Downmodulation of CXCL8/IL-8 receptors on neutrophils after recruitment in the airways

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Background: CXCL8/IL-8 is the most significant chemokine for neutrophils, and CXC chemokine receptor (CXCR) 1 and 2 are its 2 receptors, which are downmodulated by CXCL8/IL-8 and endotoxin on activated neutrophils.
Objective: We sought to evaluate the expression of the CXCL8/IL-8 receptors and the activation marker CD11b on neutrophils in peripheral blood and in the site of airway inflammation.
Methods: The flow cytometric expression of CXCR1, CXCR2, and CD11b was evaluated on peripheral blood and induced sputum neutrophils in patients with nonsevere asthma with greater than 60% sputum neutrophils, in patients with chronic obstructive pulmonary disease (COPD), and in healthy control subjects.
Results: Asthmatic patients and patients with COPD had comparable expressions of CXCR1, CXCR2, and CD11b on peripheral blood and sputum neutrophils. Compared with control subjects, the peripheral neutrophil expression of CXCR2 was lower in patients with COPD ($P = .03$) and that of CD11b was higher in asthmatic patients and patients with COPD ($P < .02$ and $P < .002$). The expression of the CXCL8/IL-8 receptors on sputum neutrophils was markedly lower than on peripheral blood neutrophils ($P < .0001$). The downmodulation of CXCL8/IL-8 receptors was also present in healthy control subjects but less than that seen in asthmatic patients. The difference between peripheral blood and sputum expression of CXCL8/IL-8 receptors correlated with serum CXCL8/IL-8 levels. In asthmatic patients the expression of CXCR1 and CXCR2 on sputum neutrophils negatively correlated with sputum neutrophils.
Conclusion: In neutrophilic asthma the expression of CXCL8/IL-8 receptors on peripheral and sputum neutrophils is similar to COPD and negatively correlated with the inflammatory infiltrate in the airways. The downmodulation of CXCL8/IL-8 receptors detected in the airways should be taken into account for an eventual therapeutic inhibition of these receptors.
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Key words: *Asthma, chronic obstructive pulmonary disease, neutrophils, CXC chemokine receptor 1, CXC chemokine receptor 2, CXCL8/IL-8*

Neutrophils play a key role in innate immunity, protecting individuals against infectious agents, and can also be responsible for significant damage when they accumulate in the site of inflammation, particularly in the airways. The activation of peripheral blood neutrophils results in their intravascular margination, adhesion to the endothelium, and migration to the site of inflammation. The last phase of this process is regulated by small chemotactic proteins called chemokines. Among the chemokines responsible for neutrophil chemotaxis, such as granulocyte chemotactic protein 2 (GCP-2), neutrophil-activating protein 2, epithelial cell–derived neutrophil attractan 78, and growth-related oncogene α-β-γ, CXCL8/IL-8 is the most significant and well-characterized chemotactic and activating factor for neutrophils.[1] The biologic activity of CXCL8/IL-8 is mediated by 2 specific membrane receptors: CXC chemokine receptor (CXCR) 1 and 2.[2] Because the chemokine system is often promiscuous, CXCR1 and CXCR2 are not CXCL8/IL-8–specific receptors, but all the neutrophil-chemotactic molecules bind to CXCR2, and only GCP-2 binds to CXCR1, although with lower affinity than CXCL8/IL-8.[3]

Asthma is a chronic airway disease characterized by infiltration of inflammatory cells into the airway lumen, particularly eosinophils. However, a great number of reports have recently described the infiltrate of neutrophils in the airways of asthmatic patients, shedding light on the role of these cells.[4-8] Subjects with neutrophilic asthma have higher sputum CXCL8/IL-8 levels compared with those of patients with eosinophilic asthma and control subjects.[5] Moreover, increased numbers of neutrophils have been reported in the lumen, the airway wall, and the lung interstitium of patients with corticosteroid-resistant asthma.[9] Neutrophil recruitment in the airways is a well-described and well-documented feature in patients with chronic obstructive pulmonary disease (COPD). Neutrophil accumulation is present on the mucosal surface of large airways, epithelium, and lumen of patients with COPD.

Recently, specific inhibitors of the CXCL8/IL-8 receptors, particularly CXCR2 inhibitors, underwent experiments designed to block the excessive neutrophil recruitment and accumulation in the airways.[10,11] Therefore the evaluation of the CXCL8/IL-8 receptor expression could be useful to the application of these new therapeutic strategies.

