

Neonatal Cytokine Profile in the Airway Mucosal Lining Fluid Is Skewed by Maternal Atopy

Nilofar V. Følsgaard¹, Bo L. Chawes¹, Morten A. Rasmussen², Anne L. Bischoff¹, Charlotte G. Carson¹, Jakob Stokholm¹, Louise Pedersen¹, Trevor T. Hansel³, Klaus Bønnelykke¹, Susanne Brix⁴, and Hans Bisgaard¹

¹Copenhagen Prospective Studies on Asthma in Childhood, Health Sciences, University of Copenhagen, Copenhagen University Hospital, Gentofte, Denmark; ²Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark; ³Imperial Clinical Respiratory Research Unit, National Heart and Lung Institute, Imperial College, London, United Kingdom; and ⁴Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Søtofts Plads, Lyngby, Denmark

Rationale: Heredity from mother or father may impact differently in complex diseases, such as atopy. Maternal atopy is a stronger risk factor than paternal atopy for the development of atopy in the offspring. We hypothesized that mother's and father's atopy would have a differential imprinting on the cytokines and chemokines in the upper airway mucosal lining fluid of healthy neonates.

Objectives: To study parental atopic imprinting on the cytokines and chemokines in the upper airway mucosal lining fluid of healthy neonates.

Methods: Eighteen cytokines and chemokines were quantified in nasal mucosal lining fluid in 309 neonates from the novel unselected Copenhagen Prospective Study on Asthma in Childhood (COPSAC) birth cohort.

Measurements and Main Results: Maternal, but not paternal, atopic status (asthma, hay fever, or eczema with or without sensitization) was associated with general down-regulation of all 18 mediators assessed by principal component analysis (overall $P = 0.015$).

Conclusions: Maternal atopy, but not paternal atopy, showed a strong linkage with a suppressed mucosal cytokine and chemokine signature in asymptomatic neonates, suggesting imprinting by the maternal milieu *in utero* or perinatal life.

Keywords: neonatal airways; parental atopic disease; skewed immunology

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Maternal atopy is a stronger risk factor than paternal atopy for the development of atopy in the offspring.

What This Study Adds to the Field

Maternal atopy, but not paternal atopy, showed a strong linkage with a suppressed mucosal cytokine signature in asymptomatic neonates, suggesting imprinting by the maternal milieu *in utero* or perinatal life.

Heredity from mothers or fathers may impact disease development in the child differently in complex diseases, such as diabetes, inflammatory bowel disease, and atopy (1–4). Thus, atopic hereditary disease linkage in the offspring is stronger for maternal than paternal atopy (3–5). This suggests imprinting (i.e., an inheritance process independent of the classical mendelian inheritance) of the maternal immunologic status or milieu on the child during pregnancy or perinatal life, which is a period of immune immaturity.

The airway mucosa is exposed to the mother's milieu before birth and to allergens, pollutants, and the microbiome after birth. The ability of the newborn to mount a balanced and appropriate local immune response at the mucosal surfaces in response to the exposome (i.e., the totality of exposures received by a person during life [6]) is essential for maintaining healthy airways because atopic diseases presumably develop from an inappropriate immune response caused by complex interactions between genes, the exposome, and the host immune system. Indeed, a recent large-scale, genome-wide study of asthma showed strong associations to genes controlling innate and adaptive immune components (7).

The cytokines and chemokines involved in the pathogenesis of asthma and allergic rhinitis have been studied extensively *in vitro* in stimulated cord blood or peripheral blood from neonates (8–11), whereas there is no available *in vivo* evidence from the target organ, the airway mucosa. It is not known how parental atopy may affect early immunity in the target organ, the respiratory mucosa.

We hypothesized that atopic heredity from mother and father would have a differential imprinting on the cytokines and chemokines in the upper airway mucosa lining fluid of healthy neonates. To investigate this hypothesis, we studied subjects enrolled in the novel unselected Copenhagen Prospective Study on Asthma in Childhood (COPSAC₂₀₁₀) birth cohort. Some of the results of this study have been previously reported in the form of an abstract (12).

