ORIGINAL ARTICLE

The Effect of Inhaled Corticosteroids on the Concentration of Soluble CD163 in Induced Sputum of Allergic Asthma Patients

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Abstract

Background and Objective: CD163 is a monocyte/macrophage-specific molecule whose expression is induced by corticosteroids and IL-10. The aim of this study was to evaluate the concentration of soluble CD163 (sCD163) in the induced sputum of asthmatic patients before and after therapy with inhaled corticosteroids (ICSs).
Patients and Methods: The study was performed in 24 patients with mild allergic asthma (AAs) and 10 healthy controls (HCs). In 18 AAs, induced sputum and serum samples were obtained before ICS therapy (T_0) and 7 days later (T_7). In the 6 AAs not treated with ICSs the procedures were performed at T_0 and T_7. The concentration of sCD163 in sputum and serum samples was evaluated using ELISA.
Results: There was no significant difference in mean (SD) baseline serum sCD163 concentration between AAs (1030 [449] ng/mL) and HCs (930 [334.5] ng/mL, P=.530). However, at T_0 the mean sputum sCD163 concentration was significantly greater in AAs (4.78 [3.34] ng/mL) than in HCs (1.8 [0.41] ng/mL, P=.009). Treatment with ICSs resulted in a significant increase in sCD163 concentration in sputum (P<.0001) but not in serum (P=.679). No change in sputum or serum sCD163 concentration was detected in AAs who were not treated with ICSs. The change in sputum sCD163 concentration inversely correlated with changes in sputum eosinophilia or exhaled nitric oxide concentration.
Conclusions: ICS therapy leads to local upregulation of sCD163 expression, which in turn may participate in the anti-inflammatory effects of ICS therapy.

Key words: CD163. Asthma. Inhaled corticosteroids. Induced sputum.

Resumen

Antecedentes y Objetivo: El CD163 es una molécula específica de monocitos/macrófagos cuya expresión es inducida por los corticosteroides y la interleucina 10 (IL-10). El objetivo de este estudio fue evaluar la concentración de sCD163 en el esputo inducido de pacientes asmáticos, antes y después del tratamiento con corticosteroides inhalados (ICS).
Pacientes y Métodos: El estudio se realizó en 24 pacientes con asma alérgica leve (AA) y 10 controles sanos (HC). En 18 AA, se obtuvieron muestras de esputo y suero antes (T0) y a los 7 días (T7) tras la introducción del tratamiento con ICS. Asimismo, en 6 AA no tratados con ICS se llevaron a cabo los mismos procedimientos en T0 y T7. Se evaluó la concentración de sCD163 en esputo y suero de las muestras mediante ELISA.
Resultados: No hubo diferencia en la concentración sérica basal media de sCD163 entre AA (1.030± 449 ng/ml) y los HC (930 ± 334,5 ng/ml, p = 0,530). Sin embargo, en T0 la concentración media de sCD163 esputo fue significativamente mayor en los AA (4,78 ± 3,34 ng/ml) que en HC (1,8 ± 0,41 ng/ml, p = 0,009). El tratamiento con ICS dio lugar a un aumento significativo de la concentración en el esputo de sCD163 (p < 0,0001), pero no en el suero (p = 0,679). No se detectaron diferencias en las concentraciones de sCD163 ni en el suero ni en el esputo del grupo de AA que no fueron tratados con ICS. La concentración sCD163 en esputo se correlacionó inversamente con la eosinofilia en esputo y la concentración NO exhalado.
Conclusiones: El tratamiento con ICS induce la expresión local de sCD163, que a su vez puede mediar en el mecanismo anti-inflamatorio de este tratamiento.

Palabras clave: CD163. Asma. Corticosteroides inhalados. Esputo inducido.

Introduction

Asthma is characterized by chronic inflammation of the airways [1]. An increasing body of evidence indicates that chronic airway inflammation depends not only on upregulation of proinflammatory mechanisms but also on suppression of the anti-inflammatory response [2-4]. The anti-inflammatory pathways fail to counterbalance the activated inflammatory response, which leads to the persistence of airway inflammation [2-4]. Macrophages and $CD4^+CD25^+$ T cells, which are present in the respiratory tract, control the inflammatory response of the airways [2,5]. In asthmatic patients, alveolar macrophages are characterized by a decreased inhibitory effect on T-cell activation [2]. Similarly, decreased numbers and impaired function of pulmonary $CD4^+CD25^+$ T cells have been demonstrated in asthmatic children [4]. It was recently shown that regulatory T cells present in the airways may cooperate with monocytes/macrophages to amplify the anti-inflammatory signal [6]. Both monocyte/macrophage function and $CD4^+CD25^+$ T-cell function are profoundly affected by corticosteroids, which are commonly used in asthma therapy [7,8].

