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CHEST

Original Research

PULMONARY HYPERTENSION

Role for Interleukin-6 in COPD-Related Pulmonary Hypertension

Ari Chaouat, MD; Laurent Savale, MD; Christos Chouaid, MD; Ly Tu, BS; Benjamin Sztrymf, MD; Matthieu Canuet, MD; Bernard Maitre, MD; Bruno Housset, MD; Christian Brandt, MD; Philippe Le Corvoisier, MD; Emmanuel Weitzenblum, MD; Saadia Eddahibi, PhD; and Serge Adnot, MD

Background: **Pulmonary artery remodeling triggered by alveolar hypoxia is considered the main mechanism of pulmonary hypertension (PH) in COPD patients. We hypothesized that the risk for PH in COPD is increased by an elevation in the proinflammatory cytokines interleukin (IL)-6, monocyte chemoattractant protein-1 (MCP-1), and IL-1β, as well as by specific genetic polymorphisms of these cytokines.**

Methods: **We assessed cytokine plasma levels and the polymorphisms G(–174)C IL-6, C(−511)T IL-1β, and A(−2518)G MCP-1 in 148 COPD patients (recruited at two centers) with right heart catheterization data and 180 control subjects including smokers and nonsmokers. Human pulmonary artery smooth muscle cells (PA-SMCs) were cultured for IL-6 messenger RNA assays under normoxic and hypoxic conditions.**

Results: **Patients with PH (mean pulmonary artery pressure [PAP], ≥ 25 mm Hg) had lower Pao_2 and higher plasma IL-6 values than those without PH; there were no differences in terms of pulmonary function test results or CT scan emphysema scores. Plasma IL-6 correlated with mean PAP ($r = 0.39$; $p < 0.001$) and was included in a multiple stepwise regression analysis, with mean PAP as the dependent variable. In patients with the *IL-6* GG genotype, the mean PAP value was significantly higher and PH was more common than in CG or CC patients (adjusted odds ratio, 4.32; 95% confidence interval, 1.96 to 9.54). Exposure to 4 h of hypoxia led to an about twofold increase in IL-6 messenger RNA in cultured human PA-SMCs.**

Conclusions: **Inflammation, most likely involving IL-6, may contribute substantially to PH complicating COPD.** ***(CHEST 2009; 136:678–687)***

Abbreviations: aOR = adjusted odds ratio; BMI = body mass index; BODE = body mass index airflow obstruction dyspnea and exercise capacity index; CI = confidence interval; DLCO = diffusing capacity of the lung for carbon monoxide; 5-HTT = serotonin transporter; Hb = hemoglobin; HRCT = high-resolution CT; IL = interleukin; IQR = interquartile range; MCP-1 = monocyte chemoattractant protein-1; PAP = pulmonary artery pressure; PA-SMC = pulmonary artery smooth muscle cell; PH = pulmonary hypertension; PWP = pulmonary wedge pressure

COPD is becoming increasingly prevalent in most countries and is expected to become the third leading cause of death worldwide by 2020.[1] The development of pulmonary hypertension (PH), a well-known complication of COPD, is a major risk factor for hospitalization[2] and death.[3] Although COPD seems to be among the main causes of PH, the mechanisms underlying PH development in COPD patients are unclear.[4]

Mean pulmonary artery pressure (PAP) varies greatly across patients with COPD and fails to correlate closely with the severity of the respiratory tract disease. Factors suspected to influence pulmonary hemodynamics in COPD patients include lung parenchyma destruction with shrinkage of the pulmonary vascular bed and pulmonary artery vasoconstriction

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induced by alveolar hypoxia. However, the failure of oxygen therapy to reverse PH supports a key role for structural vascular changes in established PH complicating COPD.[5] Pathologic studies[6] of lung specimens from patients with COPD consistently showed extensive pulmonary vessel remodeling with prominent intimal thickening. The mechanisms responsi-

ble for these structural changes are incompletely understood. Chronic alveolar hypoxia may play a central role because the degree of PH usually parallels the severity of hypoxemia. However, pulmonary vascular remodeling was observed in lung specimens from patients with mild-to-moderate COPD who did not have chronic hypoxemia.[7] Because COPD is primarily an inflammatory disease,[1,8] and because inflammation may induce pulmonary vascular lesions in patients with COPD, a current hypothesis is that both alveolar hypoxia and inflammation contribute to pulmonary vascular remodeling, the extent or consequences of which may depend on individual genetic susceptibility. A previous study[9] showed that PH severity in patients with advanced COPD was closely linked to the polymorphism of the serotonin transporter (5-HTT) gene promoter, supporting a major role for pulmonary smooth-muscle hyperplasia in the development of PH complicating COPD.

The main objective of this study was to evaluate whether inflammation, and more specifically inflammatory cytokines, participated in the development of PH complicating COPD. We first evaluated consecutive patients with COPD recruited at two centers who underwent right heart catheterization, high-resolution CT (HRCT) scanning, and measurement of the diffusing capacity of the lung for carbon monoxide (DLCO). We also studied two groups of control subjects with normal lung function test results, one composed of smokers and the other of nonsmokers. In all three groups, we measured circulating levels of the inflammatory cytokines interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1, and IL-1β. Polymorphisms of the genes encoding IL-6, MCP-1, IL-1β, and 5-HTT were determined in patients and control subjects. Because these studies pointed to IL-6 as a potential contributor to PH, we performed complementary studies to assess *IL-6* expression in the lungs of patients with COPD and in cultured pulmonary artery smooth muscle cells (PA-SMCs) exposed to hypoxia. The preliminary results of this study[10] have been reported in abstract form.