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Abbreviations used
COPD: Chronic obstructive pulmonary disease
CXCR: CXC chemokine receptor
DTT: Dithiothreitol
ECP: Eosinophil cationic protein
GCP-2: Granulocyte chemotactic protein 2
MFI: Mean fluorescence intensity
MPO: Myeloperoxidase
PE: Phycoerythrin

Taking into account the pivotal role of CXCL8/IL-8 in neutrophil recruitment and the increasing evidence on the role of neutrophils in a subgroup of asthmatic patients, we aimed to evaluate the *in vivo* expression of the CXCL8/IL-8 receptors CXCR1 and CXCR2 and the activation marker CD11b on peripheral and sputum neutrophils from patients with nonsevere asthma characterized by increased sputum neutrophils. These data were compared with those obtained from patients with COPD and with data from a control group of healthy subjects.

METHODS

Patients

Thirty-four subjects were enrolled in the study: 10 patients with intermittent to moderate-persistent asthma selected to have a percentage of sputum neutrophils greater than 60%, 13 patients with COPD, and 11 healthy control subjects (control group 1). The cutoff point of 60% was chosen because it was greater than the mean ± 2 SD of the normal range we use in our laboratory (27.3% ± 13.0%).[12] The characteristics of the subjects enrolled are reported in Table I. COPD and asthma were defined according to international guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD)[13] and the National Institutes of Health/World Health Organization,[14] respectively. All asthmatic patients had newly diagnosed disease, had never received corticosteroid medications, and had no history of upper respiratory tract infection within the previous 6 weeks. Patients were examined during stable conditions (ie, at least 4 months from the last exacerbations of the disease). Patients with COPD did not take inhaled or systemic steroids in the 4 weeks before the study.

Four pools of other nonsmoking subjects were composed of 2 subjects each who were subsequently enrolled and were analyzed to verify whether the downmodulation of the expression of CXCL8/IL-8 receptors on sputum neutrophils was a physiologic event (control group 2, Table I). Cells of control subjects were mixed in the pools maintaining the same proportion between cells derived from peripheral blood and from sputum. All patients provided their informed consent to extra blood drawing during routine venipuncture. This study conformed to the declaration of Helsinki and was approved by the Internal Review Board of the Fondazione Salvatore Maugeri.

Sputum collection and processing

Sputum induction was performed as previously described.[15] Briefly, FEV_1 was measured before and 10 minutes after inhalation of salbutamol (200 μg). Sputum was induced through the inhalation of an ultrasonically nebulized (De Vilbiss 65; De Vilbiss Co, Somerset, Pa) hypertonic (4.5%) saline solution.

Sputum processing was performed with 0.1% dithiothreitol (DTT), according to what was previously reported and to international guidelines.[15,16] Cells were separated from supernatant by means of centrifugation at 300*g* for 5 minutes and diluted in 1% BSA-PBS. Cell count and viability on the basis of trypan blue exclusion were determined with optical microscopy. Cytospin preparations were stained with Diff-Quick (Dade Diagnostika GmbH, Unterscheiheim) and analyzed for differential cell counts. Only sputum samples with less than 30% squamous cells were considered acceptable.

Soluble mediators

CXCL8/IL-8 levels were determined by using a commercially available immunoassay according to the instructions provided by the manufacturer (Endogen, Woburn, Mass). The detection limit of the assay was 3.7 pg/mL of recombinant human CXCL8/IL-8. Eosinophil cationic protein (ECP) levels were determined by using CAP-FEIA (Pharmacia, Uppsala, Sweden), and myeloperoxidase (MPO) levels were determined by using RIA (Pharmacia). The detection limit was 2 μg/L for ECP and 8 μg/L for MPO.

The method for the detection of the sputum-soluble mediators considered in the study was previously validated according to international guidelines.[16]

Expression of CXCR1, CXCR2, and CD11b and the effect of DTT on these cell markers

EDTA-anticoagulated peripheral blood and induced sputum cells were stained with CD16 FITC mAb (Pharmingen, Becton Dickinson, San Jose, Calif) and CXCR1 phycoerythrin (PE), CXCR2 PE, or CD11b PE mAbs (Pharmingen). Cells were acquired with a flow cytometer (FacScan, Becton Dickinson), and data were analyzed with CellQuest software (Becton Dickinson). Sputum cell viability was assessed with 5 μg/mL propidium iodide (Sigma, St Louis, Mo) just before the acquisition step. We chose to stain neutrophils with CD16 to discriminate, in the morphologic gate of granulocytes (forward scatter–side scatter), between neutrophils (CD16 positive) and eosinophils (CD16 negative). CD16 is also expressed by sputum macrophages, and in this case the selection of sputum neutrophils was allowed by the different morphologic characteristics of the cells (forward scatter–side scatter).

