Noneosinophilic responders with occupational asthma: A phenotype associated with a poor asthma prognosis

To the Editor:

Asthma tends to be considered a syndrome comprising different phenotypes rather than a disease representing a homogeneous group of patients. There is growing evidence that the identification of asthma phenotypes could be crucial in improving not only our understanding of the disease but also its management.[1] Although several phenotypes have been identified in asthma, there is very little information on occupational asthma (OA) phenotypes in the literature.

We sought to assess whether the type of airway inflammation induced by the exposure to a sensitizer can allow the identification of distinct phenotypes of OA with a different clinical outcome.

We performed a cross-sectional study of workers whose OA was diagnosed by specific inhalation challenges at Sacre-Coeur Hospital between 2000 and 2005 and who had sputum samples collected before and 8 hours after exposure to the offending agent at the time.

These subjects were invited to attend a visit between 2010 and 2011 in which detailed medical and occupational questionnaires were administered. Questions were asked about their respiratory symptoms at work, asthma medication, smoking habits, and work environment. Asthma control was assessed by using the validated Asthma Control Questionnaire.[2] Quality of life was assessed by using the Juniper specific asthma quality of life questionnaire.[3] Skin prick testing,[4] spirometry[5] methacholine inhalation challenge,[6] and sputum induction[7] were performed. The fraction of exhaled nitric oxide was measured with chemiluminescent analyzers (280i Sievers; GE, Boulder, Colo) before performing spirometry.[8] The study was approved by Sacre-Coeur Hospital's research ethics committee. All subjects gave their written consent.

Subjects who had a less than 2% change in their sputum eosinophil count after exposure to the offending agents were defined as noneosinophilic responders, whereas subjects who showed an increase equal or greater than 2% in their sputum eosinophil count after exposure were defined as eosinophilic responders.

Paired analyses were conducted to compare the data at the time of the specific inhalation challenge (SIC) and at the time of the study. Nonparametric tests were performed for data that were abnormally distributed. A backward linear regression was performed to assess the factors associated with a change in the level of airflow obstruction (FEV_1/forced vital capacity) between SIC and the time of the study. The statistical analysis was performed with the IBM SPSS statistical software (version 19.0.0; IBM Corporation, Somers, NY). Significance was accepted when the *P* value was .05 or less.

Sputum had been collected at diagnosis in 104 subjects before and after SIC; 42 (40.4%) subjects were noneosinophilic responders. Twenty subjects refused to participate in the current study; 40 subjects could not be reached. Forty-four subjects were enrolled for further evaluation at follow-up. During the SIC performed at the time of diagnosis, 15 subjects had a change of less than 2% (median [max-min], 0.2 [−4.4 to 1.5]%) in their sputum eosinophil count after exposure to the offending agents (noneosinophilic responders) whereas 29 subjects showed an

TABLE I. Characteristics of subjects at the time of diagnosis

	Change in eosinophil counts during SIC <2%	Change in eosinophil counts during SIC ≥2%	*P* value
n	15	29	
Sex, M/F	13/2	21/8	.3
Age (y), mean ± SD	48 ± 12.3	45.1 ± 9.4	.5
Atopy, n (%)	11 (73.3%)	27 (93.1%)	.07
Smoking habits, NS, CS/exS, n (%)	4 (26.6)/4 (26.6)/7 (46.7)	16 (55.1)/4 (13.8)/9 (31.0)	.2
Pack-years, mean ± SD	24.3 ± 22.6 (n = 11)	19.1 ± 18.9 (n = 13)	.4
Agents (HMW/LMW), n (%)	4 (26.7)/11 (73.3) Isocyanates (5), wood dust (3), sanitizer (2), colophony (1), pork (1), chicken (1), insecticide (1), flour (1)	13 (44.8)/16 (55.2) Flour (10), isocyanates (9), acrylates (1), formaldehyde (2), red cedar (2), latex (1), enzymes (1), beaver fur (1), ammonium (1), rat (1)	.2
Asthma duration (y), mean ± SD	9.8 ± 3.3	13.1 ± 7.9	.1
Time from diagnosis (y)	5.6 ± 2.9	5.3 ± 2.0	.7
Duration of exposure (y)	11.9 ± 11.8	9.3 ± 6.9	.4
Treatment with ICS, n (%)	10 (66.7)	14 (48.3)	.3
FEV_1 at diagnosis (% predicted), mean ± SD	81.0 ± 20.7	92.1 ± 16.7	.06
FEV_1/FVC, mean ± SD	0.69 ± 0.1	0.76 ± 0.05	.01
PC_{20} pre-SIC (mg/mL), GM ± SD	3.0 ± 15.4	8.1 ± 7.7	.1
PC_{20} post-SIC (mg/mL), GM ± SD	0.9 ± 6.9	3.4 ± 5.8	.04
Asthmatic reaction (I, L, D)	8/7/0	19/7/3	.2
Baseline TCC (10^6 cells/g), median (IQR)	1.8 (0.66)	1.8 (1.8)	.6
Baseline Eos (%), median (IQR)	1.0 (2.7)	1.0 (2.5)	.8
Baseline Neu (%) median (IQR)	42.7 (50.5)	43.0 (39.2)	.7
TCC after SIC (10^6 cells/g), median (IQR)	3.5 (7.2)	4.2 (9.8)	.9
Eos after SIC (%), median (IQR)	0.5 (2.0)	16.3 (26.7)	<.01
Neu after SIC (%), median (IQR)	55.0 (36.9)	42.5 (39.8)	.1

CS, Current smoker; *D*, dual; *Eos*, sputum eosinophil count; *exS*, ex-smoker; *F*, female; *FVC*, forced vital capacity; *GM*, geometric mean; *HMW*, high molecular weight; *I*, immediate; *IQR*, interquartile range; *LMW*, low molecular weight; *M*, male; *Neu*, sputum neutrophil count; *L*, late; *NS*, never smoker; *SIC*, specific inhalation challenge; *TCC*, total cell count.

TABLE II. Characteristics of eosinophilic and noneosinophilic responders at the time of the study

	Change in eosinophil counts during SIC <2%	Change in eosinophil counts during SIC ≥2%	*P* value
n	15	29	
FEV_1 at follow-up (% predicted), mean ± SD	72.6 ± 23.0	89.0 ± 18.5	.04
FEV_1/FVC, mean ± SD	0.66 ± 0.1	0.77 ± 0.1	.001
PC_{20} at follow-up (mg/mL)	3.3 ± 5.5	11.9 ± 5.6	.04
TCC at follow-up (10^6 cells/g), n (%)	3.8 (3.2)	1.6 (3.1)	.1
Eos at follow-up, n (%)	0.7 (3.0)	1.0 (3.5)	.8
Neu at follow-up, n (%)	53.2 (46.5)	45.7 (47.8)	.4
FENO (ppb), mean ± SD	13.8 ± 7.5	15.9 ± 6.3	.3
ACQ at follow-up, mean ± SD	1.6 ± 1.3	1.01 ± 0.8	.07
AQLQ at follow-up, mean ± SD	5.5 ± 1.1	5.8 ± 1.1	.3
Treatment with ICS, n (%)	11 (73.3)	9 (31)	.01

ACQ, Asthma Control Questionnaire; *AQLQ*, Asthma Quality of Life Questionnaire; *Eos*, sputum eosinophil count; *FVC*, forced vital capacity; *FENO*, fractional concentration of exhaled nitric oxide; *ICS*, inhaled cortisosteroides; *Neu*, sputum neutrophil count; *TCC*, total cell count.

increase of greater than 2% (14.3 [2-69.3]%) (eosinophilic responders) in their sputum eosinophil count. Noneosinophilic responders had a slightly greater airflow limitation but did not show a greater airway hyperresponsiveness than did eosinophilic responders (Table I).

At the time of the study, noneosinophilic responders showed lower FEV_1, greater airflow limitation, and greater airway responsiveness, which were all of statistical significance, than did eosinophilic responders. They also had a poorer control of their asthma as shown by an Asthma Control Questionnaire score of greater than 0.5 than did eosinophilic responders, which indicates an important clinical difference that was close to statistical significance (Table II). Airflow limitation worsened in the noneosinophilic group (difference between FEV_1/forced vital capacity ratio at follow-up and at baseline = −0.03 ± 0.08) from diagnosis to the current study, whereas it remained stable in the eosinophilic group (0.01 ± 0.03) ($P = .01$) (see Fig E1 in this article's Online Repository at www.jacionline.org). There was also a greater decline in FEV_1 in the noneosnophilic group (−8.4% ± 12.1% predicted) than in the eosinophilic group (−3.1% ± 6.85% predicted).

A noneosinophilic response ($P = .04$) and a high inhaled corticosteroid dose ($P = .01$) were significantly associated with a greater progression of airflow limitation (see Table E1 in this article's Online Repository at www.jacionline.org).

Although they did not achieve statistical significance after Bonferroni's correction for multiple analysis, levels of IL-2, IFN-γ, and monocyte chemotactic protein-3 were higher in the eosinophilic responder group than in the noneosinophilic group (see Table E2 in this article's Online Repository at www.jacionline.org).

We believe that this study is the first to suggest that the type of airway inflammation induced by the exposure to an occupational agent may be associated with a different outcome of the disease.

Not only did the noneosinophilic responders with OA appeared to have a more severe asthma than did eosinophilic responders at the time of diagnosis, but they also had a poorer prognosis than did eosinophilic responders as shown by a greater airflow obstruction, a greater airway hyperresponsiveness, and a poorer asthma control in spite of a higher dose of inhaled corticosteroids 5 years after diagnosis.

The originality of our study was to assess the inflammatory phenotype in response to the exposure to a specific agent. Although a positive asthmatic reaction is usually associated with an eosinophilic inflammation in subjects with OA, a noneosinophilic response is not uncommon because 40% of our subjects with an asthmatic reaction had a noneosinophilic response at diagnosis. Although an eosinophilic response is identified in the majority of the cases of inhalation challenges using common allergens, there is evidence that eosinophilic or mast cells are not always involved in late asthmatic reactions.[9]

This study has limitations that should be noted. Our sample size was limited, which prevented us from studying the effect of the type of agent on airway inflammation. We did not collect supernatant at the time of the diagnosis and thus, the mediators measured in the study were not associated with the airway inflammation induced by the exposure to occupational agents, preventing us from exploring the mechanism at the time of exposure.

In conclusion, a noneosinophlic response associated with an asthmatic reaction occurring after exposure to an occupational agent seems associated with a poorer clinical outcome. Further studies are needed to assess the reproducibility of the noneosinophilic phenotypes and to determine whether alternative treatments can be offered to those subjects.

Catherine Lemiere, MD, MSc[a,b]
Simone Chaboillez, RT[a]
Abraham Bohadana, MD[a,c]
Lucie Blais, PhD[a,b]
Karim Maghni, PhD[a,b]

From [a]Centre de recherche de l'Hôpital du Sacré-Coeur de Montréal, Montreal, Quebec, Canada; [b]Université de Montréal, Montreal, Quebec, Canada; and [c]Unite Inserm 954, Vandoeuvre les Nancy, France. E-mail: catherine.lemiere@umontreal.ca.

This study was funded by the cooperative agreement number R03 OH009734 from the Centers for Disease Control and Prevention and by Institut de recherche Robert-Sauvé en santé et en sécurité au travail.

Disclosure of potential conflict of interest: C. Lemiere has consultancy arrangements with GlaxoSmithKline, Merck, and AstraZeneca; has received one or more grants from or has one or more grants pending with AllerGen and Aerocrine; has received one or more payments for lecturing from or is on the speakers' bureau for AstraZeneca and Merck; has received royalties from UpToDate; and has received one or more payments for the development of educational presentations for AstraZeneca. L. Blais has consultancy arrangements with GlaxoSmithKline, AstraZeneca, and Pfizer and has received one or more grants from or has one or more grants pending with GlaxoSmithKline, AstraZeneca, Novartis, Merck, and Pfizer. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18:716-25.

2. Juniper E, O'Byrne P, Guyatt G, Ferrie P, King D. Development and validation of a questionnaire to measure asthma control. Eur Respir J 1999;14:902-7.
3. Juniper EF, Svensson K, Mork AC, Stahl E. Modification of the asthma quality of life questionnaire (standardised) for patients 12 years and older. Health Qual Life Outcomes 2005;3:58.
4. Perrin L, Dechamp C, Deviller P, Joly P. Reproducibility of skin tests: a comparative study of the Pepys prick test and the Morrow-Brown needle and their correlation with the serum IgE level. Clin Allergy 1984;14:581-8.
5. Crapo R. Pulmonary-function testing. N Engl J Med 1994;331:25-30.
6. Juniper E, Cockcroft D, Hargreave F. Histamine and methacholine inhalation tests. 2nd ed. Lund, Sweden: Canadian Thoracic Society and Astra Draco AB; 1994.
7. Pin I, Gibson P, Kolendowicz F, Girgis-Gabardo A, Denburg J, Hargreave F, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992;47:25-9.
8. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912-30.
9. Ali FR, Kay AB, Larche M. Airway hyperresponsiveness and bronchial mucosal inflammation in T cell peptide-induced asthmatic reactions in atopic subjects. Thorax 2007;62:750-7.

Available online October 18, 2013.
http://dx.doi.org/10.1016/j.jaci.2013.09.005

Novel childhood asthma genes interact with *in utero* and early-life tobacco smoke exposure☆

To the Editor:

Complex diseases, including asthma, have genetic and environmental origins. Genome-wide association studies have identified multiple genes for the development of asthma, yet they only explain a limited proportion of asthma heritability. Interactions between genetic predisposition and exposure to passive smoking might explain in part the hidden heritability of childhood asthma. However, to date, this approach has not been reported for the discovery of interactions between genes and tobacco smoke exposure.

We performed a genome-wide interaction study (GWIS) on childhood asthma to identify genes that interact with 2 well-known environmental risk factors for childhood-onset asthma: *in utero* and childhood tobacco smoke exposure. We meta-analyzed interaction results from 9 studies participating in the GABRIEL consortium[1] including more than 6,000 subjects of European descent. We replicated our findings in 4 independent studies including more than 13,000 subjects. Childhood-onset asthma was defined as asthma diagnosed by a doctor before the age of 16 years, which is consistent with the definition in the GABRIEL consortium.[1] *In utero* tobacco smoke exposure was defined as "exposure to maternal tobacco smoking at any time during pregnancy." Childhood tobacco smoke exposure was defined as "exposure to passive tobacco smoking at any time from birth until 16 years of age." Details on the number of subjects, the design of the individual studies, and outcome and exposure definitions are provided in Tables E1 to E4 in this article's Online Repository at www.jacionline.org.

The effects of *in utero* tobacco smoke exposure and childhood tobacco smoke exposure were analyzed separately. All individual studies were analyzed by using a logistic regression model containing the genetic effect, the effect of tobacco smoke exposure, and an interaction term indicating the interaction between the genetic effect and tobacco smoke exposure. Further methodological considerations on GWISs and details on the statistical analyses are described in this article's Online Repository at www.jacionline.org.

For *in utero* tobacco smoke exposure, the discovery genome-wide meta-analysis consisted of 2,654 cases and 3,073 control subjects derived from 7 studies (see Table E1). Overall, *in utero* tobacco smoke exposure increased the risk of childhood-onset asthma (see Fig E1 in this article's Online Repository at www.jacionline.org). A total of 536,705 single nucleotide polymorphisms (SNPs) were included in the interaction meta-analysis. Fig E2 in this article's Online Repository at www.jacionline.org shows the Manhattan plot. We identified 27 SNPs in the discovery sample with a P value of less than 10^{-4} based on the fixed effect model (Table I and see Table E5 in this article's Online Repository at www.jacionline.org). Findings did not reach genome-wide significance but were consistent over all studies included, and no significant heterogeneity across studies was present (P value Q-statistic < .05). Four of these SNPs on chromosome 10 were in high linkage disequilibrium with each other in the discovery meta-analysis (r^2 = 0.82-0.96). The most prominent marker was located on chromosome 18 near *EPB41L3* (Forest plot, see Fig E3 in this article's Online Repository at www.jacionline.org). Table E6 in this article's Online Repository at www.jacionline.org shows the associations in exposed and nonexposed subjects. EPB41L3 belongs to the protein 4.1 family of membrane-associated proteins, is involved in cell-cell junctions,[2] and might play a role in apoptosis.[3] The literature shows that *in utero* tobacco smoke exposure affects the expression of genes involved in biological processes, such as cell proliferation and apoptosis, and influences lung development of the child in general.[4] Our data suggest that this effect of *in utero* smoke exposure might potentially occur through mechanisms involving *EPB41L3* (see the additional text in this article's Online Repository).

For childhood tobacco smoke exposure, the discovery genome-wide meta-analysis consisted of 3,048 cases and 3,509 control subjects derived from 9 studies (see Table E1). Overall, childhood tobacco smoke exposure increased the risk of childhood-onset asthma (see Fig E1). A total of 538,233 SNPs were included in the interaction meta-analysis. Fig E4 in this article's Online Repository at www.jacionline.org shows the Manhattan plot. We identified 35 SNPs in the discovery sample with a P value of less than 10^{-4} based on the fixed effect model. Four of these SNPs were excluded because they showed heterogeneity, and the P value of the random effect was greater than 10^{-4}. Findings did not reach genome-wide significance. Table II and Table E7 (see this article's Online Repository at www.jacionline.org) the results for the top SNPs. Seven SNPs on chromosome 5 (except rs2312164) were in high linkage disequilibrium with each other in the discovery studies (r^2 = 0.83-1.00).

The most prominent marker was located on chromosome 6 in *PACRG* (parkin coregulated gene; Forest plot, see Fig E5 in this article's Online Repository at www.jacionline.org). Table E8 in this article's Online Repository at www.jacionline.org shows the associations in exposed and nonexposed subjects. *PACRG* is located next to and has an overlapping promoter region with parkin 2 *(PARK2)*.[5] The gene has been associated with leprosy and parkinsonian diseases and has an important role in motile cilia function and cilia morphogenesis.[2,6] *PACRG* is relatively highly

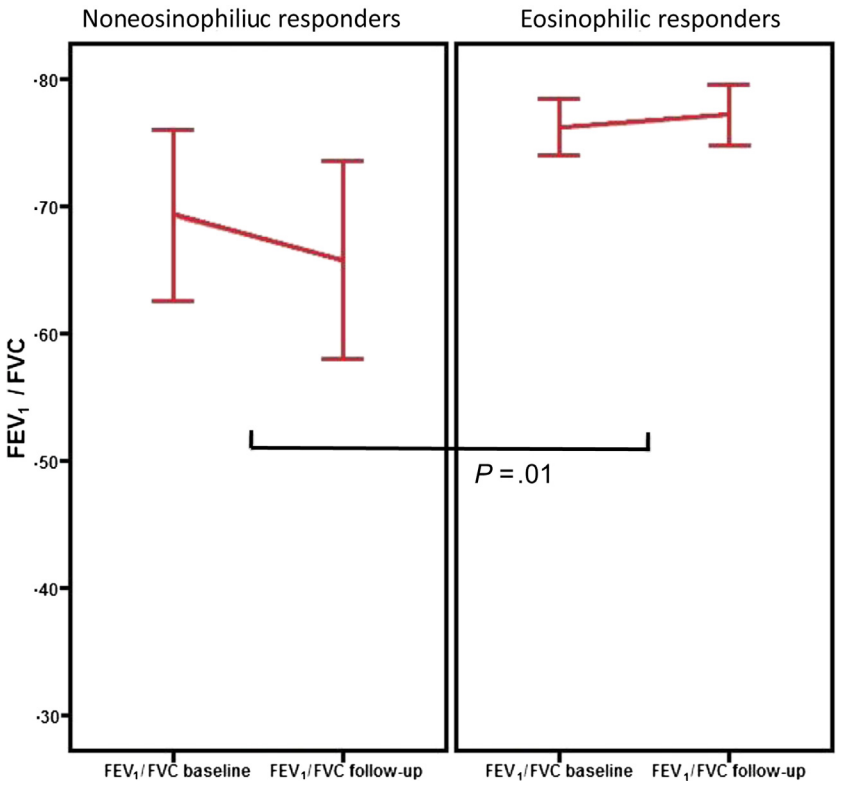

FIG E1. Outcome of FEV_1/FVC over time in eosinophilic and noneosinophilic responders. *FVC,* Forced vital capacity.

TABLE E1. Backward regression model assessing the determinants of the difference between FEV_1/FVC between diagnosis and at the time of the study

Dependent variable	β	SE (β)	*t*	*P* value
Eosinophilic response	0.034	0.02	2.1	.04
Inhaled corticosteroid dose (μg)	-4.4×10^{-5}	0.0	−2.5	.01

FVC, Forced vital capacity.

TABLE E2. Inflammatory markers in eosinophilic and noneosinophilic responders assessed at the time of the study

	Noneosinophilic responders (n = 15)	Eosinophilic responders (n = 29)	*P* value
MMP-1 (pg/mL)	78.2 ± 81.2	71.22 ± 75.3	.8
MMP-2 (pg/mL)	1,042.4 ± 1,246.7	832.9 ± 596.9	.5
MMP-3 (pg/mL)	197.1 ± 136.1	269.5 ± 292.9	.4
MMP-8 (pg/mL)	59,824.4 ± 2.1×10^4	58,346.6 ± 5.7×10^4	.9
MMP-9 (pg/mL)	1.4×10^5 ± 1.9×10^5	1.8×10^5 ± 2.6×10^5	.6
MMP-12 (pg/mL)	66.4 ± 33.9	91.9 ± 111.5	.4
TIMP-1 (ng/mL)	119.9 ± 65.6	124.3 ± 69.8	.8
MMP-9/TIMP-1	0.3 ± 0.4	0.5 ± 0.6	.4
IL-4 (pg/mL)	0 ± 0	0.3 ± 1.2	.3
IL-5 (pg/mL)	1.7 ± 3.6	4.0 ± 7.6	.3
IL-13 (pg/mL)	0 ± 0	0.02 ± 0.1	.4
IL-2 (pg/mL)	0.4 ± 0.4	1.0 ± 0.9	.02
IFN-γ (pg/mL)	0.2 ± 0.3	0.5 ± 0.5	.03
IL-8 (pg/mL)	415.9 ± 113.3	475.8 ± 435.2	.6
MCP-3 (pg/mL)	6.9 ± 3.7	10.4 ± 5.7	.04
ICTP (ng/mL)	0.04 ± 0.05	0.04 ± 0.04	.7
PICP (ng/mL)	16.2 ± 32.2	5.7 ± 7.3	.1

ICTP, Carboxyterminal telopeptide of type I collagen; *MCP-3*, monocyte chemotactic protein-3; *MMPs*, matrix metalloproteinases; *PICP*, procollagen type I C peptide; *TIMP-1*, tissue inhibitor of metalloproteinase-1.