RAGE Haplodeficient Mice are Partially Protected From Allergen Induced Type 2 Inflammation

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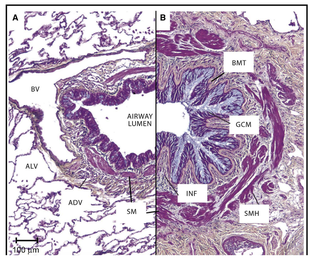
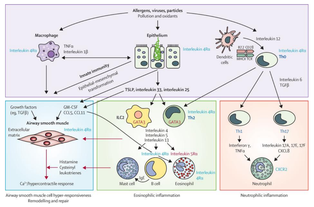
Background

Asthma





Lambrecht *et al. Immunity 2019*

Asthma

- Chronic disease of the airways:
  - Persistent inflammation
  - Mucus hypersecretion
  - Airway hyper-reactivity
- Caused by environmental and genetic factors.
- Pathologically heterogeneous
- Affects ~400 million people worldwide

Severe Asthma

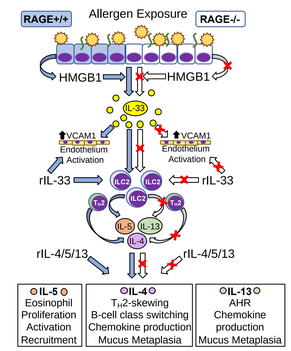
- Nearly 10% of cases are *severe*
- Accounts for 50% of healthcare burden of asthma in the US
- Half of severe asthmatics present with “Type 2-high” (T2hi) phenotype
- T2hi asthma is driven by type 2 cytokines: Interleukin(IL)-4, IL-5, and IL-13
- The mechanisms of T2hi asthma remain incompletely understood

RAGE

- DAMP and PRR
- Most abundantly expressed in the lungs
- Binds several ligands
- Activates an array of signaling pathways
- SNPs are associated with variation in lung function

RAGE in Asthma

- RAGE-deficient (heterozygous global knockout) mice are protected in several models of T2hi asthma/allergic airway disease (AAD)
- RAGE is required at several steps in the pathologic process of T2hi AAD
- Here we examine the effects of RAGE haplodeficiency in multiple models of AAD



Perkins et al. *Allergy* 2021

Methods

Experimental models of asthma
We utilized three established mouse models of T2hi asthma/ allergic airway disease:
1) Alternaria alternata
2) House dust mite (HDM) and
3) rIL-33
Allergens (Alternaria/HDM) were purchased from Greer Labs and rIL-33 from Biolegend

Animals
Wild-type (C57BL/6NTac), RAGE-Het (+/-) and RAGE-KO (-/-) were used in each experimental model

Inflammation
Bronchoalveolar lavage (BAL) cellular inflammation was determined by total cell counts and differential counts by cytospin analysis

BAL cytokines were measured by multiplex assay (Eve Technologies)

Results

Haplodeficiency of RAGE reduces airway inflammation in mouse models of *Alternaria* and House Dust Mite induced allergic airway inflammation

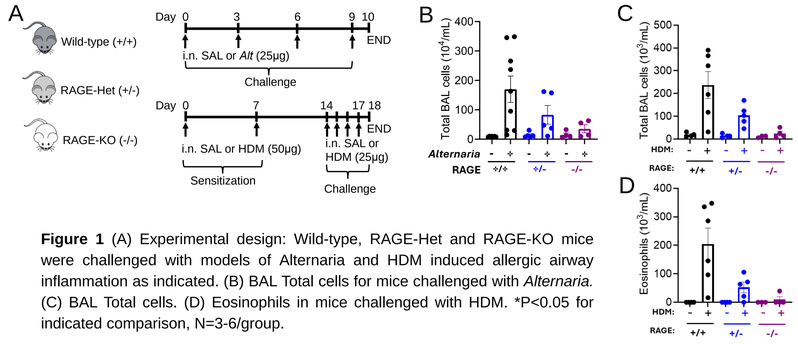


Figure 1 (A) Experimental design: Wild-type, RAGE-Het and RAGE-KO mice were challenged with models of Alternaria and HDM induced allergic airway inflammation as indicated. (B) BAL Total cells for mice challenged with *Alternaria.* (C) BAL Total cells. (D) Eosinophils in mice challenged with HDM. *P<0.05 for indicated comparison, N=3-6/group.

RAGE haplodeficient mice exhibit reduced airway inflammation in response to rIL-33 challenge

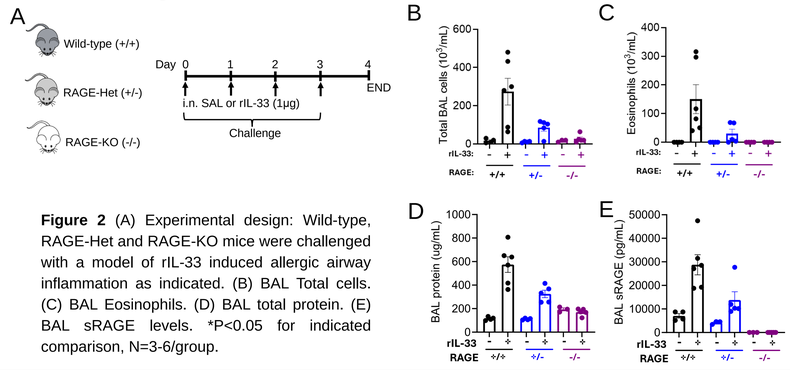


Figure 2 (A) Experimental design: Wild-type, RAGE-Het and RAGE-KO mice were challenged with a model of rIL-33 induced allergic airway inflammation as indicated. (B) BAL Total cells. (C) BAL Eosinophils. (D) BAL total protein. (E) BAL sRAGE levels. *P<0.05 for indicated comparison, N=3-6/group.

rIL-33 induced type 2 cytokine responses are reduced in RAGE haplodeficient mice

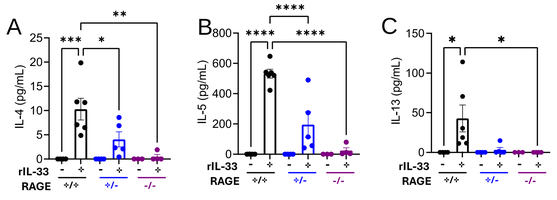


Figure 3 BAL type 2 cytokine levels. (A) Interleukin-4 (IL-4), (B) Interleukin-5 (IL-5) and (C) Interleukin-13 (IL-13) *P<0.05 for indicated comparison, N=3-6/group.

rIL-33 induced monocyte/macrophage chemokines are reduced in RAGE haplodeficient mice

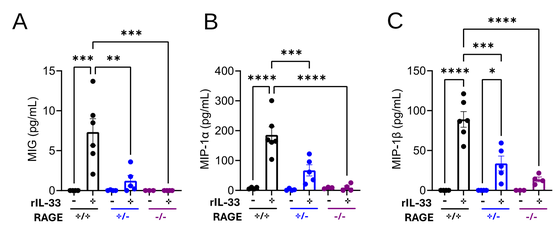


Figure 4 BAL Monocyte, macrophage chemokines. (A) Monokine-induced by gamma interferon (MIG), (B) Macrophage inflammatory protein-1α (MIP-1α) and (C) Macrophage inflammatory protein-1β (MIP-1β). *P<0.05 for indicated comparison, N=3-6/group.

Results

Type 1 and Type 17 cytokines are reduced in rIL-33 challenged haplodeficient mice

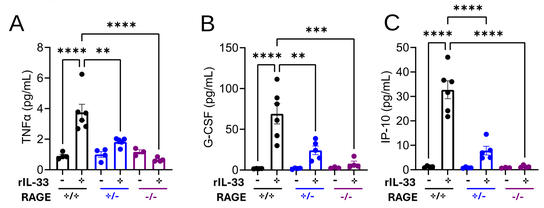


Figure 5. BAL Th1/Th17 cytokines. (A) Tumor necrosis factor α (TNF α), (B) Granulocyte-colony stimulating factor (G-CSF) and (C) Interferon gamma induced protein 10 (IP-10) *P<0.05 for indicated comparison, N=3-6/group.

Summary

Progressive reduction in RAGE expression correspondingly reduces airway inflammation in well established models of asthma

Discussion

- Our previous studies have demonstrated that full genetic knockout of RAGE (global) significantly protects mice in several experimental models of asthma
- Here we demonstrate that haplodeficiency also partially protects mice several models of T2hi asthma
- We have also recently shown that RAGE-deficient mice are protected in a model of severe T2lo asthma. However, studies are needed in additional models to assess the effects of haplodeficiency
- Current data suggests that RAGE is a promising target for asthma treatment, however, studies are needed to define the mechanisms by which RAGE promotes airway inflammation and asthma

Future Directions

- Determine the effects of RAGE haplodeficiency on airway hyperresponsiveness by FlexiVent analysis
- Determine the effects of RAGE haplodeficiency on airway mucus metaplasia and tissue inflammation
- Examine which RAGE-expressing cells promote immune responses to allergens (e.g. structural or inflammatory)

Acknowledgements

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