Monocytes/macrophages represent a heterogeneous population and differ in their pro- and anti-inflammatory response [9]. CD163 was recently proposed as a specific marker of monocytes/macrophages with strong anti-inflammatory properties [10,11]. Monocytes/macrophages treated with corticosteroids or IL-10 strongly upregulate CD163 expression [7,12-16]. In fact, in experiments that analyzed 19 upregulated genes using gene-chip technology, *CD163* showed the strongest response to IL-10 [15]. Corticosteroids are at least equally potent inducers of CD163 expression [13,16]. The magnitude of upregulation depends on the potency of the corticosteroid; those with the greatest affinity for the corticosteroid receptor are the most potent upregulators of CD163 expression [16]. It has been seen that the concomitant application of dexamethasone and IL-10 to macrophages cultured in vitro exerts an additive effect on CD163 expression [13].

CD163 is expressed on the cell surface as a membrane-bound protein (mCD163), but it also exists as a soluble protein (sCD163) that is detectable in the plasma of healthy persons and also in some bodily fluids, such as synovial fluid [11,12,17]. The main mechanism responsible for the appearance of sCD163 in bodily fluids is thought to be the shedding of CD163 from the cell surface of mononuclear phagocytes [18,19]. At least 2 enzymes have been implicated in this process: matrix metalloproteinase-9 (MMP-9) [18] and TNF-α-converting enzyme (TACE/ADAM17) [19]. Both mCD163 and sCD163 participate in anti-inflammatory processes but their mode of action is different [11,12,20-22]. Binding of mCD163 by hemoglobin-haptoglobin complexes leads to an increased synthesis of IL-10 and carbon monoxide, 2 potent anti-inflammatory molecules [20-22]. sCD163 inhibits phorbol ester–induced T-cell proliferation in vitro in a dose-dependent manner [23,24]. This anti-proliferative function of CD163 seems to be restricted to its soluble form, as membrane-bound CD163 has not been found to exert such an effect [25]. In vivo observations provide data suggesting an anti-inflammatory function for CD163. In patients with rheumatoid arthritis CD163 expression was inversely correlated with markers of T-cell proliferation in the histologic analysis of inflamed joints [26]. Moreover, lymphoid follicle macrophages, which are located at sites of intense lymphocyte proliferation, express little or no CD163 [27].

Bearing in mind the strong anti-inflammatory action of inhaled corticosteroids (ICSs) and the ability of corticosteroids to strongly induce CD163 expression, we decided to evaluate whether therapy with (ICSs in asthmatic patients leads to a local increase of sCD163 in the airways.

Material and Methods

The study was performed in 24 corticosteroid-naïve allergic asthma patients (AAs) and in 10 nonatopic healthy controls (HCs). The AAs reported episodes of dyspnea, cough, and wheezing and had positive skin prick test results to at least 1 common aeroallergen. In AAs with a baseline forced expiratory volume in the first second (FEV_1) of below 80% of predicted, an improvement of at least 12% was demonstrated 15 minutes after inhalation of 400 mcg of salbutamol. In those with a baseline FEV_1 above 80% of predicted, significant bronchoconstrictive response to inhaled histamine was demonstrated. All patients who had received anti-asthma medication other than short-acting β-agonists used as needed before the initial visit were excluded from the study. Short-acting β-agonists were withdrawn at least 24 hours before the study. Other exclusion criteria included respiratory tract infection in the previous 3 months, smoking, and the presence of any systemic disease. The patients were evaluated at baseline (T_0) and after 7 days (T_7). During the 7-day period, 18 of the AAs were treated with a low dose of ICSs while the other 6 used only short-acting β-agonists as needed. The study was approved by the local ethics committee (R-I-003/188/2005) and all participants provided written informed consent.

Skin Prick Tests

All participants underwent skin prick testing with a screening panel of aeroallergens (Allergopharma) as described previously [28].