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Materials and Methods

This study was approved by the review board of the Henri Mondor Teaching Hospital. All patients and control subjects signed an informed consent document before being included in the study.

Study Population

The study included 148 consecutive unrelated patients with COPD recruited at two French centers, in Strasbourg (n = 83) and Créteil (n = 65). The inclusion criteria were a history of smoking, a FEV_1/FVC ratio of $< 70\%$, and a Pao_2 of < 80 mm Hg. Pulmonary function testing consisted of spirometry, plethysmographic measurement of static lung volumes, and single-breath DLCO. Right heart catheterization was performed in all patients before initiating long-term oxygen therapy or any therapy for PH. There was no attempt to oversample or exclude patients with severe PH. The body mass index, airflow obstruction, dyspnea, and exercise capacity index (BODE) score was calculated according to Celli et al.[11] The visual emphysema score on lung HRCT scan images described by Park et al[12] was determined by one of us (M.C.), who was unaware of the clinical and pulmonary hemodynamic data. All patients were investigated at least 8 weeks after the end of the last acute COPD exacerbation. Study participation was refused by 34 patients, mainly because they were unwilling to undergo right heart catheterization. These 34 patients were not significantly different from the 148 participants in terms of lung function test results or arterial blood gas values (data not shown). We excluded patients with factors that might influence pulmonary hemodynamics such as left heart disease, acute or chronic pulmonary embolism, diseases known to be associated with pulmonary arterial hypertension (*eg*, portal hypertension or scleroderma), and chronic lung diseases other than COPD. Of the 148 patients, 91 had a complete set of measurements including all physiologic parameters (except DLCO), plasma cytokine assays, and genotype determinations (Fig 1).

The control groups comprised 132 nonsmokers (33 women) and 48 smokers (24 women). All control subjects were free of acute or chronic illness, except for mild systemic hypertension, and had FEV_1/FVC ratios of $> 70\%$.

Laboratory Investigations

Plasma samples for assaying IL-6, MCP-1, and IL-1β were obtained from all control subjects, and from 91 patients with

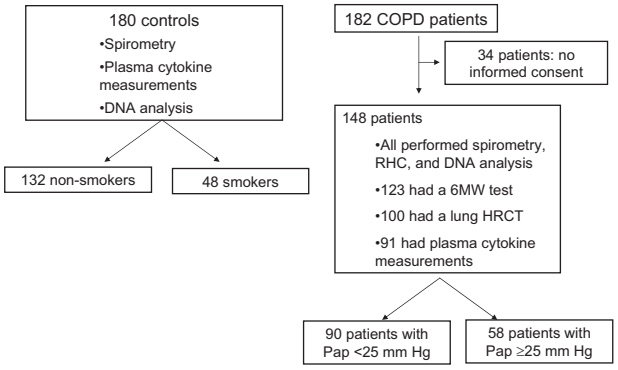

FIGURE 1. Study profile. RHC = right heart catheterization; 6MW = 6-min walk.

COPD. An enzyme-linked immunosorbent assay (R&D Systems; Lille, France) was used in duplicate to determine cytokine levels. Intraassay and interassay coefficients of variation for these cytokines were between 5% and 10%, and the mean recovery rate was > 90% in human plasma.

Genomic DNA was extracted from whole blood using a commercial kit (Qiagen; Hilden, Germany). The (−174G/C) *IL-6*, (−2518A/G) *MCP-1*, (−511C/T) *IL-1β*, and L/S *5-HTT* polymorphisms were detected as previously described[13–15] in all patients and control subjects. An enzyme-linked immunosorbent assay was used to determine tissue IL-6 levels in lung specimens from 15 patients undergoing lung surgery for localized tumors, including 9 patients who had mild COPD with an FEV_1/FVC ratio < 70%.

Human PA-SMCs were cultured from explants of pulmonary arteries (5×10^4 cells/well) as previously described.[13] After total RNA extraction, reverse transcription was performed (Biotech Ltd; Richmond, BC, Canada) in preparation for real-time quantitative polymerase chain reaction. To examine the effect of hypoxia on IL-6 messenger RNA expression, PA-SMCs were exposed to hypoxia (5% CO_2, 95% N_2) or normoxia (5% CO_2, 20% O_2, and 75% N_2) for 4 h. At the end of the procedure, IL-6 messenger RNA levels were determined in cell lysates.

Statistical Analysis

A statistical software package (SPSS, version 14.0; SPSS, Inc; Chicago, IL) was used for all statistical analyses. The data were expressed as the median and interquartile range (IQR). Because some comparisons involved small samples (n < 30), and several variables had nonnormal distributions, we mainly used nonparametric tests. When between-group comparisons using the Kruskal-Wallis test showed significant differences, we compared the groups using the Mann-Whitney *U* test. Correlation coefficients were calculated with the Spearman rank test. Multiple regression analysis with stepwise variable selection was performed, with mean PAP as the dependent variable. Because mean PAP and plasma cytokine levels had nonnormal distributions, these variables were log-transformed before the regression analysis. PH was defined according to the current standard definition as a mean PAP ≥ 25 mm Hg. Allele and genotype frequencies were compared using the χ^2 test, and the test for Hardy-Weinberg equilibrium was performed in each group. Phenotype-genotype associations were evaluated using the Mantel-Haenszel method. Odds ratios were adjusted for the potential confounding effects of PaO_2 and blood hemoglobin (Hb) level. A p value of < 0.05 was considered statistically significant.

