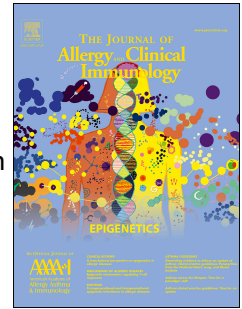


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Eric B. Brandt, Ph.D, Ian P. Lewkowich, Ph.D



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RAGE-induced asthma: a role for the receptor for advanced glycation end products in promoting allergic airway disease.

Eric B. Brandt, Ph.D,^b and Ian P. Lewkowich, Ph.D^{a,c}

^aDepartment of Pediatrics, University of Cincinnati, College of Medicine, Cincinnati, Ohio

^bDivision of Asthma Research, Cincinnati Children's Hospital Medical Center

^cDivision of Immunobiology, Cincinnati Children's Hospital Medical Center

Correspondence: Eric Brandt, Division of Asthma Research, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229.

Please send requests for reprints to Eric Brandt.

E-mail address: Eric.Brandt@cchmc.org, Tel: (513) 803 1034, Fax: (513) 636-1657.

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RAGE, the type I Receptor for Advanced Glycation End products (AGEs) belongs to the immunoglobulin superfamily and is abundantly expressed in the lungs, notably in type 1 alveolar epithelial cells.¹ As such, RAGE has been implicated in lung development, and in the pathogenesis of chronic lung diseases ranging from cystic fibrosis to asthma.¹ RAGE exists as a soluble decoy receptor, a membrane anchored pattern recognition receptor but is also present in the nucleus, where it promotes DNA double-stranded-break repair under homeostatic conditions (Figure 1A).² Oxidative stress has been shown to induce AGEs and RAGE, likely to promote cellular repair and maintain epithelial barrier integrity. In an *in vitro* scratch wound assay, inhibition of RAGE was sufficient to diminish human airway epithelial wound closure.³ *In vivo*, RAGE deficient mice are susceptible to fibrosis and are used as a model for idiopathic pulmonary fibrosis.²

Initially described as one of the receptors for advanced glycation end products, RAGE can also bind numerous endogenous alarmin-like ligands, like the S100A8/A9 heterodimer (calprotectin) or the high mobility group box 1 protein (HMGB1).¹ Both of these RAGE ligands have been implicated in the pathogenesis of allergic asthma. Calprotectin has antimicrobial activity, is produced by infiltrating neutrophils, and correlates with asthma severity in humans.⁴ However, S100A8 and/or S100A9 have been shown to either promote asthma pathogenesis or play a protective role in murine models of allergic inflammation depending upon the type of underlying inflammatory cell infiltration present.⁴ Increased HMGB1 levels have also been reported in the sputum of severe asthmatics and HMGB1 signaling through RAGE can promote allergic airway inflammation.^{5,6}

However, both S100A8/A9 and HMGB1 also signal through TLR4, a pattern recognition receptor (PRR) strongly implicated in allergic sensitization and Th2 responses. The fact that RAGE and TLR4 can bind similar ligands suggests functional redundancy between these two PRRs. Indeed, both RAGE and TLR4 mediate allergen-induced airway responses and epithelial wound closure,^{3, 7} as such, the relative contributions of both RAGE and TLR4 in S100A8/A9 and HMGB1 immune functions have been explored. In models of both house dust mite (HDM) and cockroach-induced experimental asthma-like disease, Th2 inflammation was significantly impaired in mice deficient for either TLR4 or RAGE.⁷ In a chronic model of asthma, where mice were exposed to HDM over a 7 week period, RAGE deficient mice were protected from Th2 inflammation.⁸ RAGE deficient animals given WT bone marrow demonstrated similarly reduced Th2 inflammation while WT mice given RAGE deficient bone marrow developed disease normally, suggesting that, like TLR4, RAGE-expressing stromal cells (likely type 1 alveolar epithelial cells) are responsible for promoting allergen-induced Th2 inflammation.⁸

However, more nuanced studies have suggested that HMGB1/RAGE may actually be downstream of HDM/TLR4 signaling, and thus may represent a key mediator of allergic sensitization (Figure 1A).⁷ Following a single intranasal HDM exposure, increased HMGB1 levels were observed after 48h in wild type and RAGE deficient mice but not in TLR4 deficient mice.⁷ HDM-induced IL-1 α release into the BALF also peaked one day earlier than HMGB1 and was similarly abrogated in TLR4 mice but not in RAGE deficient mice, suggesting that HDM induced IL-1 α was downstream of TLR4 signaling but upstream of HMGB1 secretion.⁷ Indeed, blocking IL-1 α resulted in a partial decrease in BALF HMGB1

67 following this single HDM exposure.⁷ Blocking HMGB1 in turn resulted in a partial decrease
68 in BALF IL-25 and IL-33 levels and subsequent Th2 responses upon HDM rechallenge.⁷

69
70 In this issue of the journal, Perkins et al examine the ability of RAGE to influence
71 signaling of cytokines typically produced during robust Th2 inflammation.⁹ Building on their
72 own work demonstrating that RAGE deficient mice did not demonstrate pulmonary
73 accumulation of ILC2s or subsequent Th2 inflammation after exposure to either *Alternaria*
74 extract or recombinant IL-33,⁸ Perkins et al here examine the capacity of IL-4 or IL-5/IL-13 to
75 induce asthma-like disease in RAGE deficient mice. While daily intranasal challenges with
76 either recombinant IL-4 or a combination of rIL-5 and rIL-13 over a 4-day period resulted in
77 BALF eosinophilia, mucus production, and robust inflammatory gene expression in WT mice,
78 this was nearly completely abrogated in RAGE deficient mice and mice treated with a RAGE
79 antagonist (FPS-ZM1). These data suggest that, in addition to the previously described role in
80 sensitization after pulmonary allergen exposures, RAGE may play a crucial role in much later
81 stages of diseases mediated by Th2 cytokines such as IL-4 and IL-13.

82 Mechanistically, Perkins et al demonstrate that reduced responsiveness to effector Th2
83 cytokines observed in RAGE deficient mice or following pharmacological inhibition of RAGE
84 was not due to altered levels of expression of the IL-4/IL-13 receptor components or protein
85 levels of the downstream signaling mediator signal transducer and activator of transcription 6
86 (STAT6).⁹ Moreover, following a single *in vivo* pulmonary challenge with rIL-13, comparable
87 levels of activated phosphorylated STAT6 (pSTAT6) were observed in the lung just after
88 exposure (10 minutes to 1 hour). However, pSTAT6 levels in IL-13-treated RAGE deficient
89 mice were markedly decreased by 8 hours and completely gone by 24 hours, in sharp contrast to

wild type mice, where sustained pSTAT6 levels were still present 24 hours later. Accordingly, when primary differentiated HBECs were stimulated with rIL13, pSTAT6 was still detected 48 hours later, whereas no pSTAT6 was detected when RAGE was blocked with the FPS-ZM1 antagonist. This suggests that signaling through RAGE is required for sustained STAT6 activation following stimulation through the IL-4/IL-13 receptor complex, but not for initial phosphorylation of STAT6 (Figure 1B).

As described above, RAGE binds many ligands that have been associated with the pathogenesis of allergic asthma. As antagonization of key intracellular signaling events associated with asthma pathogenesis would have important therapeutic implications, Perkins et al also explored the potential ligands mediating the ability of RAGE signaling to facilitate sustained signaling after Th2 cytokine exposure.⁹ Of the three RAGE ligands examined (S100A8/A9, S100B, HMGB1), only S100A8/A9 levels were elevated in wild type mice. Similar increases were not observed in RAGE deficient animals. Importantly, S100A8/A9 protein was present at low levels in the BALF during the first 8 hours post IL-13 challenge, but dramatically increased at 24 hours, when the most pronounced inhibition of STAT6 activation was observed in RAGE deficient mice, suggesting that production of S100A8/A9 is both dependent upon early RAGE signaling, and may contribute to RAGE-mediated maintenance of IL-13-induced STAT6 activation. However, given reports that S100A8/A9 may also antagonize the development of allergic asthma,⁴ formal studies to test the ability of this heterodimer to drive sustained Th2 cytokine signaling are clearly warranted.

Finally, a major unresolved question is how RAGE signaling maintains sustained STAT6 activation following type 2 cytokine stimulation. While this question was not directly examined

by Perkins et al, RAGE is known to induce robust oxidative stress, and production of reactive oxygen species (ROS). In turn, elevated ROS have been shown to inhibit some of the protein tyrosine phosphatases that are important for dephosphorylating, and thus inactivating activated STAT6 (Figure 1B; in green).¹⁰ Additional regulatory proteins have been demonstrated to negatively regulate STAT6 activation either through proteolytic cleavage (e.g. Calpains), or by blocking phosphorylation (e.g SOCS1). It is conceivable that the cascades of signaling events initiated by RAGE ligation inhibits the activity of some of these inhibitors through as yet undescribed mechanisms, thereby facilitating sustained STAT6 activation. Importantly, neither phospho-tyrosine phosphatase activity, nor expression/activity of the known negative regulators of STAT6 activity were examined here. Nonetheless, the exciting observations of Perkins et al demonstrating that blocking RAGE can, within 24 hours, effectively extinguish signals delivered by important Th2 effector cytokines like IL-4 and IL-13, warrant further investigations. Clarifying the mechanisms whereby RAGE inhibition attenuates Th2 cytokine signaling cascades and identifying the relevant ligand(s) involved in facilitating sustained signaling may allow for the development of novel therapeutics for the treatment of allergic asthma.

Figure Legend:

Role of RAGE and RAGE ligands in the development of allergic asthma. (A) RAGE ligands play an important role in lung development, epithelial repair (Nuclear RAGE expression promotes repair of double stranded DNA breaks), and allergic sensitization. Allergen-induced TLR signaling induces early IL-1 α release from lung epithelial cells, which in turn, triggers HMGB1 release. HMGB1 engagement of RAGE promotes the release of pro-Th2 cytokines like IL-33 and numerous chemokines, including the neutrophil chemokine CXCL1. Neutrophils are a major source of S100A8/A9 which also signals through RAGE and can be neutralized by the soluble RAGE decoy receptor. **(B)** Perkins et al demonstrate that Th2 effector cytokines (IL-4, IL-13) induce eosinophil chemokines, mucus production and the release of S100A8/A9, which signals back through RAGE in an autocrine manner, and may promote sustained STAT6 activation. In the absence of RAGE signaling, STAT6 activation is rapidly attenuated, and IL-13 responsiveness is abrogated. Potential mechanisms involved in RAGE-mediated maintenance of IL-4/IL-13-induced STAT6 activation are highlighted in green and include ROS-mediated inactivation of protein tyrosine phosphatases (PTPs) and/or inhibition of negative regulators of STAT6 (Calpains, SOCS1).

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