Delta (Δ) CXCR1 and CXCR2 was calculated as the difference of the receptor expression between peripheral blood and sputum neutrophils.

To evaluate the effect of DTT on the detection of CXCR1, CXCR2, and CD11b, we incubated 50 μL of peripheral blood at 37°C for 15 minutes in the presence or absence of DTT at the same dilution used for sputum samples. Then we washed out the DTT, and we followed the standard steps of the procedure to determine CXCR1, CXCR2, and CD11b expression.

Statistical analysis

Soluble mediator levels and cell counts were expressed as medians and interquartile ranges. The other results were expressed as means ± SD. Data were analyzed by using the Mann-Whitney *U* test, and the comparison between control subjects and patients regarding serum soluble mediators and the peripheral expression of CXCL8/IL-8 receptors was performed considering control group 1. The pool of healthy subjects (control group 2) was considered only for the evaluation of CXCL8/IL-8 receptor sputum downmodulation. The correlation among the CXCL8/IL-8 receptor expression and sputum cells and soluble mediators was assessed by using the Spearman rank test. *P* values of less than .05 were considered significant. Analysis

TABLE I. Characteristics of the patients

	Asthma: Peripheral blood and induced sputum (n = 10)	COPD: Peripheral blood and induced sputum (n = 13)	Control group 1: Peripheral blood (n = 11)	Control pools group 2: Peripheral blood and induced sputum (n = 4, 2 subjects each)
Age, y (range)	43.4 (15-66)	70.3 (57-82)	36.1 (26-48)	45 (30-64)
Sex (M/F)	6/4	12/1	4/7	3/5
Atopy (yes/no)	6/4	ND	ND	ND
FEV_1 (% predicted)	88.6 ± 21.4	55.1 ± 14.6	109 ± 12.5	89.0 ± 8.5
FEV_1/FVC	85.3 ± 10.9	53.3 ± 14.8	91.4 ± 6.1	103.0 ± 4.2
Po_2	77.6 ± 11.9	67.1 ± 10.9	ND	ND
Pco_2	36.1 ± 2.8	40.5 ± 4.9	ND	ND
Smoking (yes/exsmoker/no)	0/2/8	8/5/0	0/2/9	0/0/8
Packs/y*	0	68.0 ± 33.6	0	0

ND, Not done; *FVC*, forced vital capacity.
*Currently smoking subjects.

TABLE II. Soluble inflammatory mediators

		Control subjects (group 1), n = 11	Asthma, n = 10	COPD, n = 13
Serum	CXCL8/IL-8 (pg/mL)	3.0 (39.0)	4.9 (9.2)‡	16.0 (32.6)*‡
	MPO (μg/L)	348 (522)	409 (180)‡	634 (350)*†‡
	ECP (μg/L)	11.5 (47.5)	29.0 (37.9)*‡	29.2 (20.5)*‡
Sputum	CXCL8/IL-8 (pg/mL)	ND	2250 (16,950)	7000 (5000)
	MPO (μg/L)	ND	4438 (11,602)	6475 (4144)
	ECP (μg/L)	ND	506.1 (630)	557.5 (508.2)

Data are represented as medians (interquartile ranges).
*$P < .05$ versus control subjects.
†$P < .05$ versus asthma.
‡$P < .05$ versus sputum levels.

was performed with Statistica for Window software Release 4.5 (Stat Soft, Inc, Tulsa, Okla).

RESULTS

Soluble mediator levels in asthmatic patients and patients with COPD

To confirm that the asthmatic patients selected had prominent neutrophilic inflammation and eosinophilic inflammation comparable with that of patients with COPD, we evaluated CXCL8/IL-8, MPO, and ECP levels in serum and sputum supernatant. As reported in Table II, serum CXCL8/IL-8 and ECP levels were comparable between the 2 groups of patients, and serum MPO levels were lower in asthmatic patients than in patients with COPD. Compared with healthy control subjects, serum ECP levels were higher in asthmatic patients and in patients with COPD, whereas in patients with COPD, MPO and CXCL8/IL-8 levels were also higher than in healthy control subjects (Table II). The only statistically significant difference between asthmatic patients and patients with COPD was in serum MPO levels (Table II).