RESULTS

Clinical Characteristics of the Study Population

Table 1 shows the characteristics of the 148 patients with COPD. Most patients were men, and the age range was 42 to 79 years. Current smokers made up 23% of the patient group; all the other patients were ex-smokers. Current smokers had less severe airway obstruction compared to ex-smokers (data not shown). Airflow limitation was moderate to severe. PH (mean PAP ≥ 25 mm Hg) was present in 58 patients (39%). The 57 patients with no available plasma samples for cytokine assays were not significantly different from the 91 other patients regarding age, body mass index (BMI), FEV_1, or mean PAP. PaO_2 was slightly lower in these 57 patients compare to the other patients (60 mm Hg [IQR, 53 to 65 mm Hg] vs 64 mm Hg [IQR, 58 to 70 mm Hg], respectively; p = 0.012). In control nonsmokers (n = 132) and control smokers (n = 48), the median age was 60 years (age range, 55 to 65 years) and 58 years (age range, 49 to 65 years), respectively; the FEV_1 was 99% predicted (IQR, 91 to 110% predicted) and 98% predicted (IQR, 86 to 105% predicted), respectively.

PH, Physiologic Lung Parameters, and HRCT Score in Patients With COPD

Mean PAP correlated significantly with PaO_2 ($r = -0.25$, p = 0.003) and Hb ($r = 0.23$, p = 0.005)

Table 1—*Characteristics of the 148 Patients With COPD*

Variables	Patients, No.	Median	IQR
Age, yr	148	64	56–71
Gender			
Female	30		
Male	118		
BMI, kg/m²	148	26	21–29
Smoking history, pack–yr	148	50	35–70
MRC dyspnea score	148	3	3–4
6–min walking distance, m	123	360	260–475
BODE index	123	4	3–6
FEV_1, % predicted	148	36	26–49
FVC, % predicted	148	61	50–78
FEV_1/FVC ratio, %	148	45	37–56
TLC, % pred	138	110	97–124
DLCO/VA ratio, % predicted	84	58	42–81
PaO_2, mm Hg	148	63	56–69
$PaCO_2$, mm Hg	148	44	40–49
Hb, g/dL	148	14.0	13.0–15.2
PAP, mm Hg	148	23	19–28
CT score	100	15	6–24

MRC = Medical Research Council; CT score = emphysema score by CT scan of the lungs; VA = alveolar volume; TLC = total lung capacity.

