Pollen induces reactivation of latent herpesvirus and differentially affects infected and uninfected murine macrophages

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LETTER TO THE EDITOR

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To the Editor,

Aeroallergens including plant pollen are continuously inhaled and deposited in the respiratory tract and, together with their soluble components, actively interact with a variety of cells in the airways.[1] Besides epithelial cells, B cells,[2] dendritic cells,[3] and macrophages[4] play a prominent role in these interactions. Apart from causing allergic diseases, pollen exposure might also affect host-pathogen interactions. Both, pollen and pathogens including viruses, may be encountered in the respiratory tract at the same time and by the same cells. For example, it has been shown that pollen exposure weakens the innate defense against respiratory viruses.[5] Other viruses that are omnipresent in humans are herpesviruses, due to their capability to persist lifelong in the host.[6] Therefore, a co-occurrence of pollen exposure and herpesvirus infection is likely. We have recently shown that cells persistently infected with gammaherpesviruses responded to environmental nanoparticle exposure by reactivation of latent virus.[7] Therefore, we hypothesized that additional airborne factors like plant pollen might reactivate latent herpesvirus too.

To answer this question, persistently murine gammaherpesvirus 68 (MHV-68)-infected macrophages (ANA-1-MHV-68)[7] were incubated with pollen grains or aqueous pollen extracts (APEs), and lytic virus was determined in the supernatant. As shown in Figure 1, both pollen grains (Figure 1A) and APEs (Figure 1B) resulted in an increase of lytic virus in the supernatant, when compared to the control, with significant increases after stimulation with Amb-pollen grains and all tested APEs.

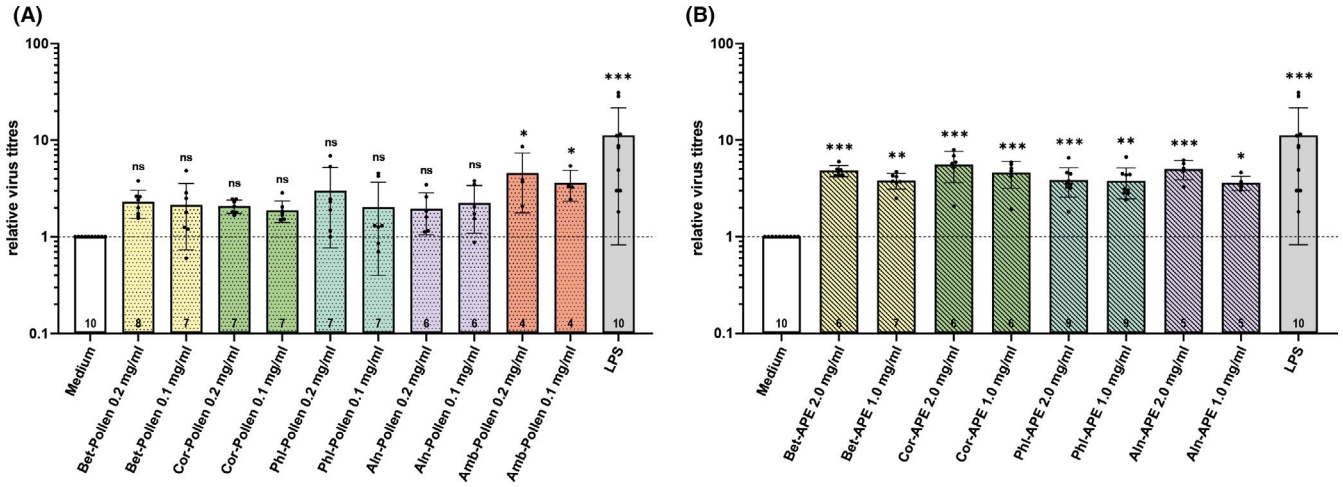

FIGURE 1 Exposure to pollen grains (A) or APE (B) induces reactivation of latent herpesvirus. ANA-1-MHV-68 were incubated with the indicated stimuli, and lytic virus was determined in supernatants by plaque assay after 72 h. Untreated cells were set as "1," and the values for cells after stimulation were calculated relative to the control. Each black dot represents the value from an individual experiment. The columns reflect the means ± SD from the number of independent experiments indicated at the bottom of each column. APE, aqueous pollen extract; Aln: alder (*Alnus*); Bet: birch (*Betula*); Cor: hazel (*Corylus*); Phl: grasses (*Phleum*); Amb: ragweed (*Ambrosia*)