Sputum CXCL8/IL-8, MPO, and ECP levels were comparable in asthmatic patients and patients with COPD and higher than in peripheral blood samples (Table II). Taken together, these data confirmed that the asthmatic patients selected in this study had prevalent neutrophilic inflammation in the airways.

CXCR1, CXCR2, and CD11b expression on peripheral blood neutrophils

No difference was detected in the percentage of CXCR1 and CXCR2 expression on peripheral blood neutrophils among asthmatic patients, patients with COPD, and healthy control subjects (>99% of neutrophils were CXCR1 and CXCR2 positive). However, the mean fluorescence intensity (MFI) of CXCR2 expression on neutrophils, which represents the amount of molecules expressed on the cell membrane, was comparable between patients with COPD and asthmatic patients and lower only for patients with COPD than for control subjects (732 ± 158 for asthmatic patients, 689 ± 215 for patients with COPD, and 862 ± 147 for control subjects; $P = .03$; Fig 1, *A*). The MFI of CXCR1 was comparable in asthmatic patients and patients with COPD and tended to be lower in patients with COPD compared with control subjects, but the differences did not reach statistical significance (665 ± 273 for asthmatic patients, 611 ± 172 for patients with COPD, and 678 ± 210 for control subjects; Fig 1, *A*).

The MFI of CD11b expression on neutrophils was higher both in asthmatic patients and patients with COPD compared with control subjects (21.8 ± 6.7 for asthmatic

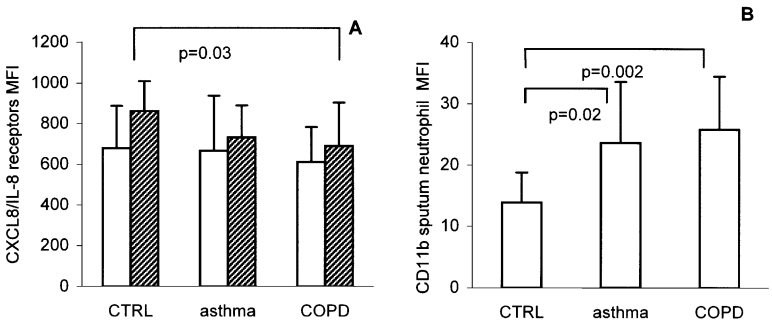

FIG 1. Expression of the CXCL8/IL-8 receptors CXCR1 *(open bars)* and CXCR2 (*shaded bars*; **A**) and CD11b **(B)** on peripheral blood neutrophils from healthy control subjects, asthmatic patients, and patients with COPD. Data are shown as means ± SD of the MFI.

patients, 20.0 ± 6.3 for patients with COPD, and 13.9 ± 4.9 for control subjects; $P < .03$ and $P < .002$; Fig 1, *B*).

Induced sputum cells and expression of CXCR1, CXCR2, and CD11b on sputum neutrophils

The differential cell count evaluated in the induced sputum samples showed that the asthmatic patients, although selected to have a high amount of sputum neutrophils (range, 63.0% to 89.4%), tended to have a lower amount of neutrophils compared with patients with COPD, but the difference was not statistically significant. A percentage of sputum eosinophils greater than 3% was found in 4 of 10 asthmatic patients and in 4 of 13 patients with COPD (Table III).

When we evaluated neutrophil activation through the expression of CXCR1, CXCR2, and CD11b on sputum neutrophils, we found that the expression of the membrane markers considered was comparable in asthmatic patients and patients with COPD (Fig 2), and for CXCL8/IL-8, receptors were markedly lower on sputum neutrophils than on peripheral blood neutrophils both in asthmatic patients and in patients with COPD ($P < .00001$, Fig 2). Furthermore, the expression of CXCR1 on sputum neutrophils correlated with the expression on peripheral blood neutrophils ($r = 0.43$, $P = .037$).