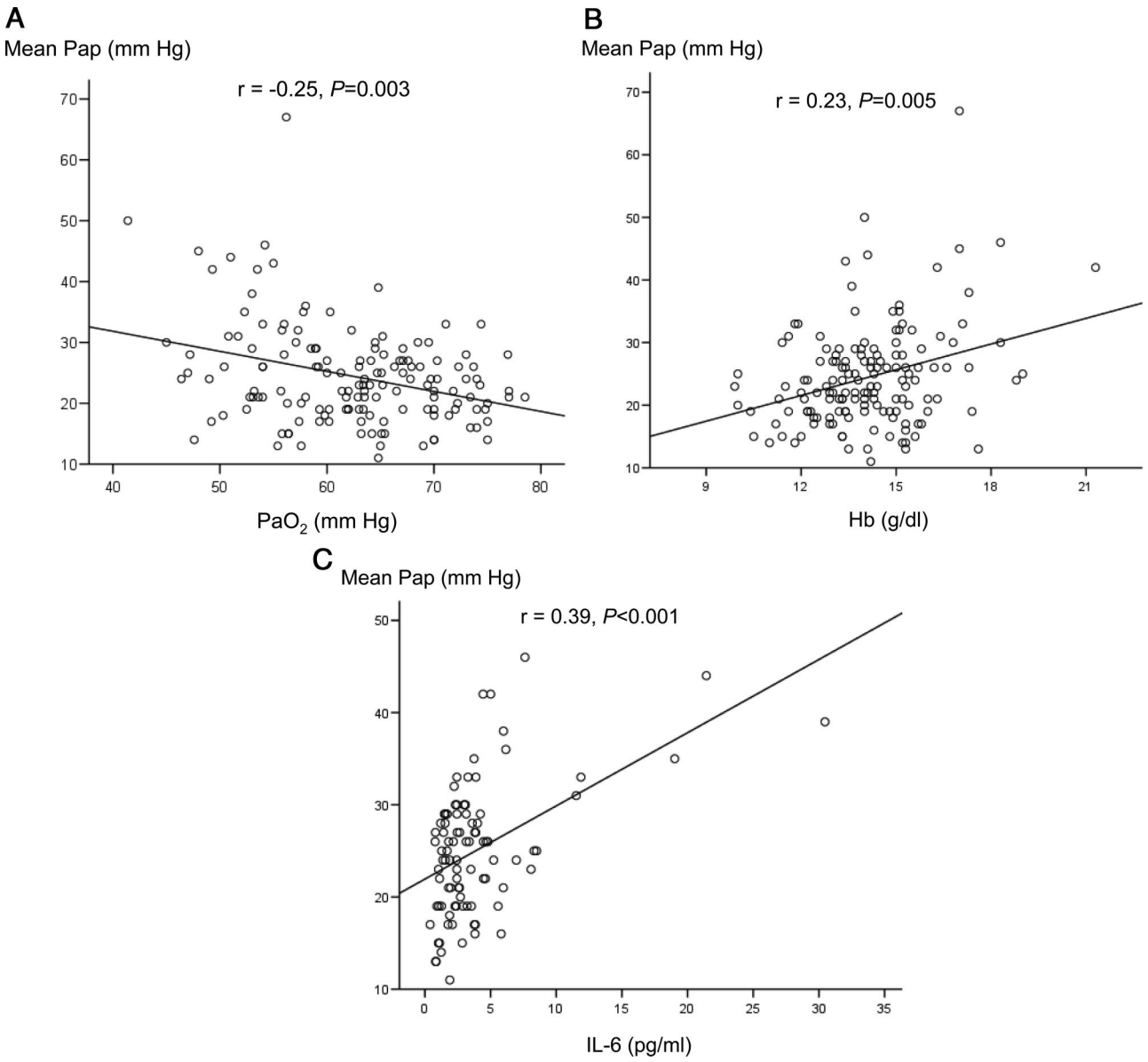

FIGURE 2. Relation between mean PAP and PaO_2 (*A*), Hb level (*B*), and IL-6 (*C*).

[Fig 2*A* and *B*, respectively). No correlations were found between mean PAP and FEV_1, FEV_1/FVC ratio, or total lung capacity. Among BODE components, neither the dyspnea score nor the 6-min walking distance correlated with mean PAP. The HRCT scan emphysema score and DLCO, two surrogates of lung and vessel destruction, correlated with each other ($r = -0.59$, $p < 0.001$) but not with mean PAP. Mean PAP values were similar in the four BODE score quartiles across the four subgroups (from the lowest to the highest BODE quartile, as follows: 21 mm Hg [IQR, 17 to 28 mm Hg]; 24 mm Hg [IQR, 19 to 27 mm Hg]; 24 mm Hg [IQR, 19 to 29 mm Hg]; and 22 mm Hg [IQR, 19 to 27 mm Hg]; $p = 0.730$).

When classified according to mean PAP levels, patients with PH (mean PAP, ≥ 25 mm Hg) had lower PaO_2 and higher Hb levels than patients without PH. No differences were found between the two groups for DLCO, the emphysema HRCT score, or other nonhemodynamic parameters (Table 2).

Circulating Cytokines in Patients With COPD and Control Subjects

In patients with COPD, plasma IL-6 levels correlated significantly with mean PAP ($r = 0.39$, $p < 0.001$) [Fig 2*C*]. MCP-1 and IL-1β levels were not correlated with mean PAP. Plasma IL-6 levels were higher in patients with PH than in

Table 2—*Comparisons of Physiologic Variables and Circulating Cytokine Levels According to the Absence or Presence of PH (PAP ≥ 25 mm Hg) in 148 Patients With COPD*

Variables	PAP < 25 mm Hg	PAP ≥ 25 mm Hg	p Value*
Age, yr	63 (56–71)	64 (56–69)	0.509
Gender			
Female	19	11	
Male	71	47	0.751†
BMI, kg/m²	25 (21–28)	26 (21–30)	0.219
Smoking history, pack–yr	50 (39–70)	45 (30–63)	0.199
MRC dyspnea score	3 (3–4)	4 (3–4)	0.302
6–min walking distance, m	370 (245–480) (n = 75)	360 (270–455) (n = 48)	0.825
BODE index	5 (3–6) (75)	4 (3–6) (48)	0.492
FEV_1, % predicted	35 (24–50)	37 (27–49)	0.621
DLCO/VA, % predicted	58 (41–82) (n = 50)	62 (43–79) (n = 34)	0.830
Pa_{O_2}, mm Hg	64 (59–70)	59 (54–67)	0.007‡
Pa_{CO_2},mm Hg	43 (39–48)	44 (41–44)	0.170
Hb, g/dL	13.6 (12.5–15.0)	14.5 (13.4–15.7)	0.002‡
PAP, mm Hg	21 (17–23)	29 (27–33)	
PWP, mm Hg	7 (5–12)	12 (9–14)	< 0.001‡
Cardiac index, L/min/m²	3.0 (2.5–3.5)	3.0 (2.6–3.3)	0.848
PVR, international units/m²	3.63 (3.01–5.36)	6.11 (4.41–8.25)	< 0.001‡
CT score	16 (8–25) (n = 65)	13 (4–22) (n = 35)	0.439
IL-6, pg/mL	2.4 (1.3–3.8) (n = 48)	3.3 (2.2–4.7) (n = 43)	0.023‡
MCP-1, pg/mL	551 (426–658) (n = 48)	549 (361–786) (n = 43)	0.862
IL-1β, pg/mL	0.68 (0.40–1.27) (n = 48)	0.65 (0.41–1.09) (n = 43)	0.642

Values are median and IQR; n = 90 patients with PAP < 25 mm Hg and n = 58 patients with PAP ≥ 25 mm Hg, unless otherwise indicated. PVR = pulmonary vascular resistance. See Table 1 for abbreviations not used in the text.
*Determined by Mann-Whitney *U* test, unless otherwise indicated.
†Determined by χ^2 test.
‡Significant.

patients without PH, and in control smokers than in control nonsmokers (Fig 3*A*). MCP-1 and IL-1β levels were significantly higher in patients than in control nonsmokers (Fig 3*B* and *C*, respectively) but did not differ between the two groups of patients with COPD classified according to mean PAP levels. In the stepwise multiple regression analysis with mean PAP as the dependent variable, pulmonary wedge pressure (PWP), Pa_{O_2}, and IL-6 plasma level were the only included covariates. They contributed to 49% of the variance of mean PAP (25% from PWP, 15% from Pa_{O_2}, and 9% from IL-6).

