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LETTER

Birch pollen extract enhances human cytomegalovirus replication in monocyte-derived dendritic cells

To the Editor,

Observational data revealed correlations between the birch pollen season and the prevalence of viral infections, including airway infections,[1] and the reactivation of latent herpesviruses.[2] One reason might be a pollen-induced disturbance of the integrity of epithelial tissues that would cause defects in its barrier function and thus allow for easier viral invasion. Importantly, the pollen matrix also contains compounds that can modulate immunity, irrespective of the allergenic traits of pollen.[3] This raises the question of whether birch pollen can directly affect antiviral immunity, to which dendritic