Because sputum samples were treated with DTT to release cells from mucous plugs, to verify whether the differences in the CXCL8/IL-8 receptor expression between blood and sputum could be due to a detrimental effect of DTT on the evaluation of these markers, we incubated peripheral blood in the absence or presence of DTT at the same dilution used in sputum processing. Results obtained showed that DTT had no effect on the expression of CXCR1 and slightly but not significantly reduced CXCR2 expression and increased CD11b expression (Fig 3). Taken together, these data showed that the difference between peripheral blood and sputum in the expression of the CXCL8/IL-8 receptors was not primarily caused by the effect of DTT but likely caused by the downmodulation of the receptors after neutrophil activation.

TABLE III. Total cells, viability, and differential cell count in induced sputum obtained from asthmatic patients and patients with COPD

	Asthma (n = 10)	COPD (n = 13)
Viability (%)	83.7 (20.5)	88 (15)
Macrophages (%)	17.0 (12.8)	9.0 (6.9)
Neutrophils (%)	73.0 (11.2)	85.7 (8.7)
Eosinophils (%)	2.5 (3.6)	2.7 (2.8)
Lymphocytes (%)	1.4 (1.0)	0.7 (1.95)
Epithelial cells (%)	1.9 (3.4)	0.7 (2.05)
Total cells/mg	22,950 (35,180)	23,950 (23,680)
Macrophages/mg	2197 (4393)	2273 (1870)
Neutrophils/mg	16,066 (26,739)	20,526 (19,886)
Eosinophils/mg	414 (2200)	259 (262)
Lymphocytes/mg	522 (620)	104 (428)
Epithelial cells/mg	506 (679)	106 (277)

Data are expressed as medians (interquartile ranges). The percentage of squamous cells was 0.9 (1.3).

Correlation among CXCR1 and CXCR2 expression on peripheral neutrophils and sputum cells

In asthmatic patients the expression of CXCR2 on peripheral neutrophils negatively correlated with the amount of total sputum cells and with sputum neutrophils (Table IV). Considering patients with COPD, the expression of CXCR1 and CXCR2 on peripheral neutrophils also negatively correlated with sputum neutrophils per milligram and with sputum lymphocytes, respectively (Table IV).

Correlation among CXCR1 and CXCR2 expression on sputum neutrophils and sputum cells and soluble mediators

Considering asthmatic patients, both CXCR1 and CXCR2 expression on sputum neutrophils negatively correlated with the amount of sputum neutrophils per milligram ($r = -0.74$, $P = .013$ and $r = -0.71$, $P = .02$, respectively) and with sputum MPO levels ($r = -0.75$, $P = .04$ for CXCR1 and $r = -0.82$, $P = .023$ for CXCR2),

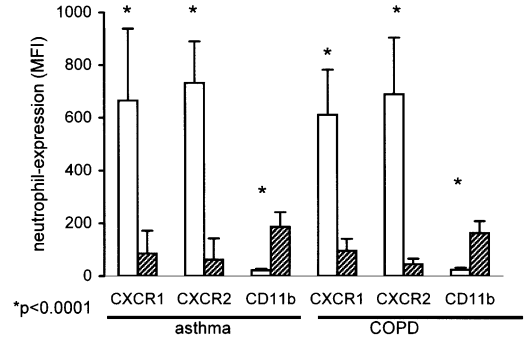

FIG 2. Expression of CXCR1, CXCR2, and CD11b on peripheral blood *(open bars)* and sputum neutrophils *(shaded bars)* in asthmatic patients and patients with COPD. Data are expressed as means ± SD of the MFI.

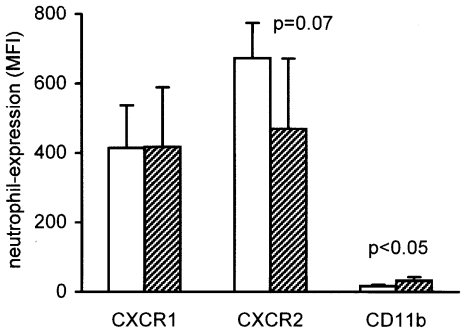

FIG 3. Effect of DTT on the expression of CXCR1, CXCR2, and CD11b evaluated on peripheral blood neutrophils. Cells were incubated in the absence *(open bars)* or presence *(shaded bars)* of DTT. Data are expressed as means ± SD of the MFI.