(Received in original form August 15, 2011; accepted in final form October 26, 2011)

Supported by the Lundbeck Foundation, the Pharmacy Foundation of 1991, the Augustinus Foundation, the Danish Medical Research Council, and the Danish Pediatric Asthma Centre. COPSAC is funded by private and public research funds all listed on www.copsac.com. The funding agencies did not have any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions: H.B. is the guarantor of the study and is responsible for the integrity of the work as a whole, from conception and design to acquisition of data, analysis and interpretation of data, and writing of the manuscript. N.V.F. contributed to acquisition of data, data analyses and interpretation, and writing of the manuscript. S.B. performed analysis of cytokines and chemokines, data interpretation, and writing of the manuscript. B.L.C. and K.B. contributed to data analyses, interpretation, and writing of the manuscript. T.T.H. contributed with data interpretation and writing of the manuscript. M.A.R. performed statistical analyses. A.L.B., C.G.C., J.S., B.L.C., and L.P. contributed to data collection. All authors made important intellectual contributions and critical final revision of the manuscript.

Correspondence and requests for reprints should be addressed to Hans Bisgaard, M.D., D.M.Sc., Copenhagen Prospective Studies on Asthma in Childhood, Health Sciences, University of Copenhagen, Copenhagen University Hospital, Gentofte, Ledreborg Allé 34, DK-2820 Gentofte, Copenhagen, Denmark. E-mail: bisgaard@copsac.com

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 185, Iss. 3, pp 275–280, Feb 1, 2012

Copyright © 2012 by the American Thoracic Society

Originally Published in Press as DOI: 10.1164/rccm.201108-1471OC on November 10, 2011
Internet address: www.atsjournals.org

METHODS

The COPSAC₂₀₁₀ Birth Cohort

The neonates were part of the novel COPSAC₂₀₁₀ cohort, an ongoing unselected prospective clinical study of a birth cohort of 700 children recruited in Zealand, Denmark, during 2009–2010. Written invitations were sent to pregnant women in Zealand. Women who were interested in participating were interviewed by telephone and subsequently attended the COPSAC clinical research unit for enrollment at gestational week 24, excluding women with chronic endocrinologic, nephrologic, or cardiac diseases. The neonates were enrolled at 1 week of age excluding any baby with severe congenital abnormality.

Measurements of Airway Inflammatory Mediators

The neonates visited the clinical research unit at 1 month of age. Upper airway mucosal lining fluid was collected with a pair of 3- × 15-mm strips of filter paper (Accuwik Ultra; fibrous hydroxylated polyester sheets, cat no. SPR0730, Pall Life Sciences, Portsmouth, Hampshire, UK; this product is no longer manufactured, but Leukosorb from Pall Life Sciences is an alternative) inserted onto the anterior part of the inferior nasal turbinate of both nostrils (*see* online video of the sampling procedure; www.copsac.com). The strips were left for 2 minutes (13). After removal, the filter papers were frozen at –80°C and stored until analysis.

The levels of IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13, tumor necrosis factor (TNF)- α , CXCL8 (IL-8), CCL11 (eotaxin-1), CCL26 (eotaxin-3), CXCL10 (IP-10), CCL2 (MCP-1), CCL13 (MCP-4), CCL22 (MDC), CCL4 (MIP-1 β), and TARC (CCL17) in the extracted upper airway mucosal lining fluid were analyzed in duplicate using the Ultrasensitive Meso Scale Discovery Multi-spot Human TH1/TH2 10-Plex cytokine assay and 9-plex chemokine assay (Meso Scale Discovery, Gaithersburg, MD). Samples were read using the Sector Imager 6000 (Meso Scale Discovery). The lower limit of detection was set as the mean signal from blanks +3 SD (*see* online supplement). For validation, IL-8 was analyzed with both cytokine and chemokine assay showing complete correlation between the two ($\rho = 0.98$). IL-8 chemokine values are presented in this article. As for IL-4, 55% of the measurements were under the set detection level (1.05 pg/ml), and 20 were undetectable or missing (i.e., 0 pg/ml) (*see* Table E2 in the online supplement). IL-4 has been included in the analysis because of its central role in Th2 skewing, but interpretation of these results must be done with caution.

Atopic Predisposition

Parents' atopic status was defined from a history of doctor-diagnosed asthma, hay fever, or atopic dermatitis independent of sensitization. This was determined by a structured clinical interview performed by the research doctors at the parents' first visit to the COPSAC research clinic during gestational week 24.

Statistical Analyses

Analyses were done by conventional statistics and by pattern recognition using principal component analysis (PCA). Mediator levels were log-transformed before analysis. In the conventional statistics, differences in cytokine and chemokine concentrations between the offspring of parents who are atopic versus parents who are not atopic were tested by Student unpaired *t* test. Our primary outcomes were mother who is atopic (irrespective of paternal atopic status) and father who is atopic (irrespective of maternal atopic status). Estimates were expressed as relative differences with the nonatopic group as reference and with corresponding 95% confidence intervals.