Bronchial Challenge

Histamine bronchial challenge was performed according to a previously described method [28]. Briefly, all patients inhaled doubling concentrations of histamine starting at a concentration of 0.62 mg/mL. The aerosol was generated using a DeVilbis #646 nebulizer attached to a Rosenthal-French dosimeter. All individuals performed 5 inspiratory-capacity breaths of histamine at the given concentration. Forced expiratory maneuvers were performed 90 seconds after each fifth inhalation. The procedure was continued until either at least a 20% fall in FEV_1 or a histamine concentration of 32 mg/mL was reached. Bronchial reactivity to histamine was expressed as the histamine concentration causing a 20% fall in FEV_1 (PC_{20}).

Exhaled Nitric Oxide Measurements

Concentration of nitric oxide (NO) in expired air was evaluated on-line using a chemiluminescence analyzer NOA 280i (Sievers). The measurements were performed according to the recommendations of the American Thoracic Society (ATS), as described previously [29]. Briefly, each patient exhaled against a fixed expiratory resistance of 16 cm H_20, which resulted in a constant flow of 50 mL/s. Both NO concentration and flow rate were displayed on the screen. A plateau of NO concentration in exhaled air at the selected exhalation rate was automatically selected by the computer software according to ATS recommendations. The NO measurements were performed 3 times and the mean value was used for analysis.

Sputum Induction

Sputum was induced as previously described [30]. Briefly, after premedication with 200 mcg inhaled salbutamol patients inhaled hypertonic saline solution (3%-5% NaCl). The volume of sputum collected was measured, mixed with an equal volume of 0.1% dithiothreitol (DTT) and then rocked at room temperature for 15 minutes. The samples were subsequently filtered through a 0.42-µm Millipore filter and centrifuged at 1500 g for 10 minutes. The supernatants were immediately aliquoted and frozen at −70ºC until further analysis. The pellets were resuspended in phosphate-buffered saline and the total number of nonsquamous cells was assessed using a Fuchs Rosenthal chamber.

Biochemical and Immunologic Assays

The concentration of sCD163 in the sputum supernatants was evaluated using ELISA (R&D Systems) according to the manufacturer's instructions. All samples were run in duplicate. Serum samples were diluted 10 times, while sputum samples were used without dilution.

To evaluate the reliability of the test in the sputum samples, calibration curves with serial dilutions of sCD163 were performed in 0.1% DTT and in supernatants of induced sputum derived from HCs and AAs.

Statistical Analysis

Continuous variables were compared using the *t* test. Correlations between individual parameters were assessed using the Pearson correlation coefficient. Data for continuous variables were expressed as means (SD). All computations were carried out using the Statistica software.

Results

There was no significant difference in sex or age distribution between HCs and AAs. Mean FEV_1 was significantly lower in AAs (100%; 95% CI, 91%-107%) than in HCs (108%; 95% CI, 100%-120%; *P*=.021), while mean NO concentration in exhaled air was significantly higher (106 ppb; 95% CI, 62-233 ppb vs 14 ppb; 95% CI, 9-20 ppb; *P*=.0001). A significantly higher mean total cell number in induced sputum was found in AAs (4.62 cells x 10^6/mL; 95% CI, 3.6-5.63 cells x 10^6/mL) as compared with HCs (2.06 cells; 95% CI, 1.44-2.66 cells x 10^6/mL; *P*=.0023). Similarly, the mean percentage of eosinophils was significantly greater in AAs (5.63%; 95% CI, 4.56%-6.69%) than in HCs (1.3%; 95% CI, 0.95%-1.65%; *P*<.0001).

sCD163 was detected in all serum and sputum samples from both HCs and AAs. However, in 3 of the 10 HCs (33.3%) the sputum concentration was below the lowest standard concentration value. Neither DTT at a concentration of 0.1% nor sputum supernatants containing 0.1% DTT significantly affected detection of sCD163 (data not shown).

At T_0 no significant difference was found between the mean (SD) serum sCD163 concentration in AAs (1030 [449] ng/mL) and HCs (930 [334.5] ng/mL; *P*=.530). However, the mean (SD) baseline sputum concentration of sCD163 was significantly higher (4.78 [3.34] ng/mL in AAs vs 1.8 [0.41] ng/mL in HCs, *P*=.009).

