EDITORIAL

The RAGE against the storm

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Among the pattern recognition receptors (PRRs), the receptor for advanced glycation end-products (RAGE) has been shown to bind many damage-associated molecular patterns (DAMPs, also known as alarmins). DAMPs are released following tissue damage due to both infectious and non-infectious trauma whereby necrotic cells lose membrane integrity, causing their intracellular contents to leak into the surrounding space. In addition, activated immune cells and stressed structural cells (*e.g.* those under oxidative stress [1]) can actively secrete alarmins. High-mobility group box 1 (HMGB1), a nuclear protein that regulates transcription, is the prototypical DAMP. Uric acid, DNA, heat-shock proteins (HSPs) and members of the calcium-binding S100 proteins also function as DAMPs [2]. Ultimately, alarmins restore homeostasis with the promotion of tissue repair by: stimulating cell proliferation and migration; recruiting progenitors from the stem cell compartment; and exerting pro-angiogenic effects [3]. By recruiting and activating PRR-expressing cells of the innate immune system, including dendritic cells, DAMPs also promote adaptive immunity [2, 3]. Indeed, danger signalling might have been the evolutionary force that shaped the immune system [4].

RAGE is a transmembrane multi-ligand receptor belonging to the immunoglobulin superfamily [5]. RAGE binds several ligands, including HMGB1, S100A8/9 and S100A12 (calgranulins), advanced glycation end-products (AGEs), and serum amyloid A (SAA). RAGE is highly expressed in the lung, where it is believed to serve a homeostatic function [6]. Indeed, RAGE is considered a marker of alveolar type I (ATI) epithelial cells and, by mediating adherence to collagen-rich surfaces like the basal lamina, it appears to determine the ATI epithelial cell flattened and spread morphology that is important for ensuring effective alveolar gas exchange [7]. RAGE is likely to play a crucial role in lung tissue organisation, since downregulation or loss of RAGE expression leads to increased fibrosis [8] and proliferation and migration of pulmonary fibroblasts and alveolar epithelial cells [9]. This is in agreement with the reduced RAGE expression observed in the lungs of subjects with idiopathic pulmonary fibrosis [8, 9] and in nonsmall cell lung carcinomas [10]. Interestingly, polymorphism of *AGER*, the gene that encodes RAGE, has been associated with increased airflow obstruction (forced expiratory volume in 1 s (FEV₁)/forced vital capacity) in a recent genome-wide association study [11].

Interest in danger signals is growing in the field of respiratory medicine, with emerging clinical evidence implicating DAMPs as potentially important drivers of chronic inflammation. Recently, increased HMGB1 and RAGE expression has been reported in the airways of smokers and smokers with chronic obstructive pulmonary disease (COPD) [12]. Bronchoalveolar lavage fluid levels of HMGB1 were also elevated and correlated positively with markers of inflammation (*e.g.* interleukin (IL)-1 β) and negatively with lung function. Similar findings have been published for asthma: significantly increased HMGB1 levels have been found in plasma and induced sputum of asthmatics, with concentrations correlating positively with disease severity and negatively with lung function (*e.g.* FEV₁) [13, 14]. Interestingly HMGB1 was also correlated with the percentage of neutrophils in both diseases, indicating that it is an important chemotactic factor for neutrophil recruitment [15]. Other DAMPs might be released at the site of stress or injury: serum levels of various HSPs were increased in COPD patients [16], and HSP70 has been found to be elevated in plasma and induced sputum of asthmatics, with levels negatively correlated with lung function and positively correlated with neutrophil counts [14]. Moreover, circulating SAA levels have been found to be elevated in COPD [17]. Together these data strongly suggest that lung and systemic levels of one or more DAMPs could be useful biomarkers of neutrophilic inflammation and respiratory function.

AGEs are also likely to be involved in COPD, as indicated by their increased expression in the airways of COPD patients [18]. Pre-clinical and clinical data suggest that the asthmatic and COPD airways are subject to increased oxidative stress [19, 20]. AGEs are irreversible adducts which form at an accelerated rate in the hyperglycaemic and pro-oxidative environment and can activate intracellular signalling through RAGE, which is known to generate reactive oxygen species (ROS), thus exacerbating the oxidative stress [21]. Recently, HMGB1 has also been shown to induce ROS release from macrophages, perhaps also through RAGE [22].

