therapeutic target" for pharmacologic interventions in IPF. Thus, at our current state of knowledge, we believe that it is a reasonable approach to try to identify mediators and pathways that are crucially involved in this fatal disease and that might serve as targets for therapeutic interventions. We also believe that, in the end, effective treatment of IPF is likely to require a combination of therapies targeting multiple mediators/signaling pathways.

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

Malgorzata Wygrecka, Ph.D.
Grazyna Kwapiszewska, Ph.D.
Ewa Jablonska, Ph.D.
Susanne von Gerlach, M.D.
Ingrid Henneke, Ph.D.
Dariusz Zakrzewicz, Ph.D.
Andreas Guenther, M.D.
Klaus T. Preissner, Ph.D.
Philipp Markart, M.D.
University of Giessen Lung Center Giessen, Germany

References

1. Kotani I, Sato A, Hayakawa H, Urano T, Takada Y, Takada A. Increased procoagulant and antifibrinolytic activities in the lungs with idiopathic pulmonary fibrosis. *Thromb Res* 1995;77:493–504.
2. Gunther A, Mosavi P, Ruppert C, Heinemann S, Temmesfeld B, Velcovsky HG, Morr H, Grimminger F, Walmrath D, Seeger W. Enhanced tissue factor pathway activity and fibrin turnover in the alveolar compartment of patients with interstitial lung disease. *Thromb Haemost* 2000;83:853–860.
3. Imokawa S, Sato A, Hayakawa H, Kotani M, Urano T, Takada A. Tissue factor expression and fibrin deposition in the lungs of patients with idiopathic pulmonary fibrosis and systemic sclerosis. *Am J Respir Crit Care Med* 1997;156:631–636.
4. Grandaliano G, Pontrelli P, Cerullo G, Monno R, Ranieri E, Ursi M, Loverre A, Gesualdo L, Schena FP. Protease-activated receptor-2 expression in IgA nephropathy: a potential role in the pathogenesis of interstitial fibrosis. *J Am Soc Nephrol* 2003;14:2072–2083.
5. Ikeda O, Egami H, Ishiko T, Ishikawa S, Kamohara H, Hidaka H, Mita S, Ogawa M. Expression of proteinase-activated receptor-2 in human pancreatic cancer: a possible relation to cancer invasion and induction of fibrosis. *Int J Oncol* 2003;22:295–300.
6. Xu KS, Li Q, Zhou X. Changes of mast cells and protease activated receptor-2 in experimental rat liver fibrosis [in Chinese]. *Zhonghua Gan Zang Bing Za Zhi* 2006;14:753–756.
7. Cederqvist K, Haglund C, Heikkila P, Hollenberg MD, Karikoski R, Andersson S. High expression of pulmonary proteinase-activated receptor 2 in acute and chronic lung injury in preterm infants. *Pediatr Res* 2005;57:831–836.
8. D'Andrea MR, Derian CK, Santulli RJ, Andrade-Gordon P. Differential expression of protease-activated receptors-1 and -2 in stromal fibroblasts of normal, benign, and malignant human tissues. *Am J Pathol* 2001;158:2031–2041.
9. Materazzi S, Pellerito S, Di Serio C, Paglierani M, Naldini A, Ardinghi C, Carraro F, Geppetti P, Cirino G, Santucci M, *et al.* Analysis of protease-activated receptor-1 and -2 in human scar formation. *J Pathol* 2007;212:440–449.
10. Gruber BL, Marchese MJ, Santiago-Schwarz F, Martin CA, Zhang J, Kew RR. Protease-activated receptor-2 (PAR-2) expression in human fibroblasts is regulated by growth factors and extracellular matrix. *J Invest Dermatol* 2004;123:832–839.
11. Howell DC, Johns RH, Lasky JA, Shan B, Scotton CJ, Laurent GJ, Chambers RC. Absence of proteinase-activated receptor-1 signaling affords protection from bleomycin-induced lung inflammation and fibrosis. *Am J Pathol* 2005;166:1353–1365.
12. Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L152–L160.
13. Borensztajn K, Bresser P, van der Loos C, Bot I, van den Blink B, den Bakker MA, Daalhuisen J, Groot AP, Peppelenbosch MP, von der Thüsen JH, *et al.* Protease-activated receptor-2 induces myofibroblast differentiation and tissue factor up-regulation during bleomycin-induced lung injury: potential role in pulmonary fibrosis. *Am J Pathol* 2010;177:2753–2764.

