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Soluble RAGE is deficient in neutrophilic asthma and COPD

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ABSTRACT: The receptor for advanced glycation end-products (RAGE) is a pattern-recognition receptor involved in the host response to injury, infection and inflammation. It is a membrane receptor, but also has soluble forms (sRAGE). Deficiencies in sRAGE are linked to heightened inflammation in various chronic conditions. We determined whether airway and systemic levels of sRAGE and the RAGE ligands HMGB1 (high-mobility group box-1) and serum amyloid A (SAA) are related to neutrophilic inflammation in asthma and chronic obstructive pulmonary disease (COPD).

Bronchial lavage fluid from subjects with moderate-to-severe persistent asthma (n=16) or COPD (n=37), or from healthy controls (n=18), was analysed for neutrophils, total sRAGE, endogenous secretory RAGE (esRAGE), HMGB1 and SAA. We also determined systemic levels of sRAGE in a separate group of asthmatic (n=101) and COPD (n=34) subjects.

Subjects with neutrophilic asthma or COPD had undetectable levels of lung sRAGE, while levels of sRAGE in asthma/COPD without neutrophilia were similar to those in controls. Systemic sRAGE was significantly decreased in subjects with neutrophilic asthma or COPD compared with those without airway neutrophilia. There was significant positive correlation between total sRAGE and esRAGE in the lung and systemically. HMGB1 levels were similar in all subject groups, while SAA was below detectable levels.

Neutrophilic airway inflammation in asthma and COPD is associated with reduced sRAGE.

KEYWORDS: Airway inflammation, endogenous secretory receptor for advanced glycation end-products, high-mobility group box-1, neutrophil, serum amyloid A, soluble receptor for advanced glycation end products

The receptor for advanced glycation end-products (RAGE) is a pattern-recognition receptor that interacts with diverse endogenous ligands involved in the host response to injury, infection and inflammation. RAGE ligands include advanced glycation end-products (AGEs), HMGB1 (high-mobility group box-1), serum amyloid A (SAA), amyloid-β peptide, S100 family proteins and the β_2-integrin CD11b. Ligation of RAGE elicits activation of immune and inflammatory responses, induction of oxidant stress and tissue remodelling responses [1]. RAGE exists as both a membrane and soluble receptor. Soluble forms of RAGE are generated through proteolytic cleavage or through alternative RNA splicing. Classically, soluble RAGE (sRAGE) functions as a natural antagonist of RAGE signalling as it sequesters RAGE ligands and inhibits RAGE-dependent cellular responses [2].

Accumulating evidence suggests that the ligand–RAGE/sRAGE axis might be crucially involved in chronic airways disease. A recent genome-wide association study identified RAGE as a genetic determinant of airflow obstruction (forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio) [3]. Moreover, increased expression of RAGE and its ligands, HMGB1 and AGEs, are observed in the lung in chronic obstructive pulmonary disease (COPD) [4, 5]. Increased HMGB1 levels are also observed in asthmatic sputum [6]. Conversely, significantly reduced levels of sRAGE, in association with increased levels of the RAGE ligand SAA, are observed systemically in COPD, suggesting that perturbation of the RAGE/sRAGE balance might contribute to heightened lung and systemic inflammation in chronic airways disease [7, 8].

For editorial comments see page 515.
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Neutrophilic inflammation is an important component of the airway inflammatory response in COPD and some phenotypes of asthma, where it is associated with more severe and treatment-refractory disease. The mechanisms underlying airway neutrophilia are not well understood but involve ongoing neutrophil influx and activation, together with impaired neutrophil clearance [9]. Since the ligand–RAGE/sRAGE axis impacts on neutrophilic inflammation [10, 11], we reasoned that perturbations in the ligand–RAGE/sRAGE balance might be linked to neutrophilic airway inflammation in chronic airways disease. Thus, we set out to determine the relationship between airway neutrophilia, sRAGE, HMGB1 and SAA in the bronchial lavage (BL) fluid and peripheral blood of asthmatic and COPD subjects.

MATERIALS AND METHODS

Study subjects and study design

Two separate groups of subjects were studied. The first group included subjects with asthma (n=16) or COPD (n=37) and healthy controls (n=18). The second group included clinically stable asthmatic (n=101) and COPD (n=34) subjects. Subjects had moderate-to-severe persistent asthma as defined by the Global Initiative for Asthma (GINA) or stage II–IV COPD as defined by the Global Intitiative for Chronic Obstructive Lung Disease (GOLD). BL fluid was collected from subjects in group 1 for analysis of differential cell counts, sRAGE and RAGE ligands. Peripheral blood and sputum samples were collected from subjects in group 2 for analysis of differential cell counts and systemic sRAGE, respectively. Details regarding clinical assessment and procedures, and subject inclusion/exclusion criteria are provided in the online supplementary material. Subsets of data collected from the second group of subjects have been reported previously [12–15]. This research was approved by the Hunter New England Human Research Ethics Committee (Hunter New England Local Health District, New Lambton, Australia) and all subjects provided written informed consent.

Classification of airway neutrophilia

Airway neutrophilia was defined as the presence of airway neutrophils ($\geqslant$65% of total cells) in BL or sputum samples. This cut-off value is two standard deviations above the mean value, and in excess of the upper limit of the 95% confidence interval of airway neutrophils in healthy control subjects [16].