For the pattern recognition analyses, PCA was used to extract underlying latent variables, called principal components, which describe the systematic part of the variation across the 18 mediators in a few uncorrelated new and latent variables. The latent variables (principal components) reflect the distribution of samples across all variables. In this way it can be seen how the different variables contribute to the principal components and thereby understand what variables dominate the dataset in biologic patterns. Scatter plots of the principal components were used to investigate differences in overall cytokine–chemokine response between the children with or without mothers who are atopic.

The data processing was conducted using MATLAB R2009b v. 7.9.0.529 (MathWorks, Natick, MA) with the statistical and PLS toolbox (*t* test, PCA, visualization). In PCA, the NIPALS algorithm (nonlinear iterative partial least squares) was used to estimate the model while dealing with missing data (14).

Additional methodologic details are given in the online supplement.

RESULTS

Baseline Characteristics

The study included the first 309 consecutive neonates from the novel COPSAC₂₀₁₀ birth cohort at the mean age of 31 days (SD, 5 d). Sampling was completed in all neonates without any complications or adverse events.

Atopic disease had been diagnosed in 173 of the mothers (56% of the study group) and 142 (47%) of the fathers, and 241 (78%) of the neonates had either a mother or a father with a history of atopy. Baseline characteristics of the neonates of mothers who are atopic (with or without fathers who are atopic) did not differ significantly from neonates of mothers who are not atopic (with or without fathers who are atopic) with respect to fathers' atopy, ethnicity, household income, antibiotics in third trimester, smoking, alcohol intake in third trimester, furred animals in the home during pregnancy, gestational age, birth weight, caesarean section, Apgar score less than 7 (15), sex, older siblings, or exclusive breastfeeding (Table 1). Neonates of fathers who are atopic (with or without a mother who is atopic) versus fathers who are not atopic (with or without mothers who are atopic) and neonates of mothers who are atopic (without fathers who are atopic) versus fathers who are atopic (without mothers who are atopic) were also comparable with respect to the previously mentioned characteristics (*see* Table E1A and E1B).

Cytokine and Chemokine Levels in Neonates with and without Parents Who Are Atopic

Mean levels and variation in cytokine and chemokine levels are shown in Table E2.

Neonates of mothers who are atopic (with or without fathers who are atopic) had lower levels of mediators compared with neonates of mothers who are not atopic (with or without fathers who are atopic) (Figure 1A). Univariate statistical analyses showed *P* less than or equal to 0.01 for eotaxin-3 and IP-10, and *P* less than or equal to 0.05 for IFN- γ , IL-13, IL-1 β , IL-2, IL-4, IL-8, TNF- α , eotaxin-1, MCP-1, MCP-4, and MDC. The mediators IL-10, IL-12p70, IL-5, MIP-1 β , and TARC exhibited a nonsignificant trend of down-regulation (*see* Table E3). The conventional statistical approach was verified in a data-driven unsupervised PCA. The loading plot for all mediators and maternal atopic status shows that all variables are strongly correlated, because the first component (PC1) describes 66.5% of the total variation (*see* Figure E1). Therefore, the significant univariate associations revealed by conventional statistics mainly reflect one general underlying inhibitory effect in the neonates of mothers who are atopic. This is confirmed by the separation plot (Figure 2) showing significantly different distribution for individuals with and without mothers who are atopic (*P* = 0.01).

Neonates with fathers who are atopic (with or without mothers who are atopic) showed similar mediator levels as fathers who are not atopic (with or without mothers who are atopic) (Figure 1B; *see* Table E3). All univariate statistical comparisons were nonsignificant except MCP-1 (up-regulated in neonates with fathers who are atopic; *P* = 0.02). This pattern was confirmed in the data-driven PCA (*see* Figure E2).

Neonates of mothers who are atopic and fathers who are not atopic exhibited significantly reduced levels of IL-10, IL-12p70, IL-2, IL-4, TNF- α , eotaxin-3, MCP-1, MCP-4, and TARC compared

