8. Siebel C, Lendahl U. Notch signaling in development, tissue homeostasis, and disease. *Physiol Rev*. 2017;97:1235-1294.
9. Hammad H, Lambrecht BN. Barrier epithelial cells and the control of Type 2 immunity. *Immunity*. 2015;43:29-40.
10. Wang W, Lyu S-C, Ji X, et al. Transcriptional changes in peanut-specific CD4+ T cells over the course of oral immunotherapy. *Clin Immunol*. 2020;219:108568.

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IL-33 prevents the enhancement of AP-N, DPP4, and ACE2 expression induced by rhinovirus HRV16 in the human lung endothelium—potential implications for coronaviral airway infections

To the Editor,

The lung vascular endothelium is a semi-permeable barrier that regulates the flow of nutrients, ions, cytokines, and immune cells between blood and lung tissues. Being a source of inflammatory mediators, it plays a crucial role in maintaining pulmonary homeostasis and in driving immune responses.[1] The main target for rhinoviruses (HRV) is the airway epithelium. However, we have recently shown that HRV16 may also infect the lung vascular endothelium *via* ICAM-1.[1] As the vascular endothelium may express coronaviral entry receptors, including aminopeptidase N (AP-N), dipeptidyl peptidase 4 (DPP4), and angiotensin-converting enzyme 2 (ACE2), it may possibly be infected by human coronavirus 229E (HCoV229E) and highly pathogenic MERS-CoV and SARS-CoV-2.[2] The latter one is responsible for currently ongoing COVID-19 pandemics.[3]

Seasonal peaks for HRV infections occur in early fall (September–November) and spring (March–May), whereas low pathogenic HCoVs which evoke mostly mild upper airway infections appear in winter and early spring (December–April). Both viruses overlap in March and April. HRV and HCoV co-infections or subsequent infections may lead to severe viral pneumonias. In such patients, the diffuse alveolar lung damage and thrombotic angiopathy in alveolar capillaries have been observed, which suggests an active involvement of the lung vascular endothelium.[4] The effect of HRV on the expression of HCoV entry receptors on the lung vascular endothelium and its modulation by IL-33 widely distributed in asthmatic airways has not been elucidated.

Firstly, we confirmed the HRV16 infection (MOI3) of human lung microvascular endothelial cells (HMVEC-L) (Figure 1A) and we observed the increase of IFN-β, RANTES (Figure 1B,C) IL-6, and TNF-α protein release (Figure S1A,B,C). Then, we noticed that HRV16 may enhance the expression of AP-N, DPP4, and ACE2 in HMVEC-L (Figures 1 and 2). The pattern of HRV16-induced ACE2 mRNA expression varied from DPP4 and AP-N. AP-N and DPP4 mRNA expression began to grow at 24 h and further increased at 72 h upon incubation with HRV16 (Figure 1D,G). In contrast, the transient enhancement of ACE2 mRNA expression occurred already at 5 hours (Figure 2A). Regardless of these differences, HRV16 led to the significant increase of AP-N, DPP4, ACE2, and TMPRSS2 surface protein expression observed by flow cytometry (Figures 1E,H and 2B,D) and confocal microscope (Figures 1F,I and 2C,E) (isotype controls and gating strategy: Figures S7 and S8). We did not notice any significant effect of HRV16 in lower MOI (0,1 and 1) on the expression of any receptor (Figures S2A–C).