Analysis of BL and blood markers

Total sRAGE was measured using an ELISA that detects cleaved and secreted forms of sRAGE (RAGE DuoSet; R&D Systems, Minneapolis, MN, USA; lower limit of detection 62.5 pg·mL^{-1}). Endogenous secreted RAGE (esRAGE) was measured using a specific ELISA for this isoform (B-Bridge International, Cupertino, CA, USA; lower limit of detection 50 pg·mL^{-1}). To determine recovery of exogenous sRAGE in BL fluid, BL samples were spiked at room temperature with recombinant human sRAGE standard, supplied in the RAGE DuoSet ELISA assay kit, immediately prior to assay using this same ELISA kit. HMGB1 (IBL International, Hamburg, Germany; lower limit of detection 2.5 ng·mL^{-1}), SAA (Anogen, Mississauga, ON, Canada; lower limit of detection 2.5 ng·mL^{-1}), CXC chemokine ligand (CXCL)8 (R&D Systems; lower limit of detection 31.25 pg·mL^{-1}) and myloperoxidase (Zen^{TM} Myeloperoxidase (MPO) ELISA, Invitrogen, Carlsbad, CA, USA; lower limit of detection 0.375 ng·mL^{-1}) were measured by ELISA.

Statistical analysis

Details regarding statistical analysis are provided in the online supplementary material.

RESULTS

Neutrophilic airway inflammation in asthmatic and COPD subjects

In the group of subjects who underwent BL, a subgroup had airway neutrophilia and significantly elevated levels of CXCL8 and MPO; markers of neutrophil recruitment and activation, respectively (fig. 1). There was a strong negative correlation between neutrophils (% BL cells) and FEV1 in all subjects with asthma or COPD (r= -0.53; p<0.001) and this was seen in both groups when examined separately (r=0.66 COPD; r=0.49 asthma; p<0.01). Subjects with neutrophilic COPD had a lower FEV1 than all other groups and there was also a trend in those with neutrophilic asthma to have a lower FEV1. Moreover, subjects with neutrophilic COPD had more frequent acute exacerbations in the preceding 12 months, though this difference was not seen in those with neutrophilic asthma (table 1). The presence of a bacterial pathogen was strongly associated with airway neutrophilia (OR 3.5, 95% CI 1.3–5.7, asthma or COPD) (table 2).

Subjects with asthma or COPD had few comorbidities; four out of 52 had a prior history of ischaemic heart disease, two out of 52 had diabetes and six out of 52 were currently using lipid-lowering therapy with a 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitor (statin). These subjects appeared to be evenly distributed between the asthma and COPD neutrophilic/non-neutrophilic groups, though the numbers were small.

sRAGE in BL fluid

We measured total sRAGE in BL fluid from the subjects described in table 1. Subjects with non-neutrophilic asthma or COPD had similar levels of sRAGE to healthy control subjects. In contrast, subjects with neutrophilic asthma or COPD had negligible levels of sRAGE (fig. 2a). In asthmatic and COPD subjects with evidence of airway bacterial colonisation, mean sRAGE levels were significantly lower compared with those without evidence of bacterial colonisation (48.6$\pm$87.4 *versus* 203.3$\pm$441.3 pg·mL^{-1}; p=0.02).

In order to establish whether the observed sRAGE deficiency in neutrophilic asthma or COPD is due to degradation of sRAGE, we determined recovery of exogenous spiking of sRAGE in BL fluid from four subjects with neutrophilic asthma or COPD, and four subjects with non-neutrophilic asthma or COPD. Recovery of exogenous sRAGE was significantly reduced in BL fluid from subjects with neutrophilic asthma or COPD compared with those with non-neutrophilic asthma or COPD (mean recovery 35.5$\pm$20.3% *versus* 80.96$\pm$31.4%; p=0.0486), indicating that degradation is the likely reason for the observed deficiency.

RAGE ligands in BL fluid

In order to determine the relationship between sRAGE and RAGE ligands in BL fluid, we measured HMGB1 and SAA. HMGB1 was detected in BL fluid from all subjects, but no