RAGE activation is known to lead to a state of sustained cellular activation, due to the prolonged presence of RAGE ligands in the tissue, and to a positive feedback loop regulating RAGE expression [23]. Thus, RAGE activation by its ligands, including DAMPs, might perpetuate and amplify airway inflammation. Moreover, HMGB1 can bind diverse molecules and present them to specific receptors, for example lipopolysaccharide [24], IL-1 β [12] and DNA [25], resulting in enhanced effects.

The inflammatory process might be arrested through the release of soluble RAGE (sRAGE) forms, which can be produced by proteolytic cleavage of the membrane-bound receptor or by alternative splicing of the RNA transcript [26, 27]. Proposed

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roles for sRAGE include functioning as a decoy receptor that competes with membrane-bound RAGE for ligand binding and, more recently, association with membrane-bound RAGE at the cell surface, resulting in hindrance of intracellular signalling [28], which has been shown to require receptor oligomerisation [29]. sRAGE levels are reduced in the plasma of COPD patients, with the reduction correlating with a decline in lung function, suggesting that sRAGE confers protective effects for respiratory function [17]. Moreover sRAGE was lower during exacerbations compared with stable disease. sRAGE expression might be upregulated in activated immune cells or stressed cells in order to interrupt the self-perpetuating RAGE expression/activation loop, a mechanism that might be overwhelmed in disease. It is not clear yet whether genetic variations of *AGER* can account for this imbalance or whether it is driven by other factors such as inflammation.

In asthma, mast cells localise in the airway smooth muscle (ASM) [30], where they might contribute to changes in ASM (e.g. increased contractility and mass). Mast cells have been shown to degranulate in response to AGEs [21] and S100A12 [31]. Calgranulins are abundant in granulocytes and are secreted at sites of inflammation [32]. Among the calgranulins, the S100A8/A9 complex has been proposed to underlie chronic airway inflammation and airway remodelling in severe steroid-refractory asthma [33]. It can be hypothesised that DAMPs/AGEs induce mast cells to release mediators that contribute to the altered ASM physiology observed in asthma [34]. Intriguingly, heparin, which is contained in mast cell granules and released at sites of injury, is an endogenous antagonist of HMGB1 [35], and might serve as another switch-off signal for this DAMP.

Systemic sRAGE had previously been proposed as a biomarker of airflow obstruction severity in COPD [17]. In this issue of the *European Respiratory Journal*, SUKKAR *et al.* [36] now show evidence of a strong association between airway neutrophils and lung sRAGE in asthma and COPD, whereas HMGB1 levels were similar across subject groups. This study implicates RAGE in airway disease and suggests that the use of sRAGE as a prognostic tool could be expanded to predict neutrophilic asthma and COPD. Inconsistencies between this and other studies need to be highlighted as others have recently reported differences in sRAGE between non-neutrophilic asthma and healthy controls and in HMGB1 levels between health and disease, but these apparent discrepancies are likely to reflect different populations and sampling [13, 14]. The finding that bronchial lavage neutrophils were the only independent predictor of airway sRAGE offers new perspectives for understanding the pathogenesis of neutrophilic airway inflammation. Interestingly, the authors also report evidence of increased sRAGE degradation in neutrophilic asthma/COPD compared with non-neutrophilic asthma/COPD. The mechanisms underlying these differences will need further investigation, but it is possible that genetic variations of the *AGER* gene are involved, suggesting this pathway may be, in part, causal in neutrophilic airway disease. Alternatively, the dysregulation of the RAGE/sRAGE balance may be a consequence of an abnormal airway environment with upregulated airway inflammation, persistence of pathogens and damage.

In his madness, Shakespeare's King Lear "raged against the storm". In asthma and COPD, there is an inflammatory storm as a consequence of the complex interactions between the

environment and host defence. We are yet to fully appreciate the complexity of these interactions, but furthering our understanding of the role of RAGE and DAMPs in asthma and COPD will help us to bring more sanity to the way we phenotype our patients and begin to personalise therapy.

STATEMENT OF INTEREST

A statement of interest for C.E. Brightling can be found at www.ersjournals.com/site/misc/statements.xhtml

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