In AAs at T_0, a significant association was found between sputum sCD163 concentration and sputum eosinophilia (r=0.49; 95% CI, 0.109-0.746; *P*=.015) or logFeNO (r=0.44, 95% CI, 0.045-0.716; *P*=.031). However, no correlation was observed between baseline sputum sCD163 and the absolute number of macrophages (r=0.201;, 95% CI, –0.220 to 0.559; *P*=.346) or the percentage of macrophages (r=-0.149; 95% CI, −0.529 to 0.279; *P*=.495) in induced sputum. There was also no correlation between the concentration of sputum and serum sCD163 (r=0.053; 95% CI, −0.358 to −0.447; *P*=.804). Moreover, no significant association was seen between sputum sCD163 and any of the other parameters evaluated.

In AAs treated with ICSs for 7 days (n=18), the mean (SD) concentration of sCD163 in sputum increased from 5.1 [3.6] ng/mL at T_0 to 31.7 [13.4] ng/mL at T_7 (*P*<.0001). By contrast, there was no significant change in the mean concentration of sCD163 (1031.5 [477.5] ng/mL at T_0 to 1043 [467.5] ng/ml at T_7; *P*=.679) (Table). However, in AAs who did not receive ICS therapy (n=6) the mean sputum concentration of sCD163 at T_7 (3.24 [1.7] ng/mL) did not differ significantly from that at T_0 (3.6 [2.2] ng/mL; *P*=.374). Similarly, no change in serum sCD163 was seen after 7 days. At T7, an increase in sputum sCD163 was seen in 100% of the patients treated with ICSs but in only 2 (33.3%) of the 6 patients not treated (Figure 1). A significant drop in sputum eosinophilia and fractional exhaled nitric oxide (FeNO) concentration was demonstrated at T_7 only in AAs treated with ICSs (Table).

The mean total cell count and the number or percentage of sputum macrophages did not differ at T_7 in comparison with T_0 in AAs, regardless of whether or not they received ICS therapy.

The magnitude of change in sputum sCD163 concentration correlated inversely with the magnitude of change in sputum eosinophilia (r=−0.840; 95% CI, −0.937 to −0.625; *P*<.0001) or FeNO concentration (r=−0.720; 95% CI, −0.885 to − 0.395; *P*=.0005) (Figure 2). No other associations between change in sputum sCD163 concentration and any other clinical or immunological parameters were demonstrated.

Discussion

To the best of our knowledge this is the first study to demonstrate that sCD163 is detectable in induced sputum, that sCD163 concentrations are elevated in AAs, and that ICS therapy further increases sCD163 concentrations in induced sputum in AAs.

Table. Changes in Selected Parameters After 7 days in Patients Treated With Inhaled Corticosteroids[a]

	T_0	T_7	T_0 vs T_7
FEV_1, % predicted	84.3 (12.2)	96.6 (9.8)	P=.0015[b]
Exhaled NO, ppb	122 (90)	32 (10)	P=.0001[b]
Sputum cell count, cells×10^6/mL	4.74 (2.45)	4.91 (1.89)	P=.892
Sputum eosinophils, %	6.0 (-2.6)	1.8 (0.9)	P<.0001[b]
Sputum macrophages, %	58.1 (8.4)	62.7 (6.9)	P=.09
Serum sCD163, mcg/mL	1.01 (0.34)	1.06 (0.39)	P=.66

Abbreviations: FEV_1, forced expiratory volume in the first second of expiration; NO, nitric oxide.
[a]Data are expressed as mean (SD).
[b]Significant.