Abbreviations: Aln-APE, aqueous extract of alder (Alnus glomerata) pollen; Amb-APE, aqueous extract of ragweed (Ambrosia artemisiifolia) pollen; ANA-I, murine macrophage cell line; APE, aqueous pollen extract; Arg 1, arginase 1 (=M2-specific marker); Bet-APE, aqueous extract of birch (Betula pendula) pollen; CCL, CC-motif family chemokine; Cor-APE, aqueous extract of hazel (Corylus avellana) pollen; CXCL, CXCL-motif family chemokine; IFN, interferon; IL, interleukin; iNOS, induced nitric oxide synthase (=M1-specific marker); LPS, lipopolysaccharide; M1, pro-inflammatory macrophage; M2, alternatively activated macrophage; MHV-68, murine gammaherpesvirus-68; MΦ, macrophage; PCA, principal component analysis; Phl-APE, aqueous extract of timothy grass (Phleum pratense) pollen; t-SNE, t-distributed stochastic neighbor embedding.

Additionally, we compared the response of ANA-1-MHV-68 to that of the respective uninfected parental macrophages (ANA-1).[7] First, we analyzed metabolic activity, apoptosis, and viability (Figure S1A-D). Overall, we did neither observe significant changes in response to stimulation nor between infected and uninfected macrophages. To evaluate whether stimulation with pollen grains or APEs leads to polarization into "M1" or "M2" macrophages, the mRNA expression of iNOS ("M1") and Arg1 ("M2") was determined. ANA-1-MHV-68 displayed an overall higher expression of iNOS (Figure 2A) and lower expression of Arg1 (Figure 2B). Within each cell line, exposure to pollen grains or APE did not significantly change the mRNA expression of the marker genes, with a few exceptions: Aln-pollen grains significantly upregulated iNOS in ANA-1-MHV-68 (Figure 2A), while Cor-APE significantly upregulated Arg1 in ANA-1 (Figure 2B). LPS increased iNOS in ANA-1 (Figure 2A). IL-4 increased Arg1 in both ANA-1 and ANA-1-MHV-68 (Figure 2B). Both Amb-pollen grains and LPS significantly downregulated Arg1 in ANA-I (Figure 2B). In addition, we determined the surface expression of CD86 ("M1") and CD206 ("M2") by flow cytometry, and the cytokine and chemokine release by multiplex assays of cell culture supernatants. CD86 was significantly higher expressed on ANA-1 (Figure 2C). CD206 was expressed less on both cell lines (Figure 2D). In both cell lines, stimulation with pollen grains or APEs did not significantly change the expression of CD86 or CD206, with the exception that Aln-pollen grains upregulated, and IL-4 downregulated, CD86 expression in ANA-1 (Figure 2C). Regarding cytokines and chemokines, uninfected cells secreted higher levels of both cytokines (Figure 2E) and chemokines (Figure 2F) than infected cells. LPS stimulation of uninfected cells induced a "M1"-specific profile, while stimulation with IL-4 led to a "M2"-specific profile (Figure S2A). Uninfected cells stimulated with APEs resembled a phenotype close