TABLE 1. BASELINE DEMOGRAPHICS

	All % (N)	Mothers Who Are Atopic % (N)	Mothers Who are Not Atopic % (N)	P Value
Baseline	309	56 (173)	44 (136)	—
Prenatal exposure				
Father who is atopic	47 (141)	43 (73)	52 (68)	0.16
Race—white	95 (293)	96 (165)	94 (128)	0.32
Household income—high*	35 (108)	31 (53)	40 (55)	0.37
Antibiotics in third trimester	18 (56)	19 (32)	18 (24)	0.85
Furred animals during pregnancy	34 (104)	35 (60)	32 (44)	0.77
Maternal smoking during pregnancy	5 (15)	3 (6)	7 (9)	0.20
Alcohol >1 unit/wk during third trimester	6 (18)	6 (10)	6 (8)	0.97
Postnatal exposure				
Gestational age†	5 (16)	5 (8)	6 (8)	0.62
Birth weight (<2,500 g)	1 (3)	2 (2)	1 (1)	0.74
Male	50 (154)	48 (83)	52 (71)	0.46
Apgar score <7 at 1 min	4 (13)	5 (9)	3 (4)	0.53
Caesarian section	12 (37)	12 (20)	12 (17)	0.26
Older sibling	48 (148)	47 (82)	49 (66)	0.84
Exclusive breastfeeding until study day	90 (279)	90 (156)	90 (123)	0.45

* High; above €110,000 a year.

† Before 37 weeks.

with neonates of fathers who are atopic and mothers who are not atopic. IL-8, IP-10, and MDC exhibited a nonsignificant trend of down-regulation (Figure 1C; *see* Table E3). The PCA confirmed the significant general down-regulation of mediators (*see* Figure E3).

DISCUSSION

Main Findings

Healthy neonates born to mothers who are atopic have down-regulated cytokines and chemokines in the upper airway mucosal lining fluid, whereas there is no association to father's atopic status. This suggests a maternal imprinting on the child's immune response developing during pregnancy or the first weeks of life.

Strengths and Limitations

We developed this noninvasive method of sampling upper airway mucosal lining fluid by filter paper with efficient absorption (wicking) properties, but with minimal protein binding capacity, so that mediators can be efficiently eluted (13, 16). This simple and noninvasive method makes it possible to assess the baseline innate and adaptive immune responses in the airway mucosa as reflected by a panel of cytokines and chemokines. The novel method was feasible in all neonates and without inconvenience to the child.

It is an advantage of this method that mediators could be measured in the upper airway mucosal lining fluid reducing the problem of dilution inherent to nasal lavage where the dilution is a significant confounder and may reduce the mediator concentration below the detection limit of the assay (17). It is a strength that the biologic sampling of mucosal lining fluid is from one of the target organs of allergic disease. A further strength of the method is that these mediators could be assessed in asymptomatic healthy neonates at baseline without stimulation, hence avoiding the excessive response accompanying challenge models (18).

It seems probable that the *in vivo* mediator concentrations are the product of networks of interactions between different host mucosal cell types reacting with the exposome, and hence this *in vivo* measure is a more complete assessment of the final common pathway of the immune response than a response to stimulated purified cells or cell lines determined *in vitro*.

The validity of the study is increased by the comprehensive prospective data collection from personal interviews in the clinic on potential confounders, including the pregnant mother's use of antibiotics, alcohol, and tobacco, mode of delivery, breastfeeding, and presence of siblings or animals at home.

The statistical evidence is strong with conventional statistics confirmed in the unsupervised, data-driven PCA (i.e., the potential issue of multiple testing by univariate analyses of each of the 18 mediators is mitigated by the overall significance in the first principal component). This PCA analysis simultaneously analyzes variation in all cytokines and chemokines, and allows testing for difference between groups by one single test. This revealed significantly different cytokine and chemokine levels for neonates with and without mothers who are atopic.

It is a limitation of the study that the definition of parents who are atopic did not include an objective measure, such as specific-IgE or skin prick test.

It may be a limitation to our conclusions that, despite being an unselected birth cohort, 78% have at least one parent who is atopic compared with the Danish population where 54% of children have been reported to have at least one parent who is atopic, reflecting a bias toward the afflicted population even in unselected cohort studies (19). It is well-known that parents with atopic disease tend to avoid certain behaviors or environments (20). Therefore, the home environment, maternal dietary habits, and the exposure to allergens in these families might differ from families without atopic predisposition, which may have affected the maternal imprinting on the child's immune response.

Interpretation

The *in vivo* airway cytokine and chemokine concentrations in neonates with mothers who are atopic presented a general down-regulation compared with mothers who are not atopic and compared with fathers who are atopic, whereas there was no influence from the fathers' atopic status compared with fathers who are not atopic. This general suppression in all mediators may be interpreted as a delayed maturation of the immune response.