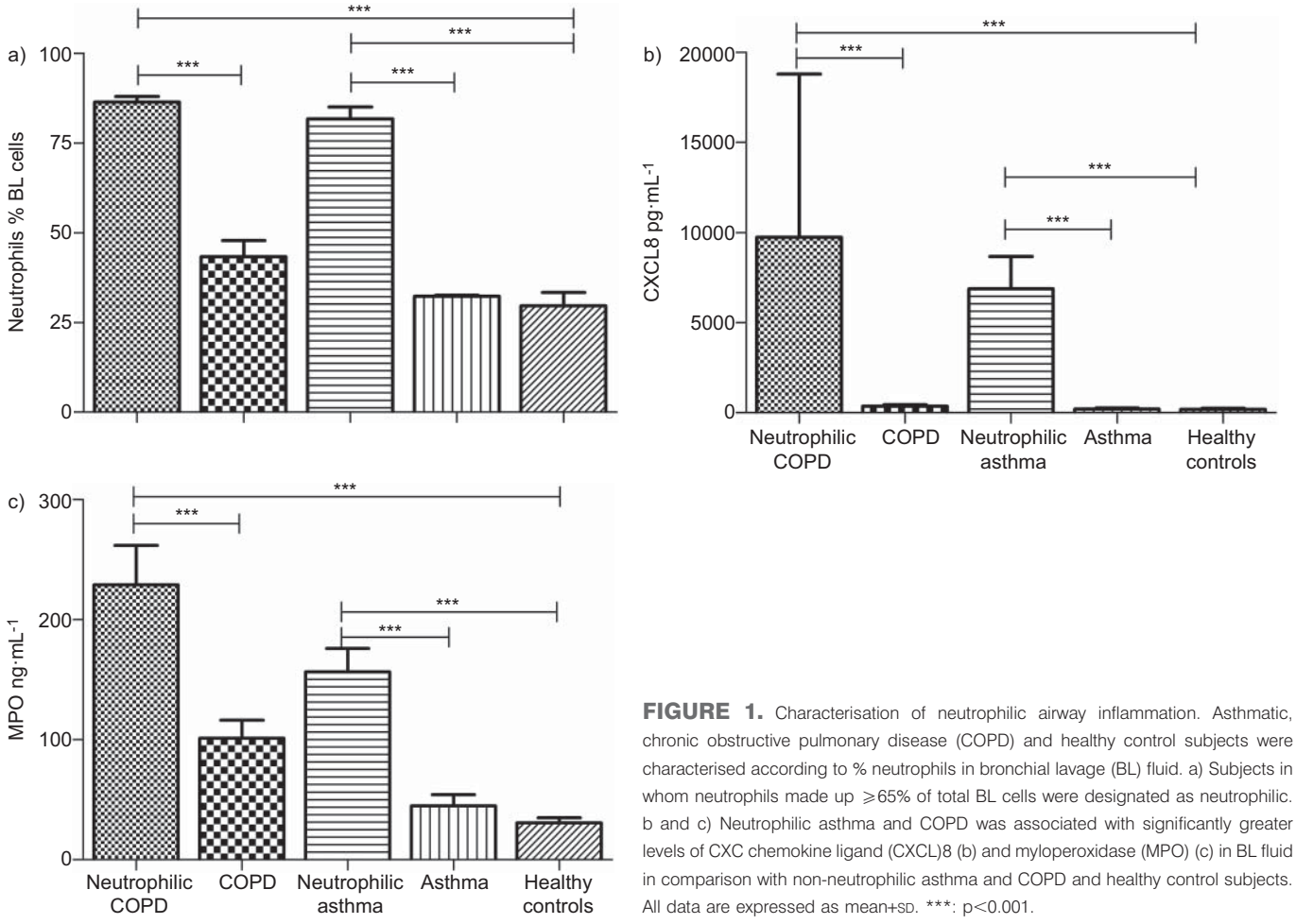

FIGURE 1. Characterisation of neutrophilic airway inflammation. Asthmatic, chronic obstructive pulmonary disease (COPD) and healthy control subjects were characterised according to % neutrophils in bronchial lavage (BL) fluid. a) Subjects in whom neutrophils made up ⩾65% of total BL cells were designated as neutrophilic. b and c) Neutrophilic asthma and COPD was associated with significantly greater levels of CXC chemokine ligand (CXCL)8 (b) and myloperoxidase (MPO) (c) in BL fluid in comparison with non-neutrophilic asthma and COPD and healthy control subjects. All data are expressed as mean+SD. ***: $p<0.001$.

significant differences were seen between the groups (fig. 2b). Levels of BL neutrophils were not correlated with HMGB1 levels ($r=0.1$, $p>0.05$). There was, however, a modest but significant increase in HMGB1 levels in those subjects with evidence of bacterial colonisation compared with those without (27.8 ± 43.8 *versus* 20.5 ± 17.5 ng·mL^{-1}; $p=0.03$). Interestingly, when COPD subjects were divided on the basis of inhaled corticosteroid (ICS) use, we observed significantly lower levels of HMGB1 in those using ICS compared with those who were not (7.464 ± 6.58 *versus* 44.7 ± 42.15 pg·mL^{-1}; $p<0.001$).

Minimal levels of SAA were detected in BL of all types of subject; indeed, in the majority of subjects, SAA was undetectable (fig. 2c).

Systemic levels of sRAGE

We measured systemic levels of total sRAGE in a separate group of asthmatic (table 3) and COPD (table 4) subjects. Subjects with neutrophilic asthma had significantly lower mean levels of systemic sRAGE compared to those with non-neutrophilic asthma (410 ± 210 *versus* 462 ± 165 pg·mL^{-1}; $p<0.01$) (fig. 3a). Subjects with neutrophilic COPD also had lower mean systemic sRAGE compared to those with non-neutrophilic COPD (285.5 ± 216 pg·mL^{-1} *versus* 496.9 ± 274 pg·mL^{-1}; $p=0.01$) (fig. 3b).

Predictors of sRAGE, HMGB1 and airway neutrophilia in asthma and COPD

Multiple linear regression analysis was performed to determine the extent to which demographic (age, sex and pack-yrs smoked), disease (airway neutrophilia, airway pathogens and lung function) and treatment (ICS use) variables impact on lung levels of sRAGE (see online supplementary table 1). Only BL neutrophils independently predicted lung sRAGE, ($t= -5.547$; $p<0.001$) in the asthmatic, COPD and healthy control subjects described in table 1, with the linear regression model explaining 53.8% of the observed variation in BL sRAGE levels. None of the variables examined were significant independent predictors of systemic sRAGE in asthmatic (table 3) or COPD (table 4) subjects. None of the variables examined were significant independent predictors of lung HMGB1 levels across all subject groups described in table 1.