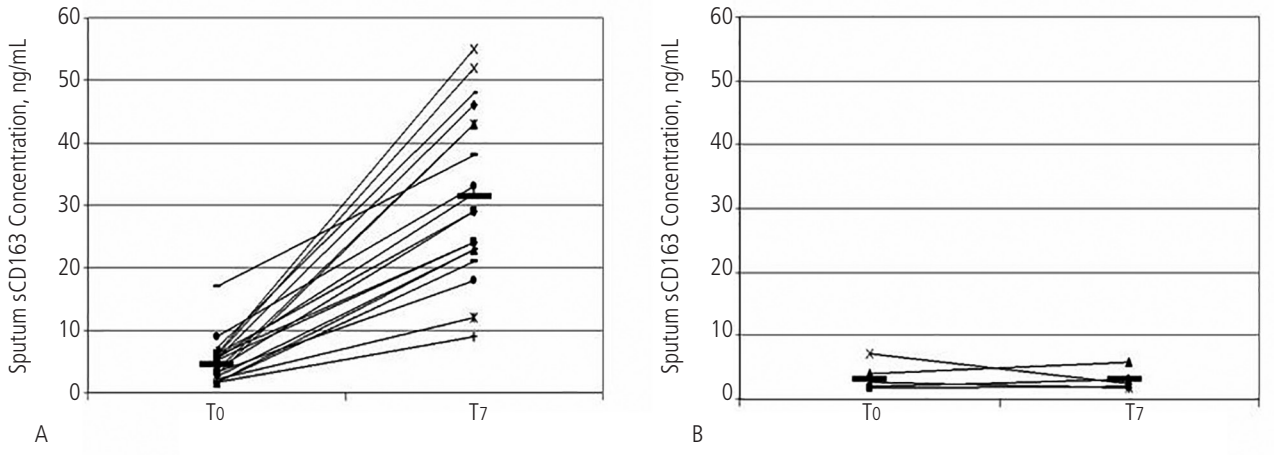

Figure 1. Concentration of soluble CD163 (sCD163) in induced sputum of allergic asthmatic patients before and after 7 days. A, Patients treated with inhaled corticosteroids. B, Patients not treated with inhaled corticosteroids.

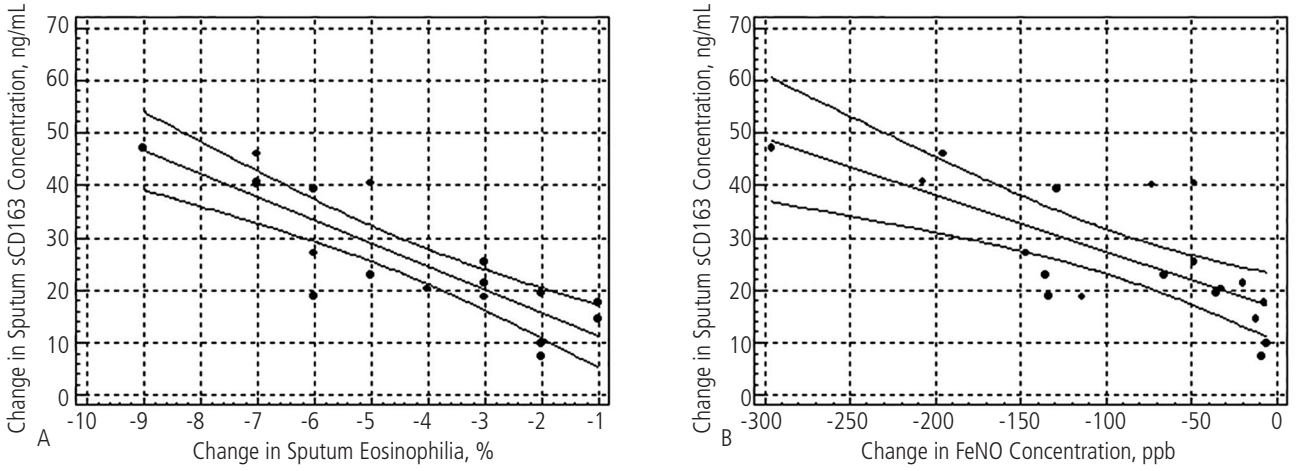

Figure 2. Correlations in individual patients treated with inhaled corticosteroids for 7 days between the change in sputum soluble CD163 (sCD163) concentration and (A) the change in sputum eosinophilia (r=–0.840; 95% CI, –0.937 to 0.625; P<.0001) or (B) change in fractional exhaled nitric oxide (FeNO) (r=–0.720; 95% CI, 0.885 to –0.395; P=.0005).