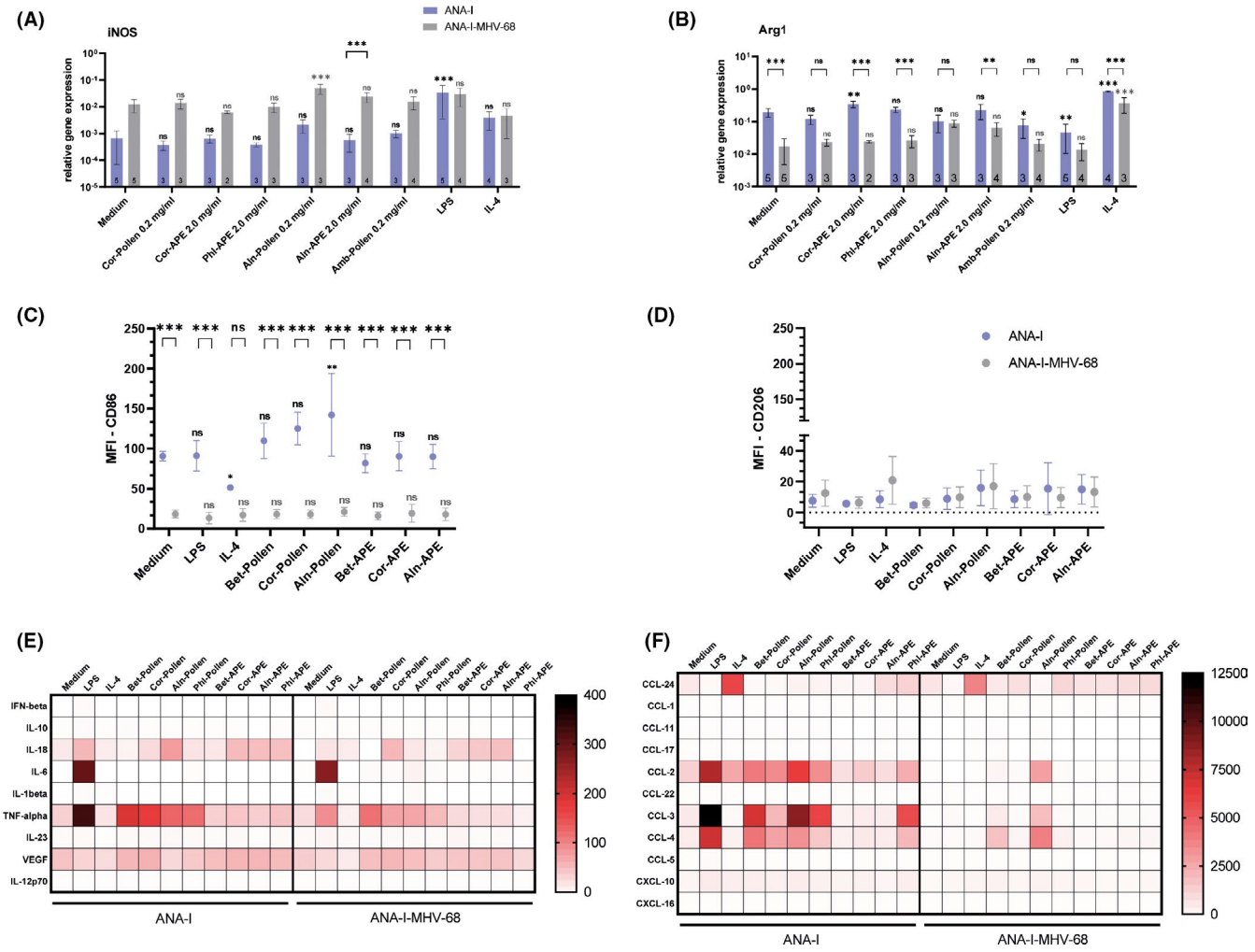

FIGURE 2 Exposure to pollen grains or aqueous pollen extract (APE) influences marker expression and cytokine and chemokine production. Cells were incubated with the indicated stimuli for 72 h, and the mRNA expression of iNOS (A) and Arg1 (B) was determined by RT-PCR. Data are expressed as relative gene expression compared to β-actin. The columns reflect the means ± SD from the number of independent experiments indicated at the bottom of each column. The surface expression of CD86 (C) and CD206 (D) was determined by flow cytometry. Shown are mean fluorescence intensities (MFI) ± SD from three independent experiments. The asterisks on top of the error bars indicate statistical significance between the indicated condition and the medium control and the asterisks above the brackets between the two cell lines. Cytokine (E) and chemokine production (F) was analyzed by multiplex assays. Data shown are means derived from 2 to 4 independent samples per condition