Associations Between Gene Polymorphisms and PH

The distributions of *IL-6* and *5-HTT* genotypes in patients and control subjects were in Hardy-Weinberg equilibrium. Plasma IL-6 levels in control smokers were related to the *IL-6* genotype: IL-6 levels were significantly higher in the GG group than in the CG and CC groups (median IL-6 levels: 2.1 pg/mL [IQR, 1.6 to 3.8 pg/mL]; 1.6 pg/mL [IQR, 0.9 to 2.1 pg/mL]; and 1.3 pg/mL [IQR, 1.1 to 1.7 pg/mL], respectively; p = 0.003). No significant differences in IL-6 levels were found across *IL-6* genotypes in patients with COPD (Table 3) or in control nonsmokers. In patients with the IL-6 GG genotype, mean PAP was significantly higher (Table 3, Fig 4*A*) and PH was more common than in CG or CC patients (adjusted odds ratio [aOR], 4.32; 95% confidence interval [CI], 1.96 to 9.54). Patients carrying the *5-HTT* LL genotype also had higher mean PAP values than LS or SS patients, and PH was more common (aOR for PH, 4.58; 95% CI, 2.00 to 10.47) [Fig 4*B*]. Homozygosity for both variants had an additive effect on the PH risk (aOR, 7.48; 95% CI, 2.40 to 23.31) [Fig 4*C*]. In an *a posteriori* analysis, aOR values for PH were significantly increased for the (−174G/C) *IL-6* and L/S *5-HTT* polymorphisms determined separately in each of the patient populations from the two recruiting centers. No significant differences in pulmonary hemodynamics were found across groups defined by the studied *MCP-1* and *IL-1β* polymorphisms or the plasma levels of these cytokines (data not shown).

Lung IL-6 Protein Levels in Patients With COPD and IL-6 Messenger RNA Levels in Cultured PA-SMCs

Of 15 patients undergoing lung resection surgery for localized tumors, 9 patients had COPD with a median FEV_1 of 65% predicted (IQR, 58 to 89% predicted) and a median FEV_1/FVC ratio of 60%

