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Farm Dust Exposure Reduces Cytokine- and Rhinovirus-Induced IL-33 Expression in Bronchial Epithelial Cells

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To the Editor,

Pre-school wheeze, attributed to respiratory viral infections, with rhinovirus (RV) being the most important risk factor, may contribute to early-onset asthma development [1, 2]. In asthmatic children, the alarmin IL-33 is elevated in the airways and is involved in the development of T helper (Th)2 immunity and in Th2-driven immune responses to RV [3, 4]. Although IL-33 is constitutively expressed, IL-33 is also increased in response to pro-inflammatory cytokines like TNF-α and IFN-γ and after RV infections in airway epithelial cells [5, 6]. Children that grow up on traditional farms develop less wheezing, allergies and asthma [7]. This is partly caused by dust exposure from cow stables [7]. Furthermore, exposure to farm dust (FD) extract or its components protects against house dust mite (HDM)–induced allergic airway inflammation in mice [7] and enhances epithelial barrier function and RV clearance in primary bronchial epithelial cells (PBEC) [7]. Currently, the effects of FD on the alarmin IL-33 in human airway epithelial cells have not been investigated. The present study aims to investigate how exposure to FD affects the expression of IL-33 in PBEC. This article's Online Repository at Zenodo (https://zenodo.org/records/10417793) contains an extended version of this letter, including supporting data and a full description of methods.

To achieve our objective, submerged (S-) cultures of PBEC were pre-treated with FD for 24 h before being infected with RV-A1B for 48 h. Alternatively, cells were stimulated with TNF-α and INF-γ in the presence or absence of FD for 6 and 8 h. Changes in gene expression and protein levels were assessed using qPCR and HEK-Blue IL-33 reporter cells. We observed that FD pre-treatment inhibited RV-mediated increase of *IL33* mRNA, without affecting viral RNA (vRNA) levels (Figure 1A). We furthermore showed that FD decreased the TNF-α-/IFN-γ-induced expression of *IL33* mRNA and protein in S-PBEC (Figure 1B). We further observed that FD reduced the TNF-α-/IFN-γ-induced expression levels of both IL-33 mRNA and protein in differentiated (ALI-)PBEC. However, effects of both TNF-α/IFN-γ-exposure and FD treatment were less prominent in ALI-PBEC, compared to S-PBEC (Online Repository).

We next explored the mechanism underlying the reduction of TNF-α-/IFN-γ-induced IL-33 by FD. We therefore pre-treated S-PBEC for 1 h with inhibitors of downstream IFN-γ signalling pathways or FD and stimulated with IFN-γ (a key driver of TNF-α-/IFN-γ-mediated IL-33 expression [8]) for 6 h to assess *IL33* mRNA expression and for 30 min for the analysis of phosphorylated (p-)STAT1, p-p38, p-EGFR and GAPDH. In addition to FD, inhibition of JAK–STAT, p38 MAPK and EGFR also decreased *IL33* expression in S-PBEC (Figure 1C). Western blot analysis showed that FD partially inhibited p-STAT1 (with a trend towards significance) in IFN-γ-stimulated S-PBEC (Figure 1D), whereas p-EGFR or p-p38 were not affected (Online Repository).

Hermelijn H. Smits and Pieter S. Hiemstra contributed equally to this work.

Altogether, this suggests that FD reduces the expression of IL-33 at least in part by interfering with IFN-γ-interferon-gamma receptor (IFNGR)-JAK-STAT1 signalling in IFN-γ-stimulated S-PBEC.

In this study, we showed that FD reduces the expression of the alarmin IL-33 in PBEC following RV infection or exposure to TNF-α/IFN-γ. The inhibitory effect involves FD interfering with IFN-γ-induced STAT1 phosphorylation. A key signalling pathway that regulates IL-33 expression. Here, we mainly focused on the effects of FD on IL-33 expression in S-PBEC since these cells are more responsive towards the effects of both IL-33-inducing stimuli and FD, compared to fully differentiated ALI-PBEC cultures (Online Repository). This can be explained by the fact that S-PBEC cultures are flat (rendering a larger surface area) and are not covered by a layer of luminal cells and therefore are more accessible to FD. In contrast to ALI-PBEC, S-PBEC solely consists of basal