to "M2," whereas stimulation with pollen grains induced a distinct, "M1-like" profile. Stimulation of infected cells with LPS resulted in a profile characterized by high levels of IL-6 and TNF-α, while IL-4 stimulation mainly resulted in a marked upregulation of CCL-24. Bet-pollen induced a mixed phenotype, and Aln-pollen induced a more "M1-like" phenotype (Figure S2B). To further visualize phenotypic similarities, we applied t-distributed stochastic neighbor embedding (t-SNE). In uninfected macrophages (Figure S2C), "M1" (LPS)- and "M2" (IL-4)-polarized cells appeared clearly separated. Unstimulated and cells stimulated with APEs appeared more similar to "M2," whereas cells stimulated with pollen appeared more similar to "M1." The phenotypic segregation of "M1" and "M2" was even more pronounced in infected macrophages (Figure S2D). To identify the factors that contribute most to these differences, we performed factorial analysis of mixed data. For uninfected macrophages, dimensions 1 and 8 explained ≈35% of the overall variance (Figure S3A). The main contributing factor within dimension 1 was "treatment" (Figure S3B). For infected macrophages, dimensions 2 and 10 accounted for ≈16% of the overall variance (Figure S3C). Within dimension 2, the main contributing factor was again "treatment" (Figure S3D). Finally, to investigate whether stimulation with pollen grains or APEs influences macrophage functions, we analyzed cAMP production and phagocytic capacity. In ANA-1-MHV-68, baseline cAMP production was higher than in ANA-1, and there was a tendency toward decreased production after stimulation with IL-4 and pollen grains (Figure S4A). In ANA-1, stimulation with pollen grains decreased cAMP production to levels below detection, while LPS and IL-4 increased cAMP production. In both cell lines, APE did not change cAMP production. Treatment with pollen grains and APEs (except Aln-APE) decreased the phagocytic capacity of ANA-1-MHV-68 (Figure S4B). However, a different response was observed in ANA-1. Here, APEs (again except Aln-APE) increased the phagocytic capacity, whereas it was not changed by stimulation with pollen grains (Figure S4B).

In conclusion, we show that pollen grains and APEs can induce reactivation of a latent gammaherpesvirus. This is in line with our recent findings[7] and identifies pollen as a hitherto unrecognized "novel" trigger of herpesvirus reactivation.[8] Constantly recurring reactivation of herpesviruses by pollen grains and their associated compounds may result in chronic inflammatory processes that may lead to immunopathology and disease. Notably, seasonal variation in reactivation and shedding of Epstein-Barr virus (EBV) in healthy adults has been described earlier, with an increase of EBV shedding in saliva during spring and autumn months.[9] The authors hypothesized that one possible explanation might be that these months represent the peak allergy seasons in the area where the study group was recruited. By causing an influx of infected immune cells, the increased presence of allergens might contribute to the increased shedding.[9] Our data provide now the first experimental evidence for this hypothesis.

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CONFLICT OF INTEREST

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REFERENCES

1. Kauffman HF. Innate immune responses to environmental allergens. *Clin Rev Allergy Immunol*. 2006;30:129-140.
2. Oeder S, Alessandrini F, Wirz OF, et al. Pollen-derived nonallergenic substances enhance Th2-induced IgE production in B cells. *Allergy*. 2015;70:1450-1460.
3. Gilles S, Mariani V, Bryce M, et al. Pollen allergens do not come alone: pollen associated lipid mediators (PALMS) shift the human immune systems towards a T(H)2-dominated response. *Allergy Asthma Clin Immunol*. 2009;5:3-5.
4. Currie AJ, Stewart GA, McWilliam AS. Alveolar macrophages bind and phagocytose allergen-containing pollen starch granules via C-type lectin and integrin receptors: implications for airway inflammatory disease. *J. Immunol*. 2000;164:3878-3886.
5. Gilles S, Blume C, Wimmer M, et al. Pollen exposure weakens innate defense against respiratory viruses. *Allergy*. 2020;75:576-587.
6. Adler B, Sattler C, Adler H. Herpesviruses and their host cells: a successful liaison. *Trends Microbiol*. 2017;25:229-241.

7. Sattler C, Moritz F, Chen S, et al. Nanoparticle exposure reactivates latent herpesvirus and restores a signature of acute infection. *Part Fibre Toxicol*. 2017;14:2.
8. Stoeger T, Adler H. "Novel" triggers of Herpesvirus reactivation and their potential health relevance. *Front Microbiol*. 2019;9:3207.
9. Ling PD, Lednicky JA, Keitel WA, et al. The dynamics of herpesvirus and polyomavirus reactivation and shedding in healthy adults: a 14-month longitudinal study. *J Infect Dis*. 2003;187:1571-1580.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Comparison of nasal allergen challenges with dissolved Timothy grass lower-case pollen tablets and aqueous extract