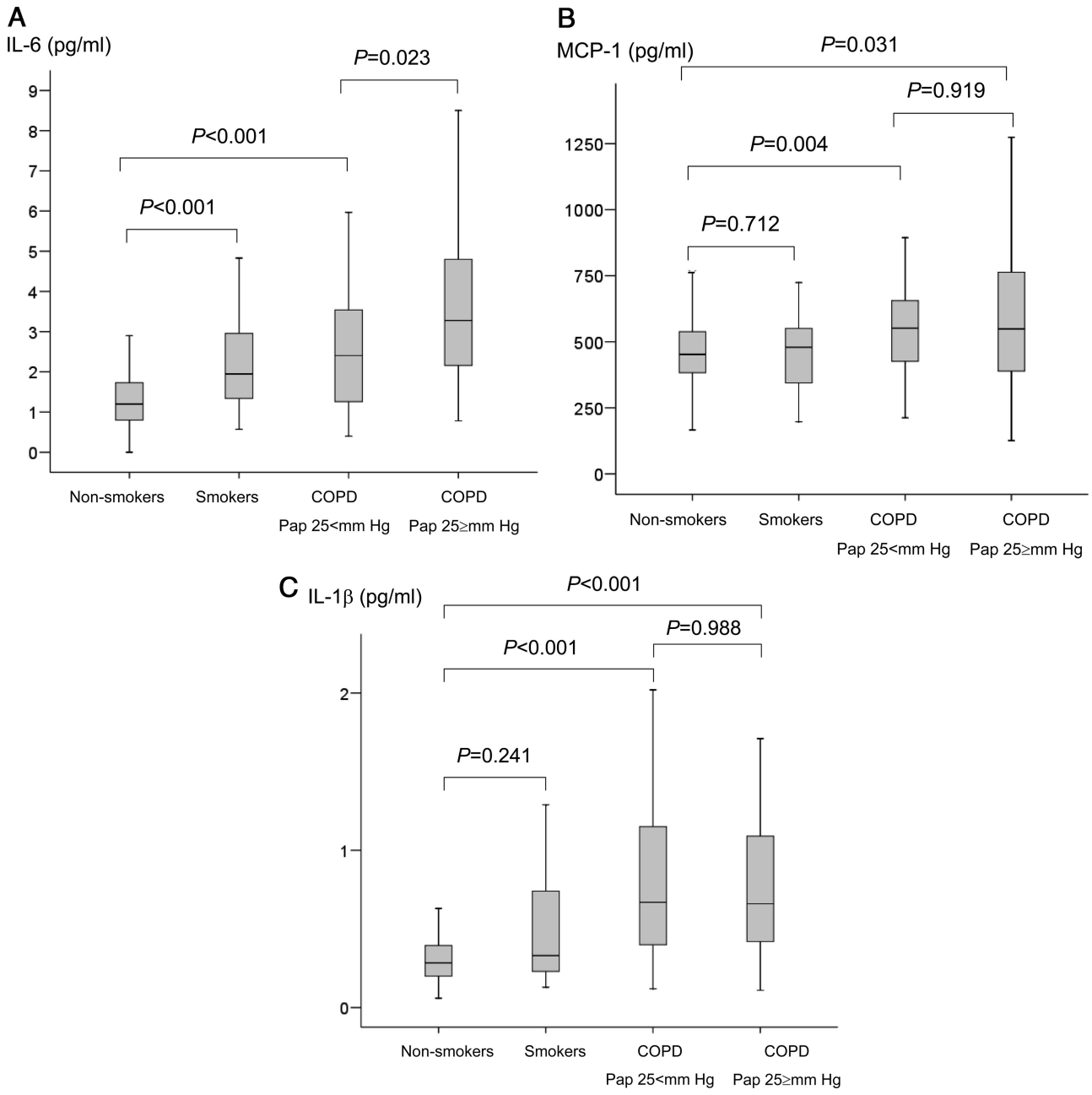

FIGURE 3. Plasma levels of IL-6 (*A*), MCP-1 (*B*), and IL-1β (*C*) in control smokers, control nonsmokers, and patients with COPD classified according to their mean PAP levels as having PH (PAP, ≥ 25 mm Hg) or not having PH (PAP, < 25 mm Hg). Plasma cytokine levels are expressed as the median, IQR, and extremes (defined as an interval of 3 IQRs).

(IQR, 53 to 68%); corresponding values in the six patients without COPD were 92% (IQR, 75 to 101%) and 75% (IQR, 73 to 77%), respectively. These 15 patients were all ex-smokers. Patients with COPD (n = 9) and without COPD (n = 6) had smoked for median duration of 37.5 pack-years (IQR, 15.0 to 60.0 pack-years) and 27.5 pack-years (IQR, 20.0 to 42.5 pack-years; $p > 0.5$), respectively. Compared to patients without COPD, patients with COPD had higher median lung IL-6 levels (82 pg/mg protein [IQR, 39 to 287 pg/mg protein] vs 37 pg/mg protein [IQR, 6.8 to 84 pg/mg protein]; $p = 0.019$). Exposure to 4 h of hypoxia led to an approximately twofold increase in IL-6 messenger RNA in cultured human PA-SMCs (Fig 5).

DISCUSSION

The results of the present study point to a role for inflammation, and more specifically for IL-6, in the

Table 3—*Comparisons of Physiologic Variables and Circulating Cytokine Levels According To CC, CG, and GG Genotypes of the* IL-6 *Gene in 148 Patients With COPD*

Variables	CC	CG	GG	p Value*
Gender, No.				0.345†
Female	6	14	10	
Male	14	50	54	
Age, yr	62 (54–73)	63 (56–70)	64 (56–72)	0.955
BMI, kg/m²	28 (22–32)	25 (21–30)	26 (21–29	0.303
FEV_1, % predicted	34 (27–49)	36 (24–51)	36 (26–47)	0.963
PaO_2, mm Hg	63 (58–74)	63 (56–69)	63 (56–68)	0.701
$PaCO_2$, mm Hg	44 (39–54)	43 (40–47)	45 (40–49)	0.454
Hb, g/dL	13.9 (12.6–15.1)	14.0 (13.0–15.2)	14.3 (13.0–15.3)	0.640
PAP, mm Hg	20 (19–24)‡	21 (17–26)§	26 (22–31)	< 0.001‖
PWP, mm Hg	12 (7–14)	9 (5–12)	10 (7–14)	0.231
Cardiac index, L/min/m²	3.3 (2.8–4.0)	3.0 (2.5–3.4)	2.9 (2.5–3.4)	0.144
PVR, international units/m²	3.18 (1.78–4.39)‡¶	4.31 (3.20–6.13)#	5.54 (4.21–6.70)	0.001‖
IL-6, pg/mL	3.1 (1.1–5.2)	2.6 (1.4–3.4)	3.1 (1.9–4.7)	0.289

Values are given as the median (IQR). See Tables 1 and 2 for abbreviations not used in the text.
*Determined by Kruskal-Wallis test, unless otherwise indicated.
†Determined by χ^2 test.
‡$p < 0.001$ (CC vs GG).
§$p < 0.001$ (CG vs GG).
‖Significant.
¶$p < 0.05$ (CC vs CG).
#$p < 0.01$ (CG vs GG).

pathogenesis of PH in patients with COPD. In patients with COPD, circulating IL-6 levels correlated with mean PAP. PAP was higher in patients who had the GG genotype of the G(−174)C IL-6 polymorphism than in patients who had the GC or CC genotype. Cultured human PA-SMCs constitutively expressed IL-6, and the expression level increased under hypoxia. Furthermore, the *IL-6* and *5-HTT* gene variants exerted additive effects on the risk of PH, leading to an aOR of 7.48 (95% CI, 2.40 to 23.31). Thus, two important conclusions from our study are that inflammation is probably an important trigger for PH development in patients with COPD, and that PH severity in patients with COPD may be related to individual genetic susceptibility.

A key issue addressed in this study was whether inflammation was involved in PH development in patients with COPD. Systemic inflammation is now recognized as a component of COPD.[1,16] Our data are consistent with this well-established concept because circulating levels of the proinflammatory cytokines IL-6, MCP-1, and IL-1β were higher in patients with COPD than in control nonsmokers (Fig 3). We chose to investigate these cytokines because basic studies have supported their involvement in PH,[17–19] and their blood levels were elevated in patients with idiopathic PH in a previous study.[20] In our population of patients with COPD, plasma IL-6 levels correlated with mean PAP and were higher in patients with PH than in patients without PH. These results are consistent with those of Joppa et al[21] showing increases in IL-6 and other proinflammatory mediators in a population of patients with COPD and a high prevalence of PH. In a histologic study[22,23] of patients with mild COPD, the pulmonary artery walls showed evidence of inflammation. Taken together, these studies support a role for inflammation in PH associated with COPD.

