Allergy – Letter to the Editor

Enzymatic activity of ACE2 regulates type 2 airway inflammation in mice

To the Editor,

Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a global pandemic. SARS-CoV-2 spike protein binds to the angiotensin-converting enzyme 2 (ACE2), a transmembrane endopeptidase on the surface of the airway epithelium, on host cells for invasion and infection; therefore, most current COVID-19 research has focused on ACE2. Patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis are reportedly at high risk of COVID-19 morbidity and mortality,[1,2] whereas those with asthma are not.[3] Asthma is an heterogeneous disease triggered by environmental factors such as house dust mites (HDM) and viruses that is typically characterized by chronic airway inflammation.[4] Exposure to these factors promotes epithelial cell damage, leading to the release of cytokines that will provoke a type 2 inflammatory response.[5] Kimura et al.[6] reported that interleukin (IL)-13 exposure reduces ACE2 expression in airway epithelium of patients with asthma, whereas interferons enhance ACE2 expression.[2] Altogether, ACE2 low expression in epithelial cells might protect asthma patients from COVID-19. However, the relationship between asthma-related allergic inflammation and ACE2 expression in the airway has not yet been assessed *in vivo*.

To explore these associations, we used an HDM-induced asthma mouse model. C57BL/6J mice were intratracheally exposed or not to HDM (4–15 mice/group) at days 0, 7, and 14. Bronchoalveolar lavage fluid (BALF) and lung tissue samples were harvested at day 3, 10, and 17 to investigate eosinophil numbers and *Ace2* expression (Figure 1A).

The eosinophil counts in BALF samples increased from day 3 to day 17 concomitantly with repeated HDM exposure in a step-wise fashion (Figure 1B; control vs day 17 [$p < 0.0001$], day 10 vs day 17 [$p = 0.0028$]), whereas lung tissue *Ace2* expression decreased (Figure 1C; control vs day 17 [$p = 0.0002$], control vs day 10 [$p = 0.0033$], day 3 vs day 10 [$p = 0.0066$]). These findings agree with Jackson et al. data[7], showing that *ACE2* was significantly reduced in human bronchial epithelial brush samples after a segmental bronchial allergen challenge. ACE2 activity was also significantly suppressed by HDM injection (Figure 1D, $p < 0.0001$). These results indicate that ACE2 expression and/or function is associated with the pathogenesis of allergic airway inflammation.