The immune response is generally immature in early life as recently demonstrated in the experiment by nature, where the H1N1v pandemic caused more severe disease in neonates and young infants than in older children, all unprotected from passive

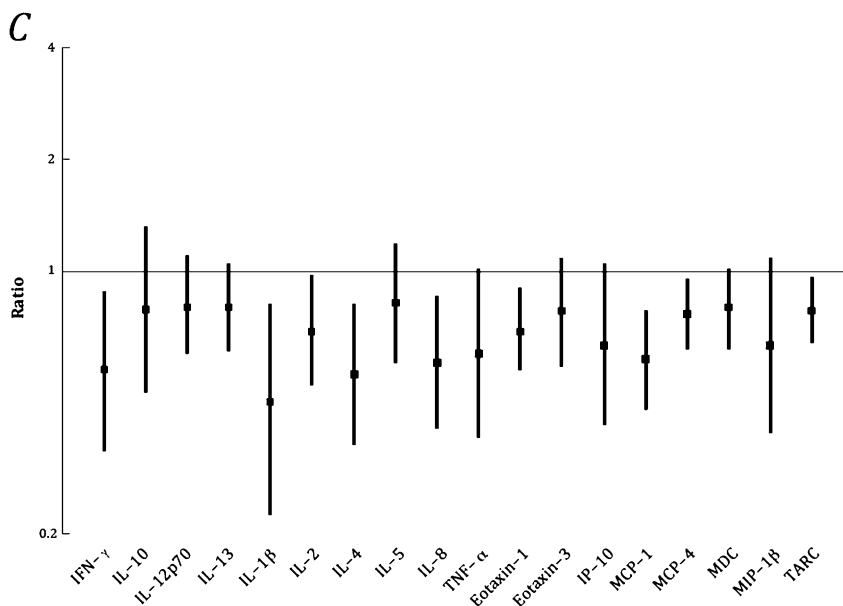
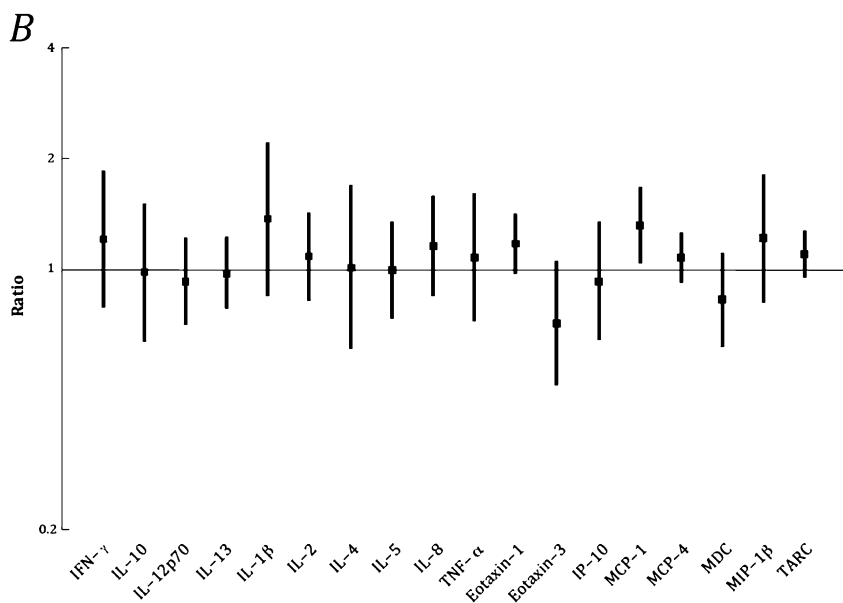
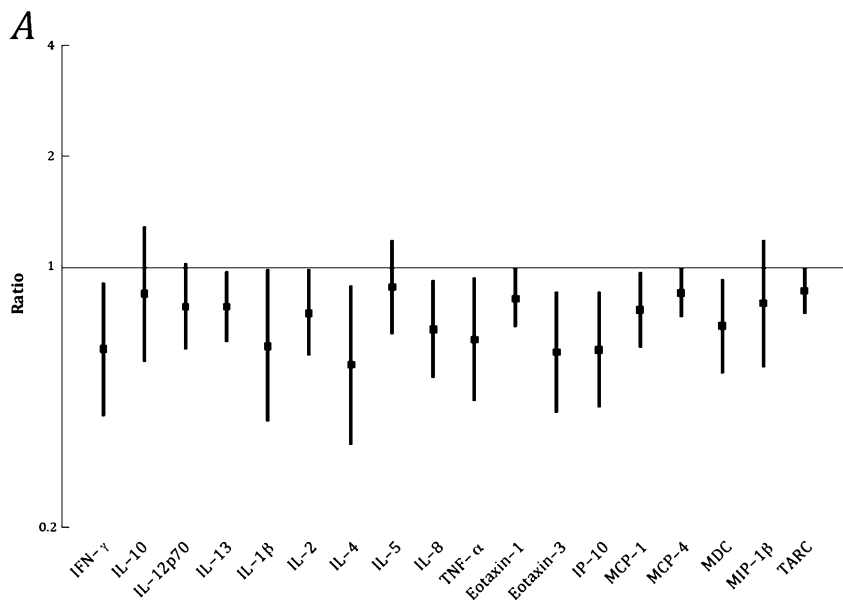