Recently, we have shown that IL-33 may enhance HRV-induced release of cytokines, chemokines, and growth factors by the lung vascular endothelium.[5] In order to analyze the effect of IL-33, HMVEC-L were pre-incubated with IL-33 (10 ng/ml) for 24 h and subsequently exposed to HRV16 (MOI 0,1; 1; 3). These results confirmed our previous findings showing that IL-33 increased the capture of HRV16 by cells (Figure 1A). Despite IL-33 prevented the induction of AP-N, DPP4, and ACE2 mRNA expression by HRV16 (Figures 1D,G and 2A). At the same time, AP-N, DPP4, ACE2, and TMPRSS2 surface expressions were significantly lower, when HMVEC-L were infected with HRV16 upon the pre-treatment with IL-33 (Figures 1E,F,H,I and 2B–E). This effect of IL-33 was partially diminished by the blockade of ST2 receptor with anti-ST2 antibodies (3 μg/ml) before the pre-treatment of cells with IL-33 (Figure S3A,B,C). To note, IL-33 alone affected neither AP-N nor ACE2 and TMPSSR2 expression, whereas it slightly decreased the surface density of DPP4 (Figure 1H). IL-33 did not significantly change the effect of HRV16 in lower MOI (0,1; 1) on the receptor expression (Figure S2A,B,C).

Our results led us to pose the question about the possible mechanism of the induction of AP-N, DPP4, and ACE2 with HRV16. First, heat-inactivated HRV16 did not exert any effect (Figure S4A,B,C,D), which indicates that the change in AP-N, DPP4, and ACE2 expression requires an active virus. Second, the pattern of ACE2 mRNA expression was similar to the expression of both HRV16 copy number and IFN-β mRNA, which suggests an activation of common cytoplasmatic