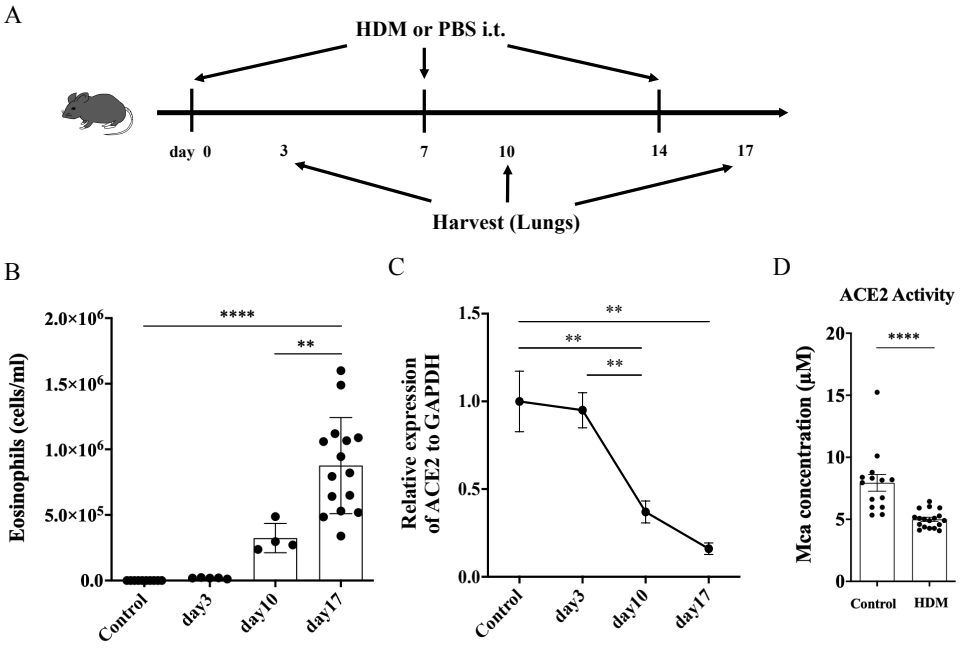

FIGURE 1. Eosinophils counts in the bronchoalveolar fluid (BALF) and *Ace2* expression in lung tissues from house dust mites (HDM)-exposed mice. (A) Experimental scheme. (B) Number of eosinophils in bronchoalveolar lavage fluid (BALF) samples. (C) Relative *Ace2* expression in lung tissues analyzed by quantitative real-time polymerase chain reaction. (D) ACE2 enzymatic activity in BALF was measured on day 14 after 6 h (14–18 mice/group from three independent experiments). Results are shown as mean ± SD (B) or SEM (C). Significance was determined by one-way ANOVA and Tukey's multiple comparisons test. $^{**}p < 0.01$ and $^{****}p < 0.0001$.

Additionally to its important role as a receptor for SARS-CoV-2, ACE2 is the main

enzyme for catalyzing angiotensin II into angiotensin$^{1\text{-}7}$ and has an anti-inflammatory effect in cardiovascular diseases.[8] Therefore, we hypothesized that ACE2 activity could modulate the type 2 inflammation of asthma. To test this hypothesis, we injected HDM-induced asthma mice with diminazene aceturate (DIZE), an activator of ACE2,[9] and examined its impact on the airway inflammatory process (Figure 2A).

DIZE treatment had no impact on *Ace2* expression (Figure 2B), whereas its activity was significantly elevated (Figure 2C, $p = 0.0148$). DIZE also significantly prevented the eosinophilia induced by HDM exposure (Figure 2D, $p = 0.0003$). Hematoxylin and eosin (HE), and periodic acid-Schiff (PAS) staining showed that DIZE markedly inhibited the HDM-induced infiltration of inflammatory cells and goblet cell hyperplasia (Figure 2E; HE [$p = 0.0002$], PAS [$p < 0.0001$]). DIZE treatment also suppressed the airway hyperreactivity in response to methacholine stimulation (Figure 2F, $p = 0.0483$). Furthermore, DIZE treatment resulted in significantly lower levels of IL-5 and IL-13 in the BALF and of IL-33 in the lungs, whereas IL-4 levels remained unaltered in the BALF (Figure 2G; IL-5 [$p = 0.0108$], IL-13 [$p = 0.0462$], IL-33 [$p < 0.0001$]). Overall, these results suggest that ACE2 activity, but not its expression, could regulate type 2 allergic airway inflammation in asthma. Interestingly, reduced IL-13 levels in BALF had no impact on *Ace2* expression in the lungs of this asthma model.