Our results indicate that the increased concentration of sCD163 in the sputum of ICS-naïve AAs is related to intensity of airway inflammation, as assessed by sputum eosinophilia or exhaled NO concentration. However, the broad distribution of individual results suggests that the association is complex. Bearing in mind that sCD163 appears to have an anti-inflammatory function, it could be speculated that elevated levels of sCD163 in induced sputum of steroid-naïve AAs may reflect a compensatory response to the airway inflammatory process. CD163 is exclusively synthesized by mononuclear phagocytes and its soluble form is shed into plasma [17]. Tissue macrophages express a high level of CD163 and are another potential source of sCD163 [26,27]. The appearance of sCD163 in sputum may therefore be caused by an influx from plasma or by in situ production by monocytes/macrophages infiltrating bronchial tissue. Both of these potential sources may be affected by airway inflammation. The enhanced production of sCD163 in the circulation, which would lead to an elevated serum sCD163 concentration in asthmatic patients, does not seem to play a major role because we did not observe an increased concentration of sCD163 in the sera of AAs in comparison with HCs. Similarly, we did not find any correlation between serum and sputum sCD163. There was also no correlation between serum sCD163 concentration and sputum eosinophilia or exhaled NO concentration, suggesting that the effect of bronchial inflammation on sCD163 production was mainly restricted to the airways in our population of AAs. Our study only included patients with mild to moderate asthma and it therefore cannot be excluded that in more severe cases of asthma, production of sCD163 in the circulation may be elevated and affect sputum levels. Nevertheless, the appearance of plasma sCD163 in the airways may result from enhanced permeability of the endothelial barrier, which would facilitate a greater influx of sCD163 into the airway compartment in AAs in comparison with HCs. In fact, enhanced leakage of plasma proteins into the airway tissues has been demonstrated in asthma [31,32]. Since sCD163 concentrations are many-fold greater in plasma than in induced sputum it seems plausible that in untreated asthmatic patients sCD163 may be directly derived, at least partially, from plasma due to enhanced endothelial permeability.

In situ production of sCD163 by bronchial mononuclear phagocytes may be another major source of sputum sCD163. Both monocytes infiltrating bronchial tissue and resident bronchial macrophages may release sCD163. In contrast to sCD163 plasma levels, peripheral blood monocytes express more CD163 in asthmatic patients, even in those with mild disease, than in healthy individuals [33,34]. Monocytes stay in the circulation for 1 to 3 days and then migrate to peripheral tissues either spontaneously or upon stimulation [9]. The migration of monocytes to bronchial tissue may be different in AAs compared with HCs [35]. Monocytes with elevated expression of CD163 seem to preferentially migrate to the lungs of asthmatic patients in response to allergen challenge [35]. Interestingly, the expression of CD163 on peripheral blood monocytes seems to be proportional to the severity of the patient's asthma [33,34]. One can therefore speculate that in AAs peripheral blood monocytes with elevated expression of CD163 are preferentially attracted to the bronchial compartment, where they release CD163, leading to elevated sCD163 levels. It has been demonstrated that bronchial macrophages express a high level of CD163 and are a potential source of sCD163 in the bronchial compartment [36]. High expression of CD163 has been demonstrated on macrophages obtained by bronchoalveolar lavage or sputum induction [37]. Moreover, elevated levels of enzymes involved in shedding of CD163, such as matrix metalloproteinase-9 (MMP-9), have been demonstrated in the sputum of asthmatic patients [38].

Observations made in asthmatic patients after ICS therapy support the major role of local sCD163 production in the airways, as a several-fold increase in sputum sCD163 concentration was not accompanied by an increase in serum sCD163 concentration. The effect of ICSs in the current study seems to be restricted to the local expression of CD163 by bronchial macrophages. It has been demonstrated that the expression of CD163 by bronchial macrophages is regulated by mediators of inflammation in vivo [36]. Inhibition of local inflammatory response by cessation of smoking is associated with upregulation of CD163 expression by bronchial macrophages [36]. Moreover, recent studies evaluating ex vivo human lung samples demonstrated that dexamethasone enhanced the expression and secretion of CD163 in a dose-dependent manner [39]. The effect was dependent on elevated de novo synthesis of CD163 by macrophages, as demonstrated by elevated levels of CD163 mRNA in lung samples [39]. Interestingly, ICSs do not affect levels of MMP-9, which is an important enzyme for CD163 shedding that leads to a microenvironment in which elevated synthesis of CD163 by macrophages is accompanied by an increased potential for releasing a soluble form of the molecule [38]. Moreover, the elevated level of sCD163 in ICS-treated AAs does not seem to be derived from plasma because ICSs decrease the permeability of the endothelial barrier and the leakage of plasma proteins into the bronchial compartment [31,32].

Finally, it seems that assessment of sCD163 in sputum might be a useful marker to evaluate the anti-inflammatory effects of ICSs. Further studies evaluating the clinical utility of this method are warranted, particularly in ICS-treated patients with noneosinophilic airway inflammation, in smokers, and in COPD patients in whom evaluation of exhaled NO concentration may not appropriately reflect airway inflammation and response to ICS therapy.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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