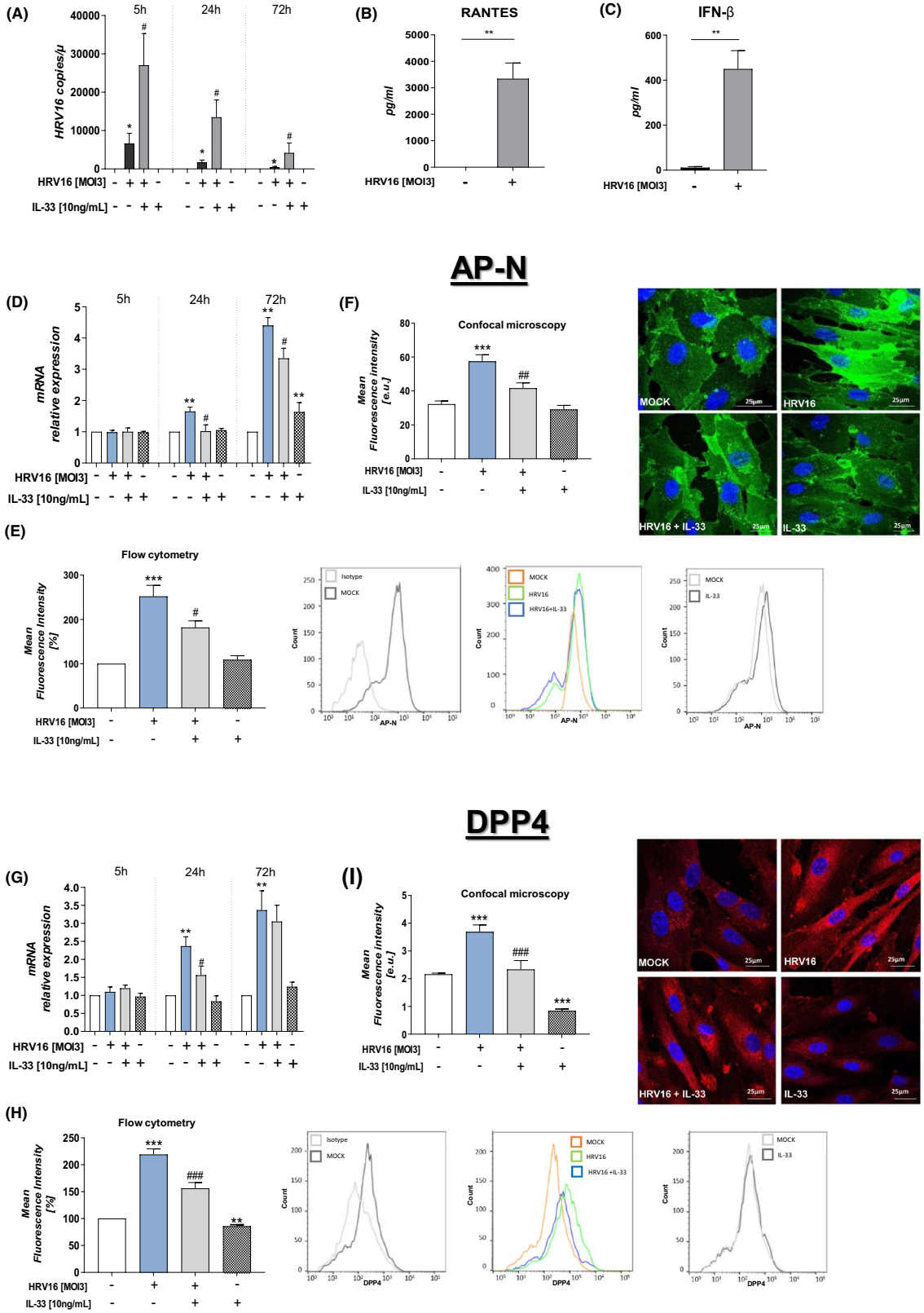

FIGURE 1 mRNA and protein expression in HMVEC-L cells incubated with HRV16 (MOI3) alone or upon 24-h pre-stimulation with IL-33 (10 ng/ml) or stimulated only with IL-33 (10 ng/ml)up to 72 h after infection. (A) The effect of HMVEC-L pre-stimulation with IL-33 (10 ng/ml) on HRV16 copies. (B, C) Release of IFN-β and RANTES by HRV16-infected HMVEC-L. (D)AP-N mRNA expression and (E, F) AP-N surface expression (green—AP-N-FITC, blue nuclei—DAPI) (confocal microscope) (G) DPP4mRNA expression and (H, I)DPP4surfaceexpression(red—DPP4—Alexa Fluor 594, blue nuclei—DAPI) (confocal microscope). The Mann–Whitney *U*-test was used to analyze differences between the two groups. Data are representatives of at least four independent experiments as means ± SEM; $n = 6–10$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ as compared to mock cells, $\#p < 0.05$; $\#\#p < 0.01$; $\#\#\#p < 0.001$ as compared to HRV16-induced cells