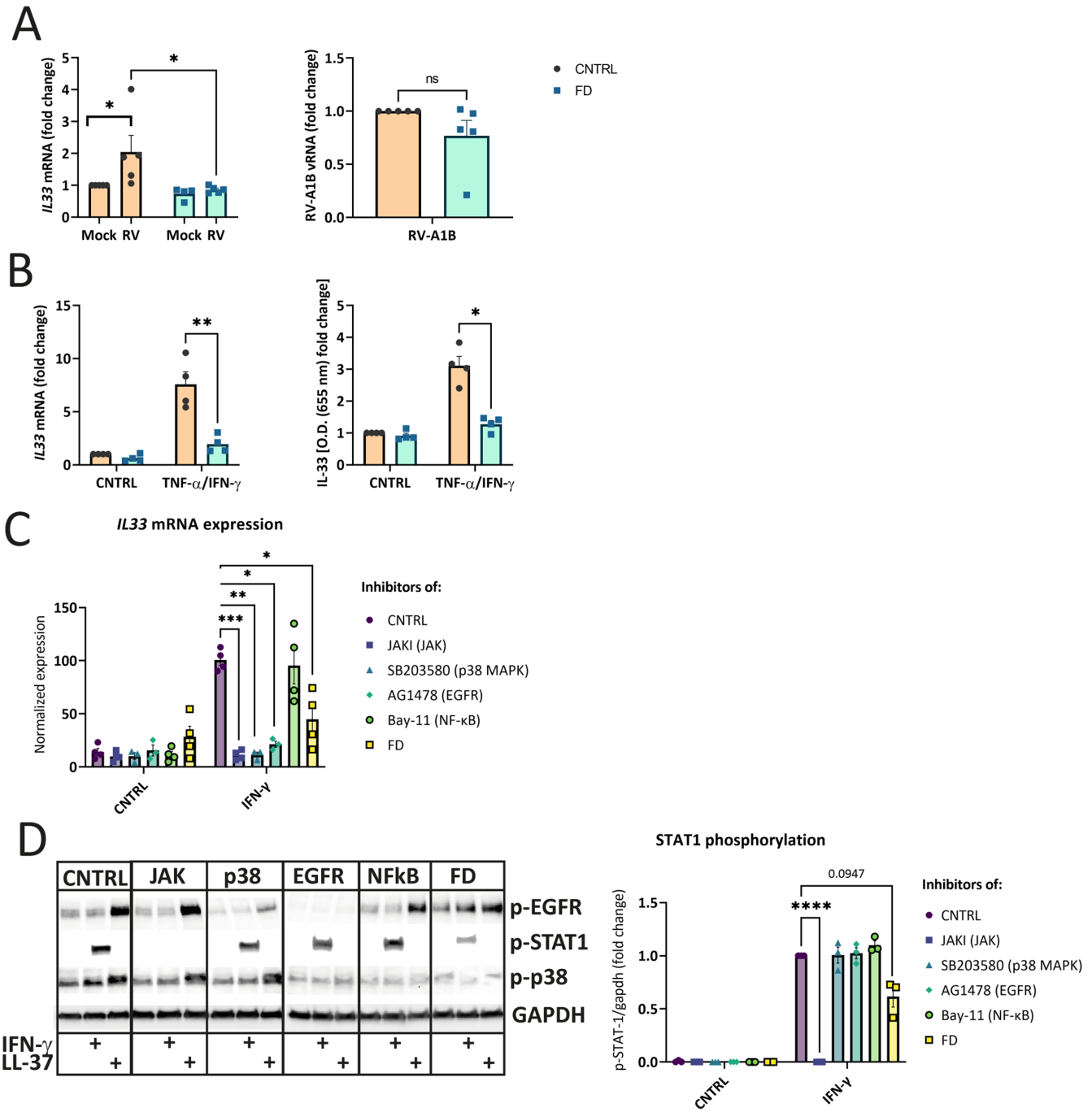

FIGURE 1 | Effects of farm dust (FD) extract on IL-33 expression and IFN-γ-downstream pathways in submerged cultures of primary bronchial epithelial cells (S-PBEC). S-PBEC were pre-incubated with FD (24 h) and infected with 0.5 MOI RV-A1B (48 h) (A) or stimulated with TNF-α/IFN-γ (6–8 h) to assess *IL33* gene expression (PCR) and IL-33 intracellular protein expression (HEK-Blue IL-33 reporter cells), respectively (B). (C, D) S-PBEC were pre-incubated with IFN-γ-signalling pathway inhibitors or with FD (1 h) and stimulated with IFN-γ for 6 h to determine *IL33* mRNA expression or for 30 min to assess phosphorylated (p)-STAT1, p-p38 and p-EGFR by western blot analysis. Data are shown as individual values or as means ± SEM and tested for significance using a Student's paired *t*-test or two-way ANOVA plus Šídák or Dunnett's post hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.001$.

Summary

- Farm dust reduces expression of IL-33 in rhinovirus-infected and in TNF-α/IFN-γ-exposed airway epithelial cells.
- This effect is in part mediated through interference with the interferon-gamma receptor-JAK–STAT1 signalling pathway.

cells, the main epithelial cell type that expresses IL-33 (Online Repository) and therefore in this context is likely the main cell type targeted by FD. So far, only studies using S-PBEC have shown that RV increases IL-33 expression in vitro [5, 6]. It would be therefore relevant to investigate the inhibitory effects of FD on RV-induced IL-33 in fully differentiated PBEC, for example, by using a co-culture model of ALI-PBEC with M2a-polarized monocyte-derived macrophages. This is based on a recent study in mice, showing that RV-induced expression of IL-33, was lower in mice lacking M2a macrophages [9]. The study's strength lies in using PBEC from multiple donors, thereby increasing the relevance of our findings. It needs to be noted that the PBEC used in this study were not derived from healthy donors but from tumour-free bronchial tissue derived from patients who underwent lung resection surgery for lung cancer. Furthermore, we were not able to show whether the active release of IL-33 was also inhibited by FD treatment, nor did we identify the active compound in FD that is responsible for inhibiting IL-33 expression. This is worth pursuing, but here beyond the scope of the study.