Multiple linear regression analysis was also performed to determine the extent to which demographics, disease and treatment, as well as lung sRAGE and HMGB1 levels, predict neutrophilic inflammation (see online supplementary table 1). Interestingly, sRAGE ($t= -4.793$; $p<0.001$), the presence of airway pathogens ($t=4.103$; $p<0.001$), age ($t=2.267$; $p=0.015$) and lung

TABLE 1 Characteristics of subjects who were evaluated for bronchial lavage levels of soluble receptor for advanced glycation end-products (RAGE) and RAGE ligands

	Neutrophilic COPD	COPD	Neutrophilic asthma	Asthma	Healthy controls	p-value
Subjects n	23	14	8	8	18	
Age yrs	69±10.8[+]	65±12	56±21	68±6	53±21	0.04
Males/females n	15/8	5/9	2/6	4/4	6/7	0.8
Atopy n (%)	6 (26)	3 (22)	2[§] (25)	5[§] (62)	0	0.02
FEV1 % pred	43±5[f]	52±8	52±10	64±6	101±2[f]	0.001
Smoking history pack-yrs	35±3.5[##]	30±4.7[##]	0	0	0	0.001
Number of exacerbations in past 12 months	2.7±0.5[¶¶]	1.5±0.5	1.3±0.8	1.5±0.6	NA	0.02
Eos % total cells	2±0.4	0.6±0.2	2.9±1	15±7[¶¶]	1.8±0.8	0.001
ICS use n (%)[#]	13 (57)	6[++] (50)	8 (100)	8 (100)	0	0.03
Total daily ICS dose μg beclamethasone equivalent[¶]	1067±177	700±247	1625±263	1500±327	NA	0.7

Data are presented as mean±SD, unless otherwise stated. COPD: chronic obstructive pulmonary disease; FEV1: forced expiratory volume in 1 s; % pred: % predicted; Eos: eosinophils; ICS: inhaled corticosteroids. [#]: significantly fewer controls used inhaled corticosteroids. [¶]: total daily ICS dose (beclamethasone equivalents), based on the assumption that 1 μg beclamethasone = 1 μg budesonide = 0.5 μg fluticasone. [+]: significantly older than healthy control subjects. [§]: a significantly greater proportion of asthma subjects were atopic. [f]: neutrophilic COPD subjects had lower pre-bronchodilator FEV1 compared with all other groups and healthy controls had significantly higher pre-bronchodilator FEV1 than all disease groups. [##]: all subjects with COPD were former smokers; there was no difference between the COPD groups. [¶¶]: significantly higher than all other groups. [++]: n=12.

function (t= -2.275; p=0.011) were all significant predictors of airway neutrophilia, with the model explaining 73.1% of the observed variation in airway neutrophilia. In the COPD subjects described in table 4, systemic sRAGE (t= -2.179; p=0.041) and pack-yrs smoked (t=2.860; p=0.011) were significant predictors of neutrophilic inflammation, with the model explaining 50.9% of the variation in neutrophilic inflammation in this group. In contrast, none of the variables examined were significant predictors of airway neutrophilia in the asthmatic subjects described in table 3.

sRAGE in BL and serum represents esRAGE

sRAGE is derived *via* proteolytic cleavage of the extracellular domain of the cell surface receptor or *via* alternative splicing of the *RAGE* gene that leads to the production of the secreted soluble isoform esRAGE [17]. We used a specific esRAGE ELISA that does not cross-react with other forms of sRAGE to determine to what extent this isoform contributes to the pool of sRAGE. We measured esRAGE in BL from healthy control subjects and a subgroup of COPD subjects with detectable levels of sRAGE (table 1; fig. 2a). In both healthy control and COPD subjects, there was a significant positive correlation between BL esRAGE and total sRAGE (fig. 4a and b). We also measured esRAGE in the serum of the neutrophilic or non-neutrophilic asthmatic subjects described in table 3. Similar to our findings in BL, there was a significant positive correlation between esRAGE and total sRAGE, irrespective of the inflammatory phenotype (fig. 4c and d).

DISCUSSION

We have demonstrated that asthmatic or COPD subjects with neutrophilic airway inflammation have a marked deficiency in lung sRAGE, and a modest but significant reduction in

TABLE 2 Bacterial pathogens isolated from bronchial lavage fluid analysed for soluble receptor for advanced glycation end-products (RAGE) and RAGE ligands

	Neutrophilic COPD	COPD	Neutrophilic asthma	Asthma	Healthy controls
Subjects in whom a pathogen was isolated n/N (%)	17/23 (74)***	2/14 (14)	6/8 (75)***	0/8	0
Pseudomonas aeruginosa	4	0	0	0	0
Haemophilus influenzae	6	0	4	0	0
Moraxella catarrhalis	3	0	0	0	0
Streptococcus pneumoniae	2	1	1	0	0
Staphylococcus aureus	2	1	1	0	0

Data are presented as n, unless otherwise stated. COPD: chronic obstructive pulmonary disease. ***: pathogen isolation was significantly more frequent in neutrophilic COPD and neutrophilic asthma than in all other groups, Chi-squared test p<0.001.