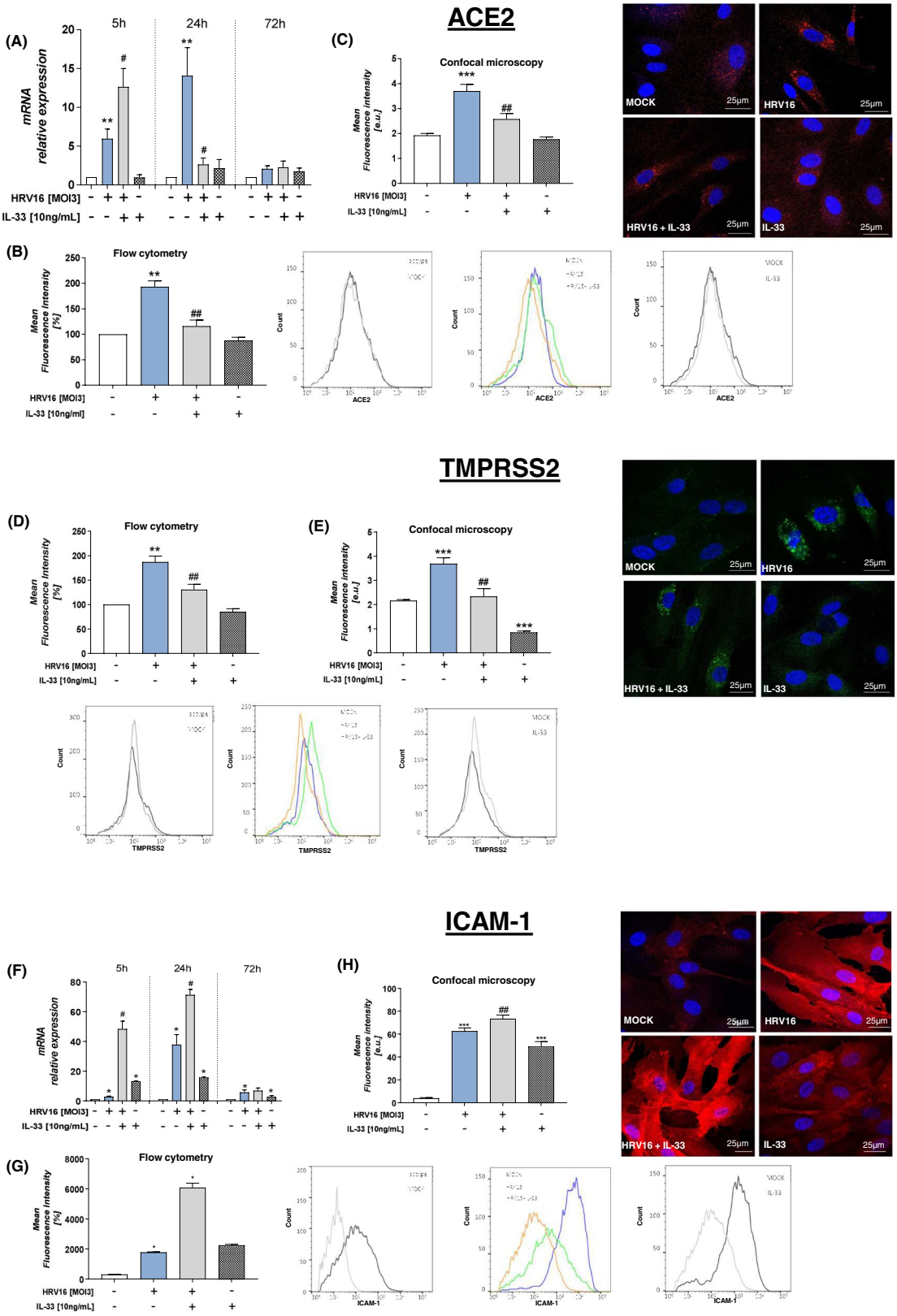

FIGURE 2 mRNA and protein expression in HMVEC-L cells incubated with HRV16 (MOI3) alone or upon cell 24-h pre-stimulation with IL-33 (10 ng/ml) or stimulated only with IL-33 (10 ng/ml) up to 72 h after infection. (A) ACE2mRNA expression (B, C) ACE2 surface expression(red—ACE2—Alexa Fluor 594, blue nuclei—DAPI) (confocal microscope) (D, E) TMPRSS2surfaceexpression(green—TMPRSS2-FITC, blue nuclei—DAPI) (confocal microscope) (F)ICAM-1mRNA expression(G,H)ICAM-1 surface expression (red—ICAM-1-PE, blue nuclei—DAPI) (confocal microscope). The Mann–Whitney U-test was used to analyze differences between the two groups. Data are representatives of at least four independent experiments as means ± SEM; $n = 6–10$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ as compared to mock cells, $\#p < 0.05$; $\#\#p < 0.01$; $\#\#\#p < 0.001$ as compared to HRV16-induced cells

pathways by the virus. ACE2 expression signatures were shown to overlap with type 1 and 2 IFN patterns. The neutralization of autocrine secreted IFN-β with anti-IFN-β antibodies (3 μg/ml) only prevented the increase of DPP4 mRNA expression (Figure S5A,B,C). Nevertheless, the increase of DPP4 and AP-N expression might be associated with autocrine action of other cytokines released by infected endothelium (i.e., IL-4,13; Figure S1D,E).[6] This matter needs further elucidation. Mechanism of the inhibition of HRV16-induced AP-N, DPP4, and ACE2 expression by IL-33 appears to be more unclear, as we confirmed that IL-33 must affect cells prior to their exposure to the virus. When IL-33 was administered together with HRV16 at the same time, the inhibitory effect of IL-33 did not occur (Figure S6).