Bronchial Nitric Oxide Flux May Be Better Associated with Inducible Nitric Oxide Synthase Promoter Methylation

To the Editor:

We read with great interest the article by Breton and colleagues (1) reporting the association between DNA demethylation of arginase (ARG)1 and ARG2, but not inducible nitric oxide synthase (iNOS), gene promoters, and fractional exhaled nitric oxide (F_{ENO}) in children with asthma. The authors are the first to link epigenetic variation in buccal DNA with increases in F_{ENO}, a biomarker associated with acute bronchial hyperresponsiveness, asthma relapse, and respiratory infection (2–4). They shed new light on biological mechanisms that may underlie NO production during asthma exacerbations. We commend the authors' collection of buccal cells as noninvasive, readily accessible cells that represent aerodigestive tract cells and can share similar patterns of gene expression with bronchial epithelial cells (5). This approach may have great advantages in longitudinal pediatric research.

Our group at the Columbia Center for Children's Environmental Health also was interested in investigating the association between buccal cell iNOS promoter demethylation and F_{ENO}, and evaluated the association between alveolar and conducting airway contributions of F_{ENO} with iNOS demethylation (6). Buccal cells were collected from 9- to 11-year-old urban children twice, 4–7 d apart ($n = 57$ subjects). Similarly, iNOS CpG-359 (corresponding to position 3 of NOS2A) was moderately methylated with a mean methylation level of 53.8% (SD 5.43; interquartile range 5.00). This intermediate level of methylation provides opportunity for changes in methylation patterns, presumably in association with environmental exposures.

We also examined the association between methylation of iNOS and F_{ENO}, and its postulated components bronchial NO flux (J_{NO}) and alveolar NO (C_{alv}) with methodology that takes advantage of multiple flow rates during collection of samples (6). Using generalized estimating equations to model the repeated measures, we found that iNOS methylation was not significantly associated with F_{ENO}. However, when compartmentalizing NO production into its proximal (bronchial) and distal (alveolar) airway components, iNOS methylation was associated inversely with J_{NO} ($\beta = -3.68$, 95% confidence interval: -6.68 to 0.67, $P = 0.016$), but not C_{alv} ($P > 0.1$). Previously we showed that J_{NO} was highly correlated with F_{ENO} and may be a better indicator of seroatopy than C_{alv}. In contrast, C_{alv} may be a better indicator of current wheeze (6).

Breton and colleagues' study and our pilot study suggest that measures of buccal cell DNA methylation may be informative biomarkers of airway inflammation in pediatric cohorts. Additionally, our pilot data suggest that iNOS demethylation may be more closely associated with proximal NO source components. Breton and colleagues may be underestimating the iNOS effect estimate because they have an NO measure from both proximal and distal sources in the lung. To strengthen these conclusions, both studies would benefit from buccal RNA expression analysis. Nonetheless, both studies reveal some new understanding on the immunopathogenesis of airway inflammation. Further studies are needed to examine the association of environmental asthma triggers with methylation patterns and clinical outcomes.

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

Julie Kuriakose, M.D.
Maria José Rosa, B.A.
Matthew Perzanowski, Ph.D.
Rachel Miller, M.D.
Columbia University
New York, New York

References

1. Breton CV, Byun HM, Wang X, Salam MT, Siegmund K, Gilliland FD. DNA methylation in the arginase-nitric oxide synthase pathway is associated with exhaled nitric oxide in children with asthma. *Am J Respir Crit Care Med* 2011;184:191–197.
2. Tossa P, Paris C, Zmirou-Navier D, Demange V, Acouetey DS, Michaely JP, Bohadana A. Increase in exhaled nitric oxide is associated with bronchial hyperresponsiveness among apprentices. *Am J Respir Crit Care Med* 2010;182:738–744.
3. Beigelman A, Mauger DT, Phillips BR, Zeiger RS, Taussig LM, Strunk RC, Bacharier LB. Network CARaEC, National Heart LnaBI: effect of elevated exhaled nitric oxide levels on the risk of respiratory tract illness in preschool-aged children with moderate-to-severe intermittent wheezing. *Ann Allergy Asthma Immunol* 2009;103:108–113.
4. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. *Thorax* 2005;60:215–218.
5. Sridhar S, Schembri F, Zeskind J, Shah V, Gustafson AM, Steiling K, Liu G, Dumas YM, Zhang X, Brody JS, *et al*. Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium. *BMC Genomics* 2008;9:259.
6. Rosa MJ, Divjan A, Hoepner L, Sheares BJ, Diaz D, Gauvey-Kern K, Perera FP, Miller RL, Perzanowski MS. Fractional exhaled nitric oxide exchange parameters among 9-year-old inner-city children. *Pediatr Pulmonol* 2011;46:83–91.

From the Authors:

We thank Kuriakose and colleagues for their insightful commentary regarding our recent publication (1). We agree with their conclusion that buccal cell DNA may be a useful biomarker for airway inflammation in pediatric research, and their attempt to further distinguish bronchial NO flux (J_{NO}) and alveolar NO (C_{alv}) is a worthwhile endeavor.

As part of the Children's Health Study, we also estimated bronchial and alveolar components of fractional exhaled nitric oxide (FE_{NO}) using six published modeling approaches (2). However, we are unable to assess the role of inducible nitric oxide synthase (iNOS) methylation on J_{NO} and C_{alv}, as buccal DNA was not collected during extended FE_{NO} measurements. In our study, we also found that FE_{NO} and J_{NO} and all model-derived J_{NO} parameters were highly correlated (all $r > 0.95$). Given this high degree of correlation, it is puzzling that Kuriakose and coworkers observed an association between iNOS methylation and J_{NO} but not FE_{NO}. We used natural log-transformation for FE_{NO} in our models. If Kuriakose and colleagues analyzed their data comparing the highest quartile with the remaining quartiles as they did earlier (3), then difference in analytic approach could also account for the difference in findings.

In fact, the arginase–nitric oxide synthase pathway is complex, and the study of a single element in relation to FE_{NO} is simplistic and liable to miss associations. More recent work in our cohort by Salam and colleagues highlights this complexity by demonstrating a complex synergy between air pollution exposures, genetic variation, and DNA methylation in iNOS in association with FE_{NO} (4). So while our original study observed no effect of DNA methylation in iNOS with FE_{NO}, once genetic variation and exposure information are taken into account, an association with FE_{NO} is observed.

The results of the investigation by Kuriakose and colleagues and our own highlight the need to delve into the complexity of underlying biological mechanisms to the extent possible in epidemiologic research. In addition to better phenotyping of the outcome or biomarker of interest (such as breaking FE_{NO} into its components), this will likely require analyzing large populations, multiple exposures, integrating genetic and epigenetic variation together in the context of environmental exposure, or some combination thereof. While both studies have contributed to a further understanding of the pathogenesis of airway inflammation, much more work lies ahead.

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

Carrie V. Breton, Sc.D.
Muhammad T. Salam, M.B.B.S., Ph.D.
University of Southern California
Los Angeles, California

References

1. Breton CV, Byun HM, Wang X, Salam MT, Siegmund K, Gilliland FD. DNA methylation in the arginase-nitric oxide synthase pathway is associated with exhaled nitric oxide in children with asthma. *Am J Respir Crit Care Med* 2011;184:191–197.
2. Linn WS, Rappaport EB, Berhane KT, Bastain TM, Salam MT, Gilliland FD. Extended exhaled nitric oxide analysis in field surveys of schoolchildren: a pilot test. *Pediatr Pulmonol* 2009;44:1033–1042.
3. Rosa MJ, Divjan A, Hoepner L, Sheares BJ, Diaz D, Gauvey-Kern K, Perera FP, Miller RL, Perzanowski MS. Fractional exhaled nitric oxide exchange parameters among 9-year-old inner-city children. *Pediatr Pulmonol* 2011;46:83–91.
4. Salam MT, Byun HM, Lurmann F, Breton CV, Wang X, Eckel SP, Gilliland FD. Genetic and epigenetic variations in inducible nitric oxide synthase promoter, particulate pollution, and exhaled nitric oxide levels in children. *J Allergy Clin Immunol* 2012;129:232–239.

Sarcoidosis Mortality

To the Editor:

Pointing out the limitations of a sarcoidosis mortality analysis (1), Baughman and Lower emphasized the contribution of black incidence to its computation (2). Thus, assuming prognostic equality and a black:white (B:W) incidence of 12:1 (3), the computed mortality in blacks would be 12 times that in whites. (The white incidence and B:W ratios cited by the authors [1] are marked outliers [4].) To circumvent this distortion, sarcoidosis mortality should be replaced by the incidence-independent, cumulative case-fatality rate—sarcoidosis deaths/100 cases—which have shown no B:W distinction (5).

In contrasting the favorable, single-institution mortality (population-based settings [PS]) with that experienced in tertiary clinics (TCs), Baughman and Lower, citing our metaanalysis (5), pointed out that the latter are "frequently populated by patients with more advanced disease." However, the plausible inference that unfavorable selection for TC referral accounts for the marked discrepancy in sarcoidosis mortality in the two settings is insufficient to account for its magnitude. A systematic review (5) of sarcoidosis mortality showed that the proportion of (prognostically highly favorable) stage I was high and similar in both settings: TCs, 49% versus PS, 59%; that sarcoidosis mortality in TCs was 10 times that in PS and 7.5 times after correction for prognostically unfavorable, advanced (III, IV) stage. The difference appeared to be largely ascribable to a seven times greater propensity to