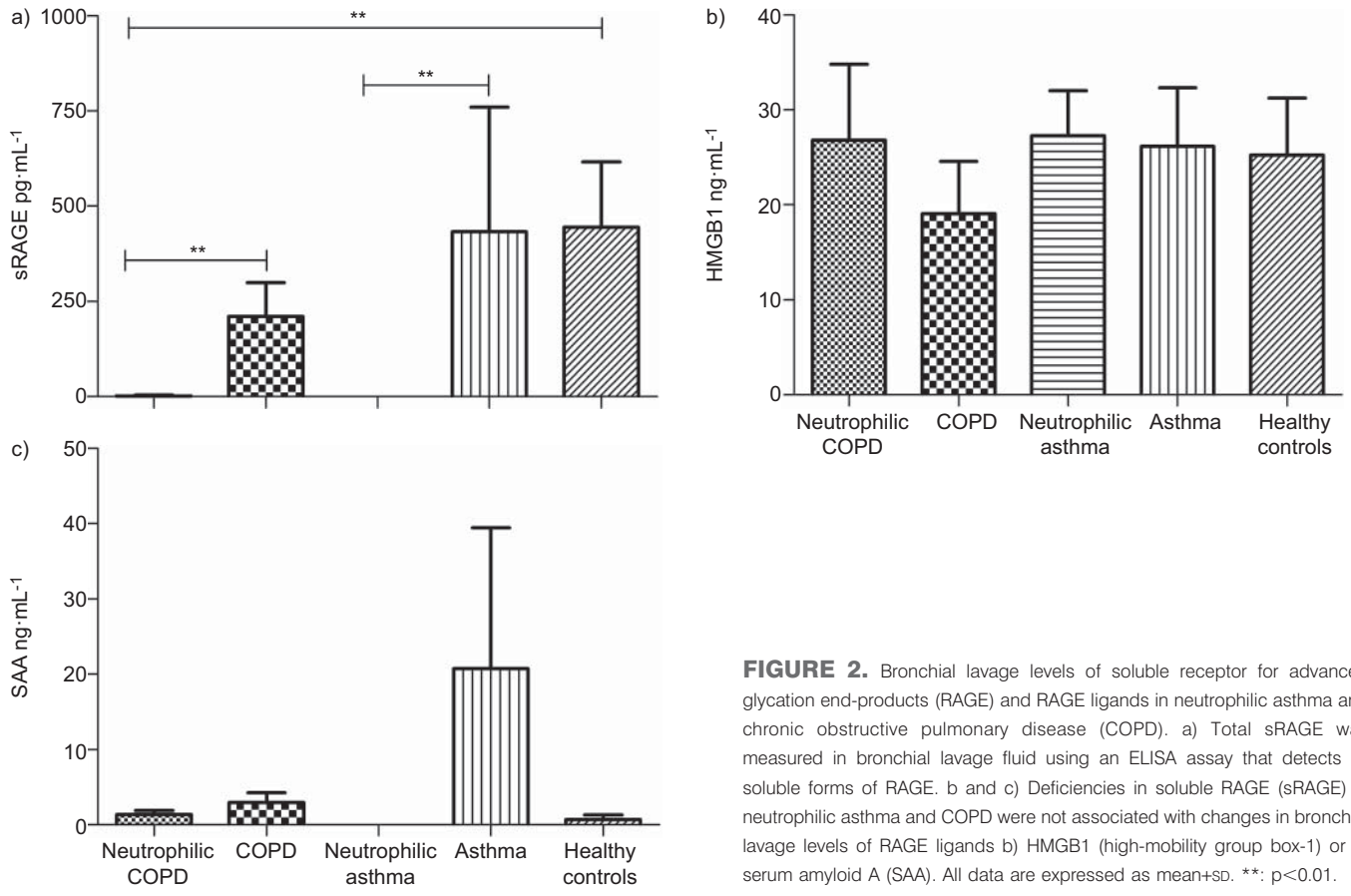

FIGURE 2. Bronchial lavage levels of soluble receptor for advanced glycation end-products (RAGE) and RAGE ligands in neutrophilic asthma and chronic obstructive pulmonary disease (COPD). a) Total sRAGE was measured in bronchial lavage fluid using an ELISA assay that detects all soluble forms of RAGE. b and c) Deficiencies in soluble RAGE (sRAGE) in neutrophilic asthma and COPD were not associated with changes in bronchial lavage levels of RAGE ligands b) HMGB1 (high-mobility group box-1) or c) serum amyloid A (SAA). All data are expressed as mean+SD. **: $p<0.01$.

systemic sRAGE. Moreover, we have shown that, in addition to older age, lower lung function and the presence of airway bacterial colonisation, lung sRAGE is an independent predictor of neutrophilic airway inflammation. We have also shown that, in addition to smoking history, systemic sRAGE is an independent predictor of neutrophilic airway inflammation in COPD subjects. These findings have important implications for understanding the mechanisms of neutrophilic airway inflammation in airways disease, and also identify sRAGE as a potential biomarker for prognosis or management.

RAGE is a multi-ligand pattern-recognition receptor which is highly expressed in the lung. In human lung, the *RAGE* gene generates ~20 alternative mRNA splice variants that encode

TABLE 3 Characteristics of asthmatic subjects who were evaluated for systemic levels of soluble receptor for advanced glycation end-products

	Neutrophilic asthma	Asthma	p-value
Subjects n	28	73	
Age yrs	61 (53.46–68.29)	59 (47.85–66.39)	0.417
Males/females n	12/16	28/45	0.679
Atopy n (%)	15 (54)	55 (75)	0.034
Pre-bronchodilator FEV_1 % pred	79.96 (4.01)	81.56 (2.14)	0.708
Smoking status current/ex/never n	0/16/12	0/28/45	NS
Smoking history pack-yrs	2.5 (0–11.0)	0.0 (0–18.0)	0.117
ICS use %	67.86	60.27	0.481
Total daily ICS dose μg beclamethasone equivalent#	1000 (500–2000)	1000 (500–1750)	0.614

Data are presented as median (interquartile range) or mean±SD, unless otherwise stated. FEV_1: forced expiratory volume in 1 s; % pred: % predicted; ICS: inhaled corticosteroids; NS: nonsignificant. #: total daily ICS dose (beclamethasone equivalents), based on the assumption that 1 μg beclamethasone = 1 μg budesonide = 0.5 μg fluticasone.