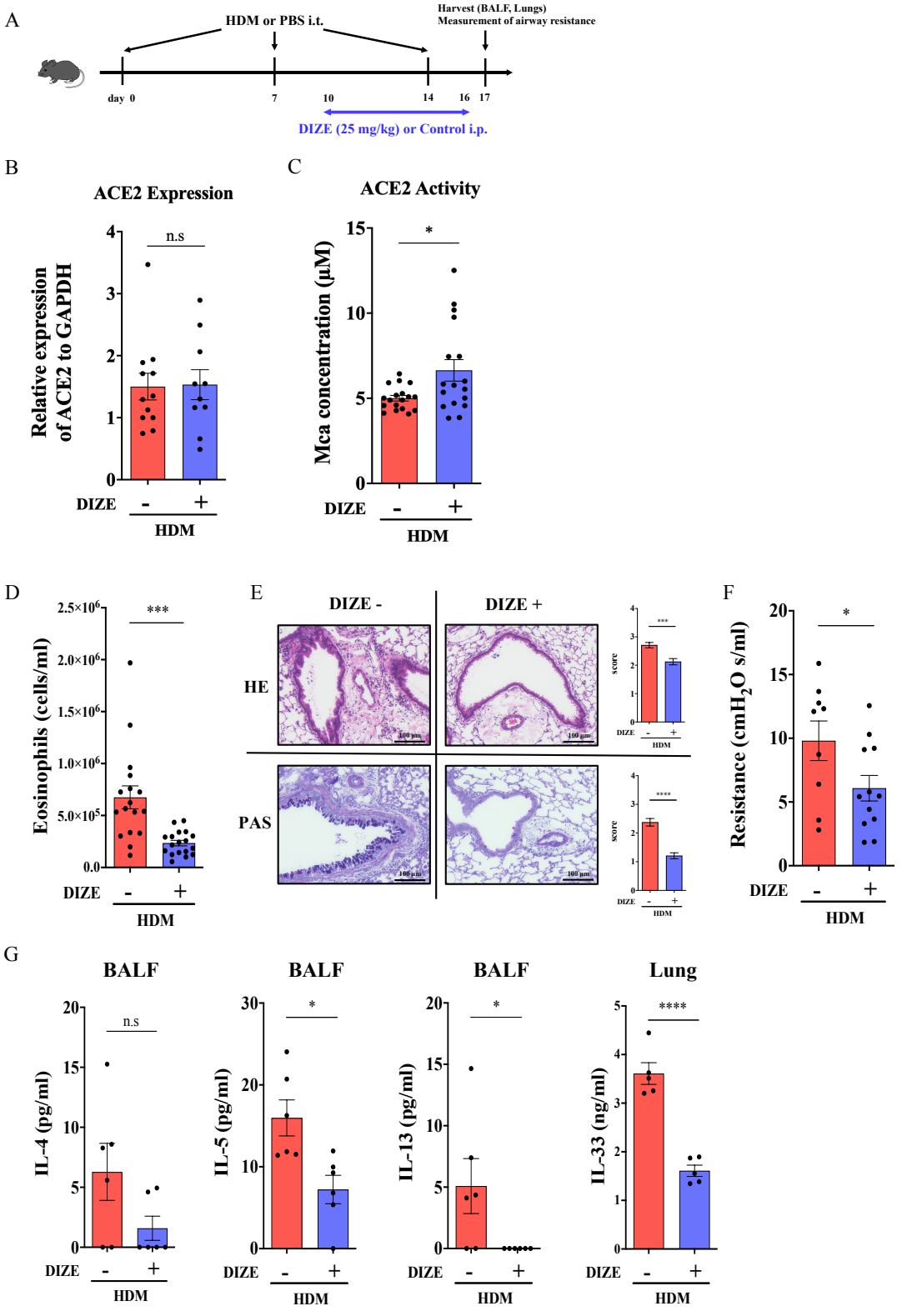

FIGURE 2. ACE2 activation by diminazene aceturate (DIZE) reduces type 2 inflammation in house dust mites (HDM)-exposed mice. (A) Experimental scheme. (B), *Ace2* expression in the lung determined by quantitative reverse-transcription polymerase chain reaction (10–12 mice/group from two independent experiments). (C) ACE2 enzymatic activity in bronchoalveolar lavage fluid (BALF) was measured on day 14 after 6 h (14–18 mice/group from three independent experiments). (D) Eosinophils count in BALF (5–6 mice/group from three independent

experiments). (E) Hematoxylin and eosin, and periodic acid-Schiff staining in the lung were blindly scored by five individuals on a scale of 0–3 for peribronchial inflammation (6 mice/group). (F) Bronchial hyperresponsiveness to 50 mg/mL methacholine (4–6 mice/group from two independent experiments). (G) IL-4, IL-5, and IL-13 protein levels in BALF and IL-33 in lung tissue was measured by enzyme-linked immunosorbent assay (5–6 mice/group). Results are shown as mean ± SEM (B–G). Significance was determined by One-way ANOVA and Bonferroni (B,C) and Student's *t*-test (D–G). *p<0.05, **p<0.01, ***p<0.001, and **** p<0.0001.

IL-33 is released by the lung epithelia and evokes type 2 cytokine production by group 2 innate lymphoid cells (ILC2).[5] Since DIZE treatment significantly reduced the IL-33 concentration in the lung, DIZE may inhibit IL-33 production by lung epithelial cells, leading to the suppression of ILC2-mediated cytokine release.

It has been speculated that suppression of IL-13 by asthma treatment may cancel out any resistance against SARS-CoV-2 infection due to the reduction of ACE2 levels. Our findings suggest that potentiation of ACE2 activity can prevent asthma-associated type 2 allergic airway inflammation without affecting ACE2 expression in the lungs. This effect offers new possibilities to treat asthma patients infected by SARS-CoV-2. Although tissues from type 2 cytokine-high allergic patients reportedly showed significantly lower ACE2 expression, which were inversely correlated with the Th2 cytokine levels and signature molecule expression,[6] further investigations are required to clarify whether reduction of IL-13 in the asthmatic airway after treatment of asthma directly affects ACE2 expression in the airway epithelia.

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