Figure 1. Ratios of cytokines and chemokines in the upper airway mucosal lining fluid in healthy neonates. Means with 95% confidence interval. (A) Ratio: Neonates with mothers who are atopic versus mothers who are not atopic. (B) Ratio: Neonates with fathers who are atopic versus fathers who are not atopic. (C) Ratio: Neonates with mothers who are atopic (fathers who are not atopic) versus neonates with fathers who are atopic (mothers who are not atopic). eotaxin-1 = CCL11; eotaxin-3 = CCL26; IFN- γ = interferon- γ ; IL = interleukin; IL-8 = CXCL8; IP-10 = CXCL10; MCP-1 = CCL2; MCP-4 = CCL13; MDC = CCL22; MIP-1 β = CCL4; TARC = CCL17; TNF- α = tumor necrosis factor- α .

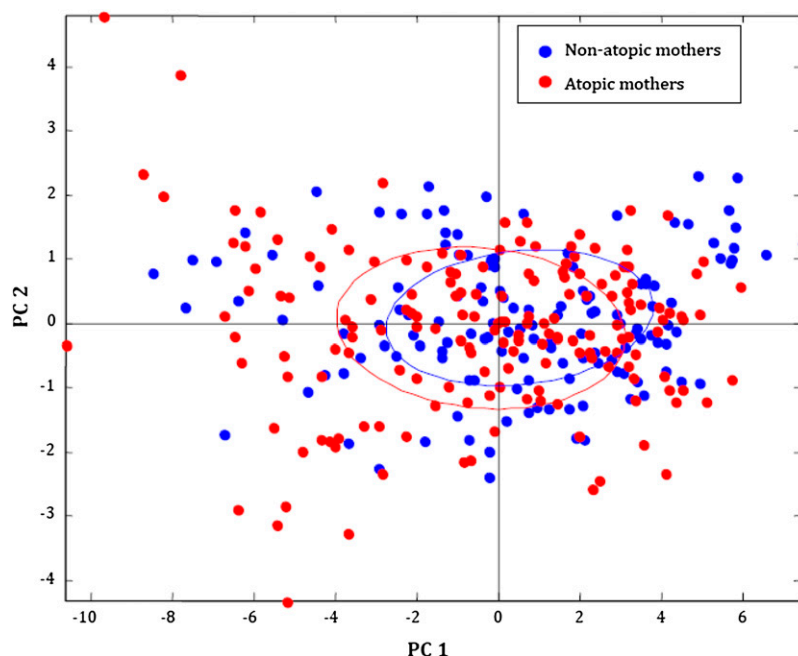


Figure 2. Separation plot for neonates with mothers who are atopic (with or without fathers who are atopic) versus mothers who are not atopic (with or without fathers who are atopic). For separation in PC1 ($P = 0.035$, Mann-Whitney U test; $P = 0.015$, t test) and PC2 ($P = 0.38$, Mann-Whitney U test; $P = 0.24$, t test). Ellipsoids are centered at the mean and rotated according to correlation with latent variables (PC1 and PC2) using standard deviation as semiaxis.

immunity to this novel virus (21). This illustrates the neonatal period as a period of immune immaturity and it is our interpretation that the milieu of the atopic mothers causes a further delay of the child's immune maturation.

Previous reports on the effect of maternal atopic status on neonatal immune profiles are ambiguous probably because of the limitation of *in vitro* studies including use of different stimulation regimens, problems with the harvesting of cells, use of single-cell lines with absence of epithelial-immune cell cross-talk, and different laboratory practices. In agreement with our findings, *in vitro* studies stimulating the innate immune cells from cord blood reported a reduced response of the Th1-type cytokine IFN- γ in children of mothers who are atopic (8, 9, 22–24). Likewise, regulatory T cells and their suppressive function and associated cytokines (IL-10) were found suppressed (24), together with reduced levels of Toll-like receptor (TLR) 2, TLR4/CD14, and TLR9 expression in cord blood from high-risk infants (10, 25). A linkage between maternal atopy and reduced Th1 (IFN- γ) response toward fetal histocompatibility complex antigens was previously reported (26), thus indicating that maternal atopy may impact fetal immune programming by immune regulatory processes at the maternofetal interface. Together these *in vitro* studies support our *in vivo* data suggesting that that maternal heredity delays the immune maturation in the offspring. One previous *in vivo* study assessed cytokines directly in the cord blood from 20 high-risk infants and 36 control subjects reporting detectable levels of the chemokines IP-10, I-TAC, MDC, and TARC with no significant correlations to the atopic status of the mother, whereas other Th1- and Th2-associated cytokines were undetectable (11). Other studies found no association between atopic heredity and the *in vitro* immune response to nonspecific stimulation of cord blood cells (27, 28). Studies have even reported enhanced levels of IFN- γ and IL-12 in cord blood from high-risk infants on stimulation of the innate immune system (29), and enhanced IL-13 response in stimulated cord blood (28).