TABLE IV. Correlation among CXCR1, CXCR2, and CD11b expression on peripheral blood neutrophils and sputum cells in asthmatic patients and patients with COPD

Peripheral blood neutrophils			*P* value	*r* value
Asthma (n = 10)	CXCR2 (MFI)	Sputum cells/mg	.037	−0.66
		Sputum neutrophils/mg	.038	−0.66
COPD (n = 13)	CXCR1 (MFI)	Sputum cells/mg	.004	−0.64
		Sputum neutrophils/mg	.009	−0.59
	CXCR2 (MFI)	Sputum lymphocytes/mg	.0003	−0.75

suggesting that the expression on sputum neutrophils of CXCR1 and CXCR2 negatively correlated with the inflammatory infiltrate in the airways. No significant correlation among CXCL8/IL-8 receptors on sputum neutrophils and sputum cells was observed in patients with COPD.

The difference between peripheral blood and sputum expression of CXCL8/IL-8 receptors (Δ MFI) correlated with serum IL-8 levels ($r = 0.61$, $P = .002$ for Δ CXCR1 and $r = 0.50$, $P = .01$ for Δ CXCR2).

Expression of CXCL8/IL-8 receptors on sputum neutrophils in healthy control subjects

To verify whether the downmodulation of the CXCL8/IL-8 receptors on sputum neutrophils observed in asthmatic patients and in patients with COPD could be a physiologic event caused by neutrophil extravasation, we analyzed 4 pools obtained from the cells of healthy subjects. As shown in Fig 4, the downmodulation of CXCR1 and CXCR2 on sputum neutrophils also occurred in healthy control subjects, but the downmodulation of CXCL8/IL-8 receptors evaluated as the difference between peripheral blood and sputum expression (Δ MFI) appeared to be greater in asthmatic patients than in healthy control subjects (Fig 4).

DISCUSSION

In this study we analyzed and compared the *in vivo* membrane expression of CXCL8/IL-8 receptors and CD11b, a marker of neutrophil activation, on peripheral blood and sputum neutrophils in asthmatic patients with a high amount of neutrophils in the airways and in patients with COPD, for whom there is a great body of evidence in the literature reporting activated airway neutrophils. These data were compared with those obtained in healthy control subjects. To the best of our knowledge, this is the first study evaluating and comparing the *in vivo* expression of CXCL8/IL-8 receptors on peripheral and airway neutrophils.

We found that peripheral blood and sputum expression of CXCR1 and CXCR2 was comparable between asthmatic patients and patients with COPD, and the expression on sputum neutrophils correlated with the expression on peripheral blood neutrophils (particularly for CXCR1) and was markedly lower than that on peripheral blood neutrophils in both groups of patients. CXCR2 expression on peripheral neutrophils negatively correlated with sputum inflammatory infiltrate in asthmatic patients and in patients with COPD, and in asthmatic patients the expression of CXCR1 and CXCR2 on sputum neutrophils also negatively correlated with the amount of sputum inflammatory cells. The downmodulation of CXCL8/IL-8 receptors was also present on sputum neutrophils of a pool of healthy control subjects, but the magnitude of the downmodulation appeared higher in asthmatic patients than in healthy control subjects.

The downmodulation of CXCR1 and CXCR2 expression that we found associated with the neutrophil recruitment in the airways could account for different causes. First is the high CXCL8/IL-8 level detected in these patients. It has been reported that CXCL8/IL-8

induces the internalization of both CXCL8/IL-8 receptors, with a restored CXCR1 expression after the stimulation and only a partially restored expression of CXCR2.[17,18] Second is the possible presence in the induced sputum of endotoxin and TNF-α, which have been reported to downmodulate CXCL8/IL-8 receptors.[19,20] Furthermore, it was recently demonstrated that the LPS activation of Toll-like receptor 2, part of a family of molecules called the Toll-like receptors involved in the recognition of microorganisms, induces the downregulation of CXCR2.[21] Third is the effect of DTT used in sputum processing. We have demonstrated that DTT had no effect on CXCR1 expression and slightly decreased CXCR2 expression, but it could not be considered the main responsible factor for the marked decrease of CXCL8/IL-8 receptors expression in sputum.