Cytokine gene polymorphisms have been shown to play a role in various inflammatory diseases, as well as in the development of atherosclerosis.[24] More specifically, the (−174C/G) *IL-6*, (−511C/T) *IL-1β*, and (−2518 A/G) *MCP-1* gene polymorphisms influence the inflammatory response.[14,15,25] In our study, genotypes resulting from polymorphisms of the genes encoding IL-6, IL-1β, or MCP-1 were equally distributed between patients with COPD and control subjects, indicating there was no relationship between these polymorphisms and COPD (data not shown). In contrast, PH severity in our patients with COPD was related to the *IL-6* GG polymorphism. Of note, the relationship between the *IL-6* GG genotype and PH severity was found in two separate populations of patients with COPD, who were recruited at two different centers in France. Interestingly, comparisons of CC, CG, and GG patients showed no differences regarding arterial blood gas variables, smoking history, or airflow limitation. The *IL-6* genotype was related to plasma IL-6 levels in the control smokers but not in the patients with COPD. The reason for the absence of a relationship in patients with COPD is unclear but may involve

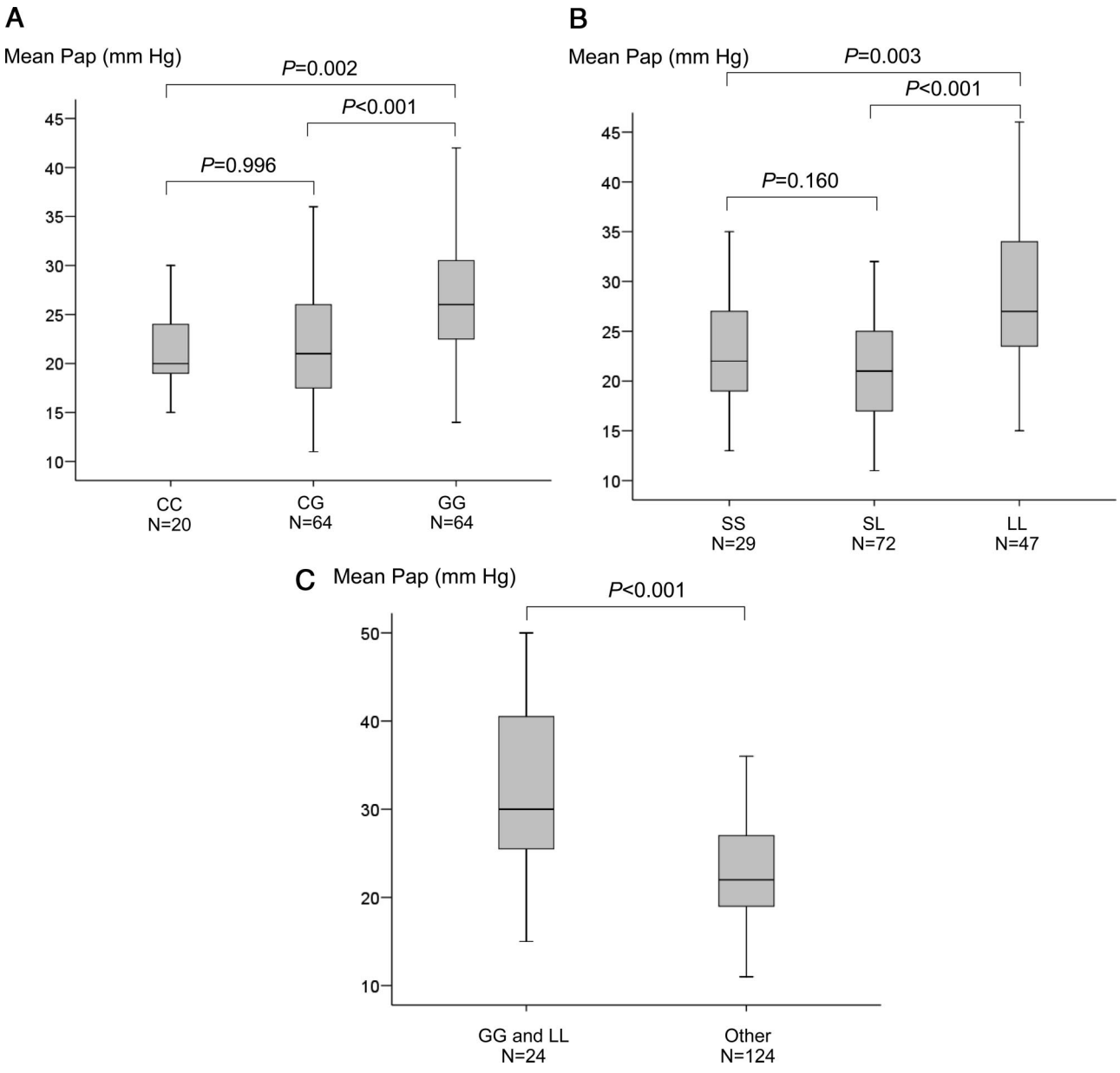

FIGURE 4. Mean PAP in 148 patients with COPD classified according to the *IL-6* (−174C/G) genotype (*A*) and to the *5-HTT* (−1444L/S) genotype (*B*). PAP in the 24 patients carrying *IL-6* GG and *5-HTT* LL compared to the other patients (*C*). PAP is expressed as the median, IQR, and extremes.

day-to-day variability in the clinical condition, which may have influenced cytokine levels. Plasma IL-6 levels are influenced by many factors, and confounders such as current smoking and possible modifier genes involved in the inflammation process may have influenced plasma IL-6 levels in our COPD patients.