To the Editor,

We and others have used nasal allergen challenge (NAC) to investigate the clinical and immunological effects of allergen exposure[1-3] and the efficacy of immunotherapy.[4] The procedure is safe and well tolerated.[5] For NAC to be a valid tool, it is essential that the allergen extracts used are standardized. The availability of these extracts is limited in some areas. We have previously used an aqueous extract of Timothy Grass (*Phleum pratense*) pollen, Aquagen® (ALK-Abello), supplied as a dry powder, to be reconstituted prior to use by dissolving in an albumin-based diluent, to 100 000 SQ-U/mL. Aqueous extract of Timothy Grass (*Phleum pratense*) pollen production has been discontinued, but the identical allergen is available as Timothy Grass lyophilisate tablets, Grazax® (ALK-Abello), 75 000 SQ-T, an approved, widely available and licensed product for treatment of severe seasonal allergic rhinitis. Timothy Grass lyophilisate tablets consists of purified, freeze-dried Timothy Grass pollen extract, plus three excipients, (fish) gelatine, mannitol and sodium hydroxide. We anticipated that the clinical effect when dissolved and used for NAC would be equivalent to the use of aqueous extract of Timothy Grass (*Phleum pratense*) pollen for a given allergen concentration. We therefore conducted a randomized-order, cross-over trial of NAC with the two allergen extracts.

Grass pollen allergic adults (18-65 years) and nonatopic controls were recruited from the Allergy Clinic at Royal Brompton Hospital, London. For inclusion/exclusion criteria, see Appendix S1. Following screening, participants underwent two NACs, at least 4 weeks apart, between September 2019 and January 2020. Participants were randomized to have either NAC using aqueous extract first, dissolved tablet second, or vice versa. Nonatopics underwent a single NAC with the dissolved tablet. Participants gave written, informed consent; the study was approved by regional ethics committee (ref.19/LO/1346) and registered with clinicaltrials.gov (NCT04078009). NACs were performed with aqueous extract/dissolved tablets diluted in 0.9% normal saline to 16 667 SQ-U/mL or 16 667 SQ-T/mL (equivalent to 5000 BU/mL or approximately 1 mcg major allergen (Phl p 5)/mL, see Appendix S1 for further details). Total nasal symptom scores (TNSS, 0-12) and peak nasal inspiratory flow (PNIF) were recorded before NAC and at 5, 15, 30 and 60 minutes. Peak expiratory flow (PEF) was recorded before and at 60 minutes. The primary endpoint was the area under the curve for TNSS 0-60 minutes (TNSS-AUC). The primary comparison was between NAC with aqueous extract and NAC with dissolved tablet. Secondary comparisons were between the first NAC and second NAC visit, and by first and second visit according to challenge extract.

Twenty-two volunteers were screened, of which 20 were included (Table S1). All 20 participants completed both challenges except one who did not undergo visit 2 due to an upper respiratory tract infection. Ten nonatopic individuals underwent dissolved tablet NAC only. Mean TNSSs were similar during NACs with each extract at baseline, 5, 15, 30 and 60 minutes (Figure 1A). Mean TNSS AUC was no different between the two ($P = .19$, paired t test; Figure 1B). Mean change from baseline PNIFs was also similar at 5, 15, 30 and 60 minutes (Figure 1C), and AUC was not different ($P = .99$; Figure 1D). Pearson's coefficient showed correlation between TNSS AUC following each challenge ($r = .65$, $P = .002$; Figure 1E). Bland-Altman plot of the difference between the TNSS AUC showed 18 of 19 (94.7%) paired results lying within ±1.96 standard deviations of the mean (Figure 1F). NAC in nonatopic individuals had no effect on TNSS or PNIF (Figure 1A, C). Individual TNSS following nasal challenge with aqueous extract of Timothy Grass (*Phleum pratense*) pollen and Timothy Grass lyophilisate tablets are depicted in Figure S3.

Whilst peak (5 minute) TNSS was equivalent at visits 1 and 2, mean scores at 15, 30 and 60 minutes were lower at visit 2 than visit 1, without reaching statistical significance (Figure 2A). Mean TNSS AUC was greater at visit 1 than visit 2 ($P = .03$; paired t test, Figure 2B). A similar trend was seen for ΔPNIF (Figure 2C, D). This effect appeared to be predominantly driven by participants who