TABLE 4 Characteristics of chronic obstructive pulmonary disease (COPD) subjects who were evaluated for systemic levels of soluble receptor for advanced glycation end-products

	Neutrophilic COPD	COPD	p-value
Subjects n	18	16	
Age yrs	69 (57.58–79.61)	70 (59.69–82.65)	0.8
Males/females n	7/11	9/7	0.5
Atopy n (%)	6 (33)	4 (25)	0.3
Pre-bronchodilator FEV_1 % pred	45.1±14.6	51.4±16.5	0.2
Smoking status current/ex/never n	0/12/6	1/10/5	0.8
Smoking history pack-yrs	30.3 (0–71.6)	37.7 (0.2–102.5)	0.8
ICS use %	83.33	93.75	0.8
Total daily ICS dose μg beclamethasone equivalent#	1000 (500–2000)	1000 (500–1750)	0.6

Data are presented as median (interquartile range) or mean±SD, unless otherwise stated. FEV_1: forced expiratory volume in 1 s; % pred: % predicted; ICS: inhaled corticosteroids; NS: nonsignificant. #: total daily ICS dose (beclamethasone equivalents), based on the assumption that 1 μg beclamethasone = 1 μg budesonide = 0.5 μg fluticasone.

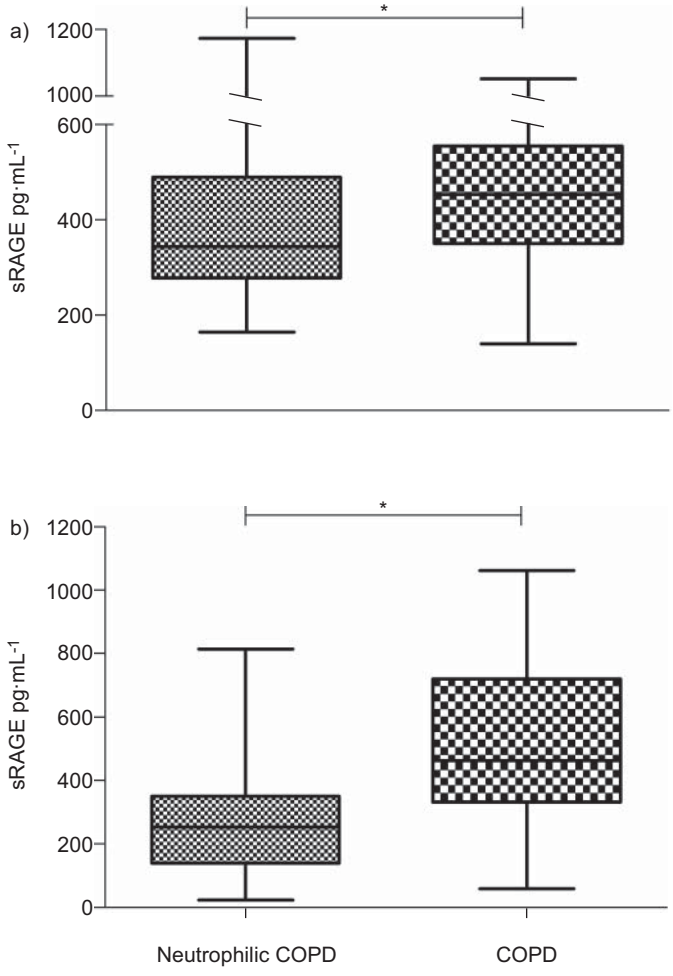

FIGURE 3. Systemic levels of total soluble receptor for advanced glycation end-products (RAGE) in neutrophilic and non-neutrophilic a) asthmatic and b) chronic obstructive pulmonary disease (COPD) subjects. Total soluble RAGE (sRAGE) was measured in plasma (asthma) or serum (COPD) using an ELISA assay that detects all soluble forms of RAGE. The central line represents the median, the boxes represent the interquartile range and the whiskers represent the 5–95% range. *: $p<0.05$.

membrane-associated and soluble forms of the receptor. Approximately 80% of lung RAGE is in the form of the membrane-associated receptor, while about 7% is a soluble form generated through alternative RNA splicing, known as esRAGE [17]. Soluble forms of RAGE may also be generated through proteolytic cleavage of the extracellular domain of the receptor [2], but the extent to which proteolysis contributes to the circulating pool of sRAGE is unclear [18, 19]. We observed significant positive correlations between levels of total sRAGE and esRAGE in BL fluid and peripheral blood, irrespective of the presence or type of airways disease, suggesting that esRAGE is likely to be the major component of lung and systemic sRAGE.