In order to assess if HRV16 and IL-33 specifically regulate the expression of AP-N, DPP4, and ACE2 in HMVEC-L, we analyzed their effect on the secretion of inflammatory cytokines, including IFN-β, IL-4,6,13, and TNF-α. We observed that HRV16 alone increased IFN-β, IL-4,6,13, and TNF-α production. However, the pre-stimulation with IL-33 enhanced HRV16-induced secretion of IL-4,6,13, whereas it did not affect any release of IFN-β and TNF-α (Figure S1A–E). Moreover, HRV16 increased ICAM-1 expression, while the pre-stimulation of cells with IL-33 enhanced it further (Figure 2F–H). Blockade of ST2 receptor by anti-ST2 antibodies prevented the effect of IL-33 (Figure S3D). Interestingly, we noticed that the neutralization of IFN-β by anti-IFN-β antibodies decreased HRV16-induced ICAM-1 expression (Figure S5D). These results suggest that IL-33 may variously modulate the effect of HRV on the lung vascular endothelium. However, precise mechanisms need to be elucidated in further research.

To sum up, our data indicate that rhinovirus may upregulate ACE2, DPP4, and AP-N surface expression on the lung vascular endothelium, thus leading to the increased susceptibility to infections with human coronaviruses. Interestingly, IL-33 may prevent the induction of the expression of coronavirus entry receptors by HRV on the endothelium. Thus, the protective feature of IL-33 might underlie the lower occurrence of coronavirus associated as compared to rhinovirus-related asthma exacerbations.[3] Finally, we intend to emphasize that these data refer to the lung vascular endothelium although the airway epithelium is a prime target for both HRV and HCoV. However, the closeness of airway epithelium to endothelium lining mucosal vessels and alveolar capillaries enables the viral transmission between these tissues. Therefore, the possible modulation of coronavirus entry receptors by rhinovirus on the lung vascular endothelium and its clinical significance needs further research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Izabela Gulbas involved in planning of experiments, cell cultures, real-time PCR, flow cytometry assessments, data analysis, and manuscript writing. Adrian Gajewski involved in BioPlex and ELISA. Mateusz Gawrysiak involved in virus propagation and preparation for cultures, help in flow cytometry assessments. Robert Szewczyk involved in sample staining for confocal analysis. Aleksandra Likońska involved in mRNA isolation. Sylwia Michlewska involved in confocal microscope analysis. Marek L. Kowalski involved in co-supervision and advisory role. Maciej Chałubiński involved in conceptualization, co-planning of experiments, data analysis, manuscript co-writing, and supervision.

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REFERENCES

1. Gawrysiak M, Gajewski A, Szewczyk R, et al. Human rhinovirus HRV16 impairs barrier functions and regeneration of human lung vascular endothelium. *Allergy*. 2021;76(6):1872-1875. doi:10.1111/all.14671
2. Liu F, Han K, Blair R, et al. SARS-CoV-2 infects endothelial cells in vivo and in vitro. *Front Cell Infect Microbiol*. 2021;11:701278. doi:10.3389/fcimb.2021.701278
3. Chałubiński M, Gajewski A, Kowalski ML. The relationship between human coronaviruses, asthma and allergy-an unresolved dilemma. *Clin Exp Allergy*. 2020;50(10):1122-1126. doi:10.1111/cea.13718
4. Daisley H, Rampersad A, Daisley M, et al. Coronavirus 229E with Rhinovirus co-infection causing severe acute respiratory distress syndrome with thrombotic microangiopathy and death during Covid-19 pandemic: lessons to be learnt. *Autops Case Rep*. 2020;10:e2020194. doi:10.4322/acr.2020.194
5. Gajewski A, Gawrysiak M, Szewczyk R, et al. IL-33 augments the effect of rhinovirus HRV16 on inflammatory activity of human lung vascular endothelium-possible implications for rhinoviral asthma exacerbations. *Allergy*. 2021;76(7):2282-2285. doi:10.1111/all.14806
6. Ziegler CGK, Allon SJ, Nyquist SK, et al. SARS-CoV-2 Receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. *Cell*. 2020;181(5):1016-1035.e19. doi:10.1016/j.cell.2020.04.035

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