A role for inflammation and, more specifically, for IL-6 in the pathogenesis of PH was suggested by previous studies.[17,20] A recent experimental study[19] confirms the hypothesis that IL-6 is involved in the process of pulmonary vascular remodeling. A role for IL-6 in PH is further supported by the excessive IL-6 release by cultured pulmonary artery cells from patients with idiopathic PH and by the involvement of IL-6 in experimental monocrotaline- and hypoxia-induced PH.[26] In the present study, IL-6 was increased in lungs of patients with COPD compared to patients without COPD undergoing surgery for lung tumors. This latter result must be interpreted with caution because PAP measurements were not available in this patient subgroup. Moreover, we cannot exclude that in this subgroup of patients the presence of lung tumor may have influenced lung IL-6 protein levels. However, we found that the expres-

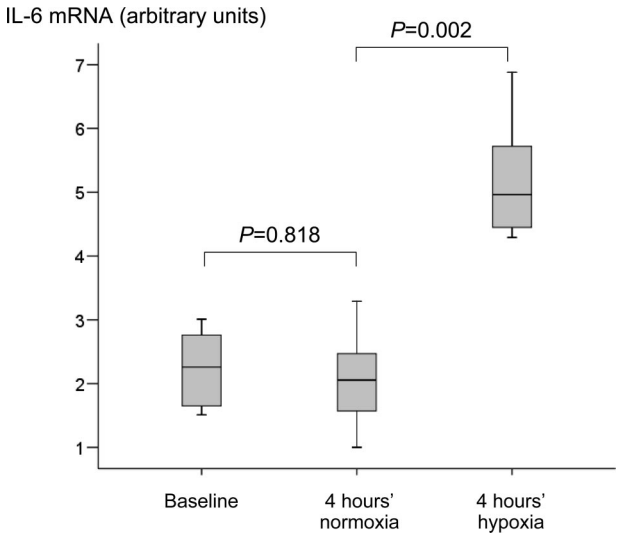

FIGURE 5. IL-6 messenger RNA levels in quiescent human PA-SMCs exposed to normoxia or hypoxia for 4 h. Each box plot shows the median, IQR, and extremes from six different experiments.

sion level of IL-6 was high in human PA-SMCs and increased further under hypoxia. One potential stimulus of IL-6 synthesis in COPD patients is hypoxia, and firm evidence[27] indicates that hypoxia can trigger IL-6 expression in various cell types. Our results are consistent with a role for IL-6 and hypoxia in the development of pulmonary vascular remodeling in COPD. Another link between IL-6 and hypoxia previously shown in animal models is that IL-6 may potentiate a pulmonary pressure response to alveolar hypoxia[17] and promote microarterial thrombosis in the lung.[28]

In previous studies of patients with COPD (including one by our group[9] and one by Ulrich et al[29]) and in the present study, PH severity was linked to the L/S 5-*HTT* polymorphism. The finding that both 5-*HTT* and *IL-6* gene polymorphisms may determine the occurrence of PH in patients with COPD has important pathogenic implications because one of these genes (5-*HTT*) is involved in pulmonary vascular remodeling and the other (*IL-6*) encodes a multifunctional cytokine involved in various inflammatory responses. Interestingly, both genes are overexpressed in response to hypoxia,[9,27] and 5-HTT expression is stimulated by inflammation and cytokines.[26]

The main limitation of the present study is the relatively small mean PAP difference between the *IL-6* GG patients and the CG and CC patients. Nevertheless, the persistence of the difference in an *a posteriori* analysis of the populations from each of the two centers supports the phenotype-genotype association. We also found that mean PAP correlated significantly with PWP, suggesting that left heart disease in patients with COPD may contribute to PH development. Another point is the relatively low value of the correlation coefficients between PAP and each covariate (IL-6, Pa_{O_2}, and Hb), suggesting weak associations. Because several factors independently contributed to increase PAP in our study, each factor might explain only part of the PAP variance. Moreover, other mechanisms not explored in the present study are probably involved, given that the multiple regression analysis explains only 49% of the mean PAP variance. Although our results do not establish that IL-6-mediated inflammation directly causes PH in COPD, we believe they constitute the first data on the role for inflammation in COPD-related PH diagnosed using right heart catheterization. Despite the limitations of our study, our results suggest that IL-6-mediated inflammation may be associated with PH development in patients with COPD.

An important point addressed in our patients with COPD was whether capillary loss due to emphysema contributed to PH development. We evaluated whether the HRCT scan emphysema score and DLCO, used as indexes of lung parenchyma destruction or pulmonary vascular bed reduction, were linked to PH severity. Neither variable correlated with mean PAP, although the two variables correlated with one another. Thus, although the HRCT scan emphysema score and DLCO seemed to reflect the functional or anatomic status of the lungs in our patients, they were not related to the presence or severity of PH. In contrast, hypoxemia severity and Hb correlated with mean PAP, as previously reported in many studies,[30–32] supporting a role for alveolar hypoxia and polycythemia in PH complicating COPD. The loss of pulmonary vessels in a cohort of COPD patients with severe emphysema may, however, be associated with higher mean PAP values, indicating greater PH severity.[33] Thus, important new findings provided by our study are that inflammation contributes to PH development in COPD patients and that individual genetic susceptibility may be an important determinant of PH development in patients with COPD.

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