ production by $CD8^+$ T cells and early sensitization among infants at high risk of atopy. J Allergy Clin Immunol 113:710–716

Sheikh A, Smeeth L, Hubbard R (2003) There is no evidence of an inverse relationship between TH2-mediated atopy and TH1-mediated autoimmune disorders: lack of support for the hygiene hypothesis. J Allergy Clin Immunol 111:131–135

Smart JM, Kemp AS (2002) Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease. Clin Exp Allergy 32:796–802

Storey JD (2002) A direct approach to false discovery rates. J Royal Stat Soc B 64:479–498

Tang M, Kemp A, Varigos G (1993) IL-4 and interferon-γ production in children with atopic disease. Clin Exp Immunol 92:120–124

Tang ML, Kemp AS, Thorburn J, Hill DJ (1994) Reduced interferon-γ secretion in neonates and subsequent atopy. Lancet 344:983–985

Umetsu DT, Akbari O, DeKruyff RH (2003) Regulatory T cells control the development of allergic disease and asthma. J Allergy Clin Immunol 112:480–487

Ungerer MC, Halldorsdottir SS, Purugganan MD, Mackay TF (2003) Genotype-environment interactions at quantitative trait loci affecting inflorescence development in *Arabidopsis thaliana*. Genetics 165:353–365

von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, Waser M, Nowak D (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 30: 1230–1234

von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Reitmeir P, Thiemann HH (1994) Skin test reactivity and number of siblings. BMJ 308:692–695

Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO (1994) Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? Clin Exp Allergy 24:423–430

Weiss LA, Abney M, Cook EH Jr, Ober C (2005) Sex-specific genetic architecture of whole blood serotonin levels. Am J Hum Genet 76:33–41

Weiss ST (2002) Eat dirt—the hygiene hypothesis and allergic diseases. N Engl J Med 347:930–931

Werner M, Topp R, Wimmer K, Richter K, Bischof W, Wjst M, Heinrich J (2003) TLR4 gene variants modify endotoxin effects on asthma. J Allergy Clin Immunol 112:323–330

Wickens KL, Crane J, Kemp TJ, Lewis SJ, D'Souza WJ, Sawyer GM, Stone ML, Tohill SJ, Kennedy JC, Slater TM, Pearce NE (1999) Family size, infections, and asthma prevalence in New Zealand children. Epidemiology 10:699–705