According to data in the literature reporting a decrease in CXCR1 and CXCR2 expression *in vitro* associated with neutrophil activation[17-19,21] and *in vivo* in sepsis,[20] we demonstrated that in asthmatic patients the peripheral blood and sputum expression of the CXCL8/IL-8 receptors negatively correlated with the inflammatory infiltrate in the airways (neutrophils), that the expression on sputum neutrophils correlated with the expression on peripheral neutrophils (particularly for CXCR1), and that the difference between peripheral and sputum expression correlated with serum CXCL8/IL-8 levels. Taken together, these observations suggest that the decrease in the expression of these cell markers reflects the state of airway inflammation and that there is a strict correlation between airway and peripheral blood inflammation. Recently, an increase in mRNA of CXCR1 and CXCR2 has been described in the bronchial biopsy specimens of patients with COPD with an exacerbation of the disease[22] and in the nasal mucosa of patients with active allergic rhinitis.[23] These data seem to be in contrast with our results, showing a downmodulation of the membrane expression of CXCL8/IL-8 receptors in the airways after activation. This discrepancy could be explained by different mechanisms of regulation between mRNA and cell-surface protein expression because the expression of several molecules can be downmodulated by the intracytoplasmic degradation of their mRNA.

In our study we also found an increase of CD11b expression on sputum compared with peripheral blood neutrophils that could be partly due to the physiologic overexpression of this molecule after extravasation[24] and partly due to the DTT effect, which we have demonstrated and which has previously been reported by Qiu and Tan.[25]

Neutrophils are present in high amounts in the airways of asthmatic patients in different conditions, such as severe asthma or corticosteroid-resistant asthma,[9] but also in mild-to-moderate persistent asthma[5] or as a consequence of viral-microbial infections or secondary to their smoking history. A recent overview of published data has shown that, at most, only 50% of asthma cases are attributable to eosinophilic airway inflammation, and it is hypothesized that a great proportion of asthma is based on neutrophilic inflammation.[26]

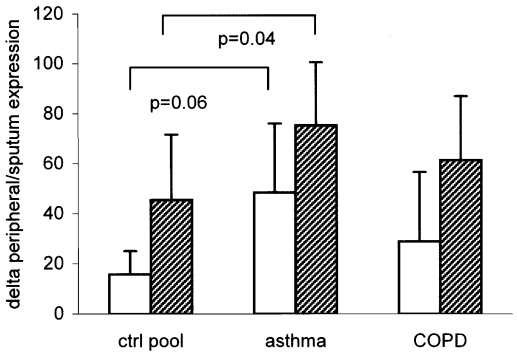

FIG 4. Difference (Δ) between peripheral blood and sputum expression of CXCR1 *(open bars)* and CXCR2 *(shaded bars)* on neutrophils from asthmatic patients and patients with COPD and from pools of healthy subjects (control group 2). Data are expressed as means ± SD of the percentage of expression.

The neutrophil accumulation in the airways of our patients with mild-to-moderate asthma appeared not to be caused by viral infections or a consequence of smoking because no patient was a current smoker. However, independently from the cause of neutrophil recruitment, the presence of a high amount of dangerous cells, such as neutrophils, in the airways could worsen the patient's condition. Moreover, the evaluation of neutrophil inflammation and the state of activation of these cells in asthmatic patients could be noteworthy for therapy and follow-up, considering that glucocorticoids are less effective in attenuating allergen-induced airway inflammation in subjects with high amounts of neutrophils.[27] Neutrophil recruitment is driven not only by CXCL8/IL-8 but also by other chemotactic factors, such as GCP-2, epithelial cell–derived neutrophil attractan 78, and growth-related oncogene α-β-γ, and thus the inhibition of a multiligand-specific receptor such as CXCR2 appears to be a more suitable therapeutic target than the inhibition of the activity of each single neutrophil chemotactic molecule. Recently, different antagonists of CXCR2 have been developed to prevent neutrophil accumulation; in particular, a selective nonpeptide antagonist of CXCR2 has been shown to exhibit significant anti-inflammatory effects in acute and chronic models of arthritis in rabbits.[10] Considering that CXCL8/IL-8 acts not only as a chemotactic but also as an activating and degranulating factor for the neutrophils already present at the site of airway inflammation, a hypothesis of therapeutic intervention could be the local inhibition of the CXCL8/IL-8 receptors rather than a dangerous systemic inhibition of neutrophil recruitment. We should also take into account that some studies have demonstrated that in asthmatic subjects after the administration of an anti-IL-5 mAb, the few eosinophils remaining in the airways failed to be inhibited in their activity, probably because they downmodulate their IL-5 receptor.[28,29] In this view the downmodulation of CXCL8/IL-8 receptors we found on sputum neutrophils should be considered in the application of therapeutic strategies aiming at the inhibition of CXCL8/IL-8 receptors.

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