Our findings may have implications for understanding of mechanisms underlying the protective effects of a farming environment on allergy and asthma development in children. By limiting the release of epithelial alarmins through exposure to FD, the activation of immune cells and subsequent production of Th2 cytokines could be reduced. In conclusion, our study demonstrates that FD suppresses the induction of IL-33 in human bronchial epithelial cells during RV infection and exposure to pro-inflammatory cytokines in vitro. These findings provide insight into the putative protective role of FD against allergy and asthma development in children living on a farm.

Author Contributions

Jasmijn A. Schrumpf: conceptualisation (equal), writing–original draft (lead), investigation (lead), formal analysis (lead), writing–review and editing (equal), visualisation (lead). **Dennis K. Ninaber**: resources (equal), investigation (supporting). **Christoph Müller**: resources (equal). **Bettina Rankl**: resources (equal). **Mikaela Tham**: investigation (supporting). **Erika von Mutius**: resources (lead), conceptualisation (supporting), funding acquisition (equal). **Hermelijn H. Smits**: conceptualisation (equal), writing–review and editing (equal), funding acquisition (lead), supervision (equal). **Pieter S. Hiemstra**: conceptualisation (lead), resources (lead), writing–review and editing (lead), funding acquisition (equal), supervision (lead). All authors reviewed, provided input and approved the final manuscript.

Ethics Statement

Cells were isolated from macroscopically normal lung tissue obtained from patients undergoing resection surgery for lung cancer at the Leiden University Medical Center, the Netherlands. Patients from which this lung tissue was derived were enrolled in the biobank via a no-objection system for coded anonymous further use of such tissue (www.coreon.org). However, since 01 September 2022, patients are enrolled in the biobank using active informed consent in accordance with local regulations from the LUMC biobank with approval by the Institutional Medical Ethical Committee (B20.042/Ab/ab and B20.042/Kb/kb).

Conflicts of Interest

J.A.S., M.T., D.K.N., H.H.S. and P.S.H. declare no conflicts of interest related to this work. C.M. is the inventor of the following patents: PCT application number EP21189353, entitled 'Proteins identified from barn dust extract for the prevention and treatment of diseases' and PCT application, serial number PCT/EP2019/085016, entitled 'Barn Dust Extract for the Prevention and Treatment of Diseases'. B.R. is the inventor in PCT application number EP21189353, entitled 'Proteins identified from barn dust extract for the prevention and treatment of diseases'. E.v.M. report a grant form Gottfried Wilhelm Leibniz Award 2013 of the German Research Foundation. E.v.M. is inventor of the following patents: EP2361632 ('Specific environmental bacteria for the protection from and/or the treatment of allergic, chronic inflammatory and/or autoimmune disorders'), EP1411977 ('Composition containing bacterial antigens used for the prophylaxis and the treatment of allergic diseases'), EP1637147 ('Stable dust extract for allergy Protection'), PCT/US2021/016918, entitled 'Therapeutic Fractions and Proteins from Asthma-Protective Farm Dust', PCT application number EP21189353, entitled 'Proteins identified from barn dust extract for the prevention and treatment of diseases', in PCT application, serial number PCT/EP2019/085016, entitled 'Barn Dust Extract for the Prevention and Treatment of Diseases'. E.v.M. received honoraria as an expert from the Chinese University of Hongkong, the European Commission, HiPP GmbH & Co KG, AstraZeneca, Imperial College London, OM Pharma, ALK-Abello Arzneimittel GmbH and Boehringer Ingelheim International GmbH. E.v.M. received payment from Massachusetts Medical Society, Springer-Verlag GmbH, Elsevier Ltd, Boehringer Ingelheim International GmbH, European Respiratory Society (ERS), Universiteit Utrecht, Faculteit Diergeneeskunde, Universität Salzburg, Springer Medizin Verlag GmbH, Japanese Society of Pediatric Allergy and Clinical Immunology (JSPACI), Klinikum Rechts der Isar, University of Colorado, Paul-Martini-Stiftung, Astra Zeneca, Imperial College London, Children's Hospital Research Institute of Manitoba, Kompetenzzentrum für Ernährung (Kern), OM Pharma S.A., Swedish Pediatric Society for Allergy and Lung Medicine, Chinese College of Allergy and Asthma (CCAA), ALK-Abello Arzneimittel GmbH, Abbott Laboratories, Deutscher Apotheker Verlag GmbH & Co. KG, Japanese Society of Allergology.

Data Availability Statement

Raw data is available upon request. Supporting and additional data is publicly available in at Zenodo: https://zenodo.org/records/10417793.

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