Our study confirms and extends the findings of two recent studies demonstrating reduced systemic sRAGE in stable COPD [7, 8], but is inconsistent with the findings of WATANABE *et al*. [6], who reported elevated levels of esRAGE in asthmatic sputum. In the study by WATANABE *et al*. [6], the subjects had low levels of sputum neutrophils (median 24%, upper limit 47%). This is a likely reason for the difference in findings, as we have shown that the level of neutrophilic airway inflammation is a significant independent predictor of lung sRAGE, with high levels of airway neutrophils (*ie* >65% total cells) being significantly associated with lower levels of lung sRAGE.

Deficiency in sRAGE is observed in a number of chronic diseases [20], although very little is known about the mechanisms involved. In order to establish whether the observed deficiency in sRAGE in neutrophilic asthma or COPD was due to degradation, we determined recovery of exogenous spiking of sRAGE in BL fluid from subjects with neutrophilic and non-neutrophilic asthma or COPD. Intriguingly, recovery of exogenous sRAGE was approximately 45% less in samples from subjects with neutrophilic asthma or COPD, compared with those with non-neutrophilic asthma or COPD. Since sRAGE is known to have multiple protease-sensitive sites [21], these data suggest that sRAGE might be degraded by neutrophil-derived proteolytic enzymes present in BL fluid from subjects with airway neutrophilia. Furthermore, these data raise the possibility that sRAGE might be bound to ligands that interfere with its detection by ELISA in BL fluids from subjects with airway neutrophilia. We are

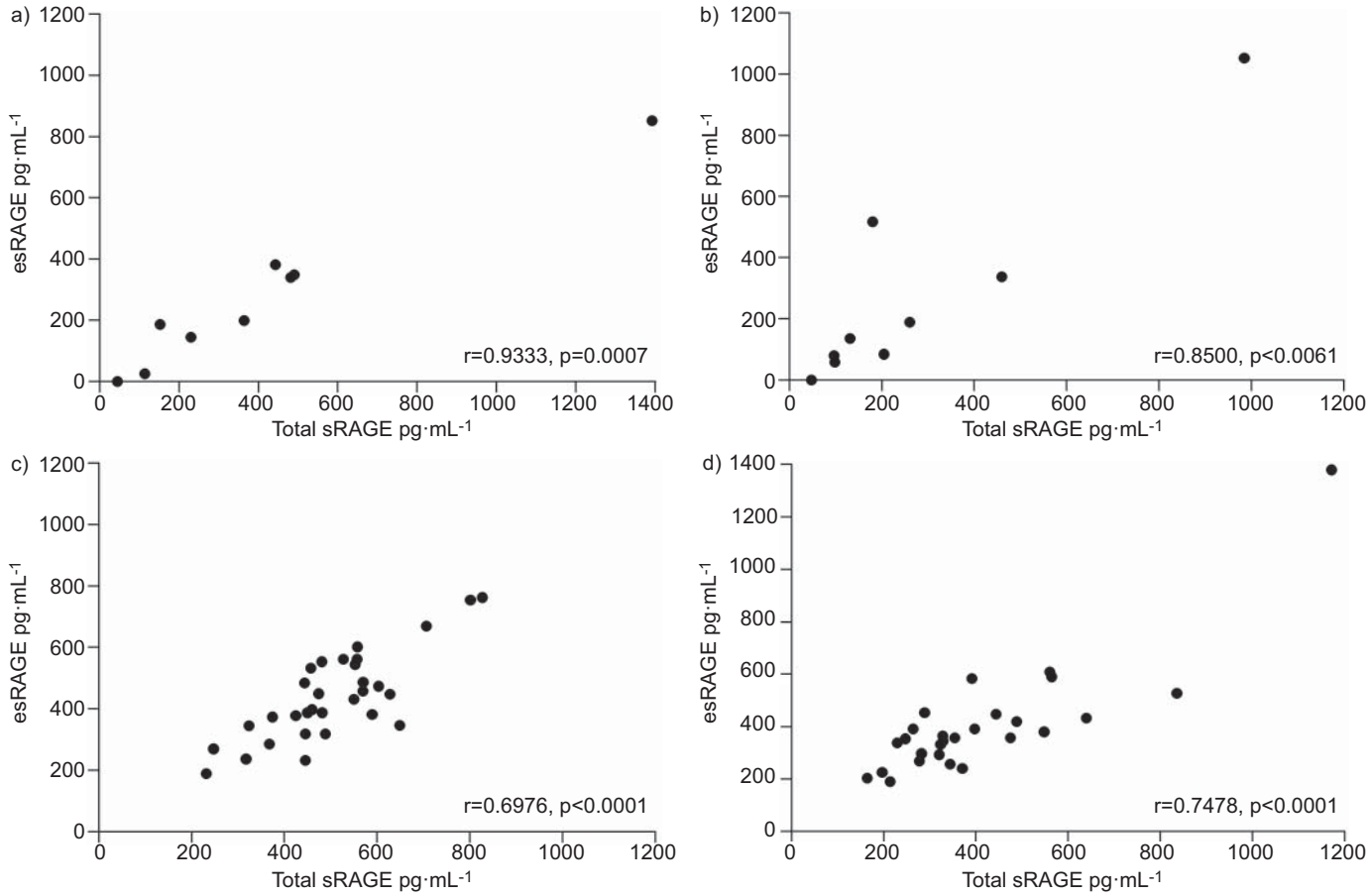

FIGURE 4. Correlation between total soluble receptor for advanced glycation end-products (sRAGE) and endogenous secretory RAGE (esRAGE) in the airways and systemically. A strong positive correlation was observed between bronchial lavage levels of total sRAGE and esRAGE in a) healthy subjects and b) chronic obstructive pulmonary disease subjects with and without neutrophilic airway inflammation. Similarly, there was a strong positive correlation between serum levels of total sRAGE and esRAGE in c) non-neutrophilic and d) neutrophilic asthmatic subjects. Total sRAGE was measured using an ELISA that detects all soluble forms of RAGE and esRAGE was measured using an ELISA that detects this specific soluble isoform, which represents a splice variant of the *RAGE* gene. Correlations were determined using Spearman's correlation.

currently employing proteomic approaches to examine the extent and nature of sRAGE proteolytic degradation and ligand binding in BL fluid from subjects with neutrophilic asthma or COPD.

While our findings clearly suggest that sRAGE degradation or sequestration are contributing factors to the observed deficiency, reduced or impaired production of sRAGE cannot be excluded as an additional causative factor. Indeed, it is known that certain single-nucleotide polymorphisms in the *RAGE* gene are associated with lower levels of plasma sRAGE in both health and disease [22, 23]. In light of evidence that RAGE is a genetic determinant of pulmonary function (FEV_1/FVC) [3], it will be important to determine whether genetic factors are predictors of sRAGE deficiency and neutrophilic-dominant inflammation in asthma and COPD.

Our findings raise the intriguing question of whether deficiency in sRAGE occurs as a consequence of neutrophilic inflammation (*e.g.* due to degradation by excessive levels of neutrophil-derived enzymes) or rather, is a causative factor that underlies neutrophilic inflammation. In cystic fibrosis, a chronic condition characterised by persistent airway neutrophilia, the complete absence of sRAGE in airway fluids is associated with increased expression of membrane RAGE (the signalling receptor) in both airway and peripheral blood neutrophils [24]. Thus, if augmentation of RAGE activity is part and parcel of the neutrophilic response, and one were to accept the prevailing view that sRAGE limits RAGE activity, it is tempting to speculate that dysregulation of the RAGE/sRAGE balance underlies airway neutrophilia, rather than sRAGE deficiency being a primary consequence of excessive neutrophilic inflammation. Interestingly, it has recently been shown that intratracheal administration of sRAGE in mice elicits neutrophilic lung inflammation, and that sRAGE is chemotactic for human neutrophils *in vitro* [11]. If this were true, then an alternative interpretation of our findings can be considered, that is: mechanisms that limit the production of sRAGE come in to play when the neutrophilic response becomes exuberant. Future systematic investigations of the relationship between RAGE/sRAGE expression and activity, and airway neutrophilia are likely to uncover new mechanisms of disease in asthma and COPD.

Although bacterial airway colonisation was not a significant independent predictor of lung sRAGE or HMGB1, we noted lower levels of sRAGE, and higher levels of HMGB1, in the airways of asthmatic and COPD subjects with bacterial airway

colonisation compared with those without. This is of interest, given that SMITH *et al*. [7], reported lower plasma levels of sRAGE in COPD subjects during disease exacerbation, which is frequently caused by respiratory bacterial and/or viral infection, compared with subjects with stable COPD. Interestingly, *RAGE*-deficient mice exhibit less lung inflammation and improved survival following intratracheal administration of *Streptococcus pneumoniae* or influenza A virus, suggesting that ligation of the RAGE pathway is detrimental to the host pathogen response [25, 26]. It is therefore possible that profound deficiency of sRAGE in the airways in neutrophilic asthma and COPD, together with enhanced expression of the membrane receptor [4, 24], provides an environment conducive to bacterial colonisation. Certainly, further investigation of the impact of the RAGE/sRAGE balance on airway bacterial colonisation, and disease exacerbations, in neutrophilic asthma and COPD is warranted.

sRAGE sequesters RAGE ligands and limits RAGE signalling [20]. Thus, we determined whether reduced sRAGE is associated with increased levels of the RAGE ligands HMGB1 and SAA, both of which are potent mediators of neutrophil inflammatory responses [1, 27]. We observed similar BL levels of HMGB1 in asthmatic and COPD subjects, with or without airway neutrophilia, and in healthy subjects. This contrasts with other studies demonstrating increased HMGB1 in the airways and systemically in stable COPD [4, 28] and in asthmatic sputum [6]. Differences in HMGB1 levels in our subjects were possibly abrogated by the use of ICS, as we observed significantly lower levels of HMGB1 in COPD subjects using ICS compared with those who were not. Indeed, in those studies reporting increased HMGB1 in COPD and asthma, the subjects were *not* using ICS.

Increased sputum levels of SAA, along with evidence of airway neutrophilia, have been reported in symptomatic asthmatic subjects [29], while SAA has been reported as a blood biomarker of acute COPD exacerbation [30]. Moreover, SMITH *et al.* [7] have recently shown that reduced levels of plasma sRAGE are associated with increased levels of plasma SAA in stable COPD and during acute COPD exacerbation [7]. In this study, SAA was undetectable in the majority of BL fluid samples, and it is possible that BL fluid is too dilute to allow quantification by ELISA.

In conclusion, deficiency in sRAGE in asthma and COPD is selectively associated with neutrophilic airway inflammation. Although further interrogation of the ligand–RAGE/sRAGE axis in asthma and COPD is necessary, correcting deficiencies in sRAGE could represent a therapeutic strategy for neutrophilic asthma and COPD.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

Statements of interest for V.M. McDonald and P.G. Gibson can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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