Maternal specific imprinting has also been reported for other complex diseases, such as diabetes (1) and Crohn's disease (2). The mechanism behind maternal imprinting cannot be determined by our data. It may be speculated to arise from the maternal immune system signaling to the developing fetus

during pregnancy or may involve epigenetic mechanisms (30). Alternatively, exposure to environmental immune activators, such as maternal nutrition during pregnancy (e.g., omega-3 fatty acids and vitamin D intake) or the microbiome of the mother, is imprinting the fetal immune response toward the atopic status. We recently reported an association between abnormal bacterial colonization of the upper airways of neonates and later development of asthma suggesting that, *post or propter*, the human microbiome is associated with the skewing of the immune balance in people with asthma (31).

CONCLUSIONS

Maternal atopic disease was associated with a general down-regulation of cytokine and chemokine levels in the upper airway mucosal lining fluid in healthy neonates. There was no paternal linkage to the mucosal immune response pattern, suggesting maternal programming of the fetus or neonate is causing an aberrant local airway immune profile in the newborn child.

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank the children and parents participating in the COPSAC₂₀₁₀ cohort and the COPSAC study team. The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and approved by the Ethics Committee for Copenhagen (H-B-2008-093) and the Danish Data Protection Agency (j.nr. 2008-41-2599). Both parents gave their informed consent before enrollment of the children.

References

1. Cerf ME. Parental high-fat programming of offspring development, health and beta-cells. *Islets*. 2011;3:118–120.
2. Akolkar PN, Gulwani-Akolkar B, Heresbach D, Lin XY, Fisher S, Katz S, Silver J. Differences in risk of Crohn's disease in offspring of mothers and fathers with inflammatory bowel disease. *Am J Gastroenterol* 1997;92:2241–2244.
3. Lim RH, Kobzik L, Dahl M. Risk for asthma in offspring of asthmatic mothers versus fathers: a meta-analysis. *PLoS ONE* 2010;5:e10134.
4. Ruiz RG, Kemeny DM, Price JF. Higher risk of infantile atopic dermatitis from maternal atopy than from paternal atopy. *Clin Exp Allergy* 1992;22:762–766.

5. Liu CA, Wang CL, Chuang H, Ou CY, Hsu TY, Yang KD. Prenatal prediction of infant atopy by maternal but not paternal total IgE levels. *J Allergy Clin Immunol* 2003;112:899–904.
6. Rappaport SM. Implications of the exposome for exposure science. *J Expo Sci Environ Epidemiol* 2011;21:5–9.
7. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, Mutius EV, Farrall M, Lathrop M, Cookson WO. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;363:1211–1221.
8. Prescott SL, King B, Strong TL, Holt PG. The value of perinatal immune responses in predicting allergic disease at 6 years of age. *Allergy* 2003;58:1187–1194.
9. Rinas U, Horneff G, Wahn V. Interferon-gamma 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* 1993;4:60–64.
10. Reece P, Thanendran A, Crawford L, Tulic MK, Thabane L, Prescott SL, Sehmi R, Denburg JA. Maternal allergy modulates cord blood hematopoietic progenitor Toll-like receptor expression and function. *J Allergy Clin Immunol* 2011;127:447–453.
11. Sandberg M, Frykman A, Ernerudh J, Berg G, Matthiesen L, Ekerfelt C, Nilsson LJ, Jenmalm MC. Cord blood cytokines and chemokines and development of allergic disease. *Pediatr Allergy Immunol* 2009;20:519–527.
12. Folsgaard NV, Chawes BL, Rasmussen MA, Bonnelykke K, Brix S, Bisgaard H. Cytokine and chemokines in the airway epithelial lining fluid are down-regulated in newborns of atopic mothers [abstract]. Amsterdam: European Respiratory Society; 2011.
13. Chawes BL, Edwards MJ, Shamji B, Walker C, Nicholson GC, Tan AJ, Folsgaard NV, Bonnelykke K, Bisgaard H, Hansel TT. A novel method for assessing unchallenged levels of mediators in nasal epithelial lining fluid. *J Allergy Clin Immunol* 2010;125:1387–1389.
14. Wold S, Esbensen K, Geladi P. Principal component analysis. *Chemom Intell Lab Syst* 1987;2:37–52.
15. Kliegman RM. Nelson textbook of pediatrics, 18th ed. Amsterdam: Saunders Elsevier; 2007.
16. Nicholson GC, Kariyawasam H, Tan AJ, Hohlfeld J, Quinn D, Walker C, Rodman D, Westwick J, Jurcevic S, Kon O, *et al.* The effects of an anti-IL-13 mAb on cytokine levels and nasal symptoms following nasal allergen challenge. *J Allergy Clin Immunol* 2011;128:800–807.e9.
17. Bisgaard H, Krogsgaard OW, Mygind N. Measurement of secretion in nasal lavage. *Clin Sci (Lond)* 1987;73:217–222.
18. Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. *N Engl J Med* 1987;316:1506–1510.
19. Benn CS, Wohlfahrt J, Aaby P, Westergaard T, Benfeldt E, Michaelsen KF, Bjorksten B, Melbye M. Breastfeeding and risk of atopic dermatitis, by parental history of allergy, during the first 18 months of life. *Am J Epidemiol* 2004;160:217–223.
20. Kummeling I, Thijs C, Stelma F, Huber M, Brandt PA, Dagnelie PC. Do parents with an atopic family history adopt a 'prudent' lifestyle for their infant? (KOALA Study). *Clin Exp Allergy* 2006;36:489–494.
21. Libster R, Bugna J, Coviello S, Hijano DR, Dunaiewsky M, Reynoso N, Cavalieri ML, Guglielmo MC, Areso MS, Gilligan T, *et al.* Pediatric hospitalizations associated with 2009 pandemic influenza A (H1N1) in Argentina. *N Engl J Med* 2010;362:45–55.
22. Liao SY, Liao TN, Chiang BL, Huang MS, Chen CC, Chou CC, Hsieh KH. 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* 1996;26:397–405.
23. Gabrielsson S, Soderlund A, Nilsson C, Lilja G, Nordlund M, Troye-Blomberg M. Influence of atopic heredity on IL-4-, IL-12- and IFN-gamma-producing cells in *in vitro* activated cord blood mononuclear cells. *Clin Exp Immunol* 2001;126:390–396.
24. Schaub B, Liu J, Hoppler S, Haug S, Sattler C, Lluís A, Illi S, Mutius EV. Impairment of T-regulatory cells in cord blood of atopic mothers. *J Allergy Clin Immunol* 2008;121:1491–1499.
25. Krauss-Etschmann S, Hartl D, Heinrich J, Thaqi A, Prell C, Campoy C, Molina FS, Hector A, Decsi T, Schendel DJ, *et al.* Association between levels of Toll-like receptors 2 and 4 and CD14 mRNA and allergy in pregnant women and their offspring. *Clin Immunol* 2006;118:292–299.
26. Prescott SL, Breckler LA, Witt CS, Smith L, Dunstan JA, Christiansen FT. Allergic women show reduced T helper type 1 alloresponses to fetal human leucocyte antigen mismatch during pregnancy. *Clin Exp Immunol* 2010;159:65–72.
27. Martinez FD, Stern DA, Wright AL, Holberg CJ, Taussig LM, Halonen M. Association of interleukin-2 and interferon-gamma production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J Allergy Clin Immunol* 1995;96:652–660.
28. Kopp MV, Zehle C, Pichler J, Szepefalusi Z, Moseler M, Deichmann K, Forster J, Kuehr J. Allergen-specific T cell reactivity in cord blood: the influence of maternal cytokine production. *Clin Exp Allergy* 2001;31:1536–1543.
29. Prescott SL, Noakes P, Chow BW, Breckler L, Thornton CA, Hollams EM, Ali M, van den Biggelaar AH, Tulic MK. Presymptomatic differences in Toll-like receptor function in infants who have allergy. *J Allergy Clin Immunol* 2008;122:391–399.
30. Kong A, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P, Besenbacher S, Jonasdottir A, Sigurdsson A, Kristinsson KT, Jonasdottir A, *et al.* Parental origin of sequence variants associated with complex diseases. *Nature* 2009;462:868–874.
31. Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bonnelykke K, Brasholt M, Heltberg A, Vissing NH, Thorsen SV, *et al.* Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;357:1487–1495.