

1 **TITLE:** Mouse eosinophil-associated ribonuclease-2 exacerbates the allergic response

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3 To the Editor,

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5 Eosinophil granule cytotoxic proteins, including eosinophil-derived neurotoxin (EDN), play
6 important roles in the pathogenesis of allergic inflammation.¹ Serum EDN concentrations are
7 considerably higher in patients with severe asthma, which suggest that EDN is also a potential
8 biomarker for diagnosis. Mouse eosinophil-associated ribonuclease-2 (mEAR2), is considered as a
9 murine ortholog of human EDN.²⁻³ mEAR2 and EDN share several common characters and
10 biological activities,²⁻⁴ which make an animal model of mEAR2 might become a potential useful
11 model to study the functional role of mEAR2 as well as EDN in allergic disease such as allergic
12 asthma.

13 To investigate the contribution of mEAR2 in allergic diseases, we generated mEAR2-
14 deficient mice (mEAR2^{-/-}) in a C57BL/6J background by removing coding exon 2 (gene also
15 contains a non-coding exon 1) (Fig.S1A-C). In line with the expected genotype (Fig.S1D), mEAR2
16 expression was abrogated in bone marrow, blood, and lung tissue (Fig.1A-B) as well as in bone
17 marrow-derived eosinophils (BMDE) (Fig.1C) from mEAR2^{-/-} mice. No alteration in the expression
18 of other mEAR family members was observed in mEAR2^{-/-} mice or mEAR2^{+/-} mice (Fig.S1E).
19 Furthermore, BMDE from mEAR2^{-/-} mice differentiated in similar numbers and had identical
20 histological morphology as those from mEAR2^{+/+} animals (Fig.1D-E). Importantly, mEAR2^{-/-}
21 BMDE displayed similar chemotaxis towards eotaxin-1 (Fig.1F) and cytokine production (Fig.1G).
22 This suggests that, at resting state, mEAR2 expression does not have a developmental or functional
23 impact on eosinophils.

24 To assess for a potential role of mEAR2 in allergic asthma, allergic airway inflammation
25 (AAI) was induced by house dust mite (HDM) administration (Fig.S2A).⁶ Upon HDM sensitization
26 and challenge, lung histological inflammation (Fig.2A), and inflammatory cell recruitment into

27 bronchoalveolar lavage fluid (BALF) (Fig.2B) significantly decreased in mEAR2^{-/-} compared to
28 mEAR2^{+/+} mice. A particularly prominent effect was observed on eosinophil recruitment suggesting
29 a direct role of mEAR2 in eosinophil activation/migration/recruitment upon antigen challenge.
30 Importantly, airway hyperresponsiveness (AHR) in sensitized and challenged animals was virtually
31 abrogated in mEAR2^{-/-} mice with lung resistance and compliance (Fig.2C-D) comparable to
32 unchallenged mice. Lung Type 2 cytokines (IL-4, -5, -13) were strongly decreased in mEAR2^{-/-}
33 mice (Fig.2E and S2B) while no effects were observed for Type 1 and Type 17 cytokines (Fig.S2B).
34 These differences only appeared after the last HDM challenge (Fig.S2C-E). Moreover, IgE
35 production was also strongly decreased in mEAR2^{-/-} mice (Fig.2F). Expectedly, while mEAR2
36 mRNA expression increased in the lungs of mEAR2^{+/+} mice upon HDM stimulation, it was not
37 detected in the lungs from mEAR2^{-/-} mice (Fig.S2F). These results demonstrate that eosinophil-
38 derived mEAR2 play a major role in AAI by acting on cellular inflammation, type 2 cytokine
39 response and IgE production upon challenge but not sensitization phase.

40 In *in vitro* experiments, BDME and/or HDM-sensitized splenocytes from mEAR2^{+/+} or
41 mEAR2^{-/-} mice were co-cultured with PBS/HDM. The protein level and mRNA expression of IL-4,
42 -5, and -13 was significantly increased in mEAR2^{+/+}/HDM compared with mEAR2^{+/+}/PBS and
43 mEAR2^{-/-}/HDM group (Fig.2G and Fig.S2G). Moreover, no increasing of type 2 cytokines was
44 observed in the individual eosinophils and splenocytes culture (Fig.S2G), suggesting that mEAR2
45 contributes to enhances type 2 immune response in cooperation with eosinophils and other
46 proinflammatory cells.

47 In conclusion, we first used a mEAR2-deficient mouse model to demonstrate that mEAR2 is
48 dispensable for eosinophil differentiation and function at resting state but essential for the
49 development of AAI including AHR and lung eosinophilia. mEAR2 increased the production of
50 Type2 cytokines with an ensuing increase in IgE production. mEAR2 exerts its effects, thereby
51 enhancing the allergic response.

52 **Clinical implications:** mEAR2, representing human EDN, might not only represent a clinical
53 biomarker for allergic asthma management and follow up but also a new therapeutic target.

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111 All the authors have no conflicts of interest to declare.

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126 **SUPPORTING INFORMATION**

127 Supporting Information section at the end of this article.

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129 **FIGURE LEGENDS**

130 **Figure 1. Unchanged characteristics of eosinophils from mEAR2-deficient mice at resting**
131 **state**

132 (A) RT-PCR of mEAR2 in the bone marrow, blood, and lung tissues. (B) mEAR2 deficiency in the
133 lung tissue. (C) mEAR2 deficiency in bone marrow-derived eosinophils. (D, E) Total cell number
134 and percentage of bone marrow-derived eosinophils and H&E staining, respectively. (F)
135 Chemotaxis assay toward eotaxin-1; chemotaxis index was calculated as migration events/baseline
136 events. (G) qPCR analysis. Data are expressed as means \pm SEM (n = 3 – 4 for each group). No
137 significant difference compared with mEAR2^{+/+} mice (all *p* value > 0.05). ND: not detected.

138 **Figure 2. Eosinophil-derived mEAR2 plays a major role in Allergic Airway Inflammation**

139 (A) Histology of lung sections. Red arrows indicate eosinophilic infiltration. (B) Bronchoalveolar
140 lavage fluid (BALF) cellularity. (C, D) Lung compliance and resistance. (E) Protein levels of type 2
141 cytokine in the BALF. (F) Total serum IgE. (G) Type 2 cytokine production in co-culture
142 experiments; Arbitrary units: percentage increase from mEAR2^{+/+}/PBS group. Data are expressed as
143 means \pm SEM (n = 3 - 4). * and ** Significant difference from mEAR2^{+/+}/HDM group, (*p* < 0.05)
144 and (*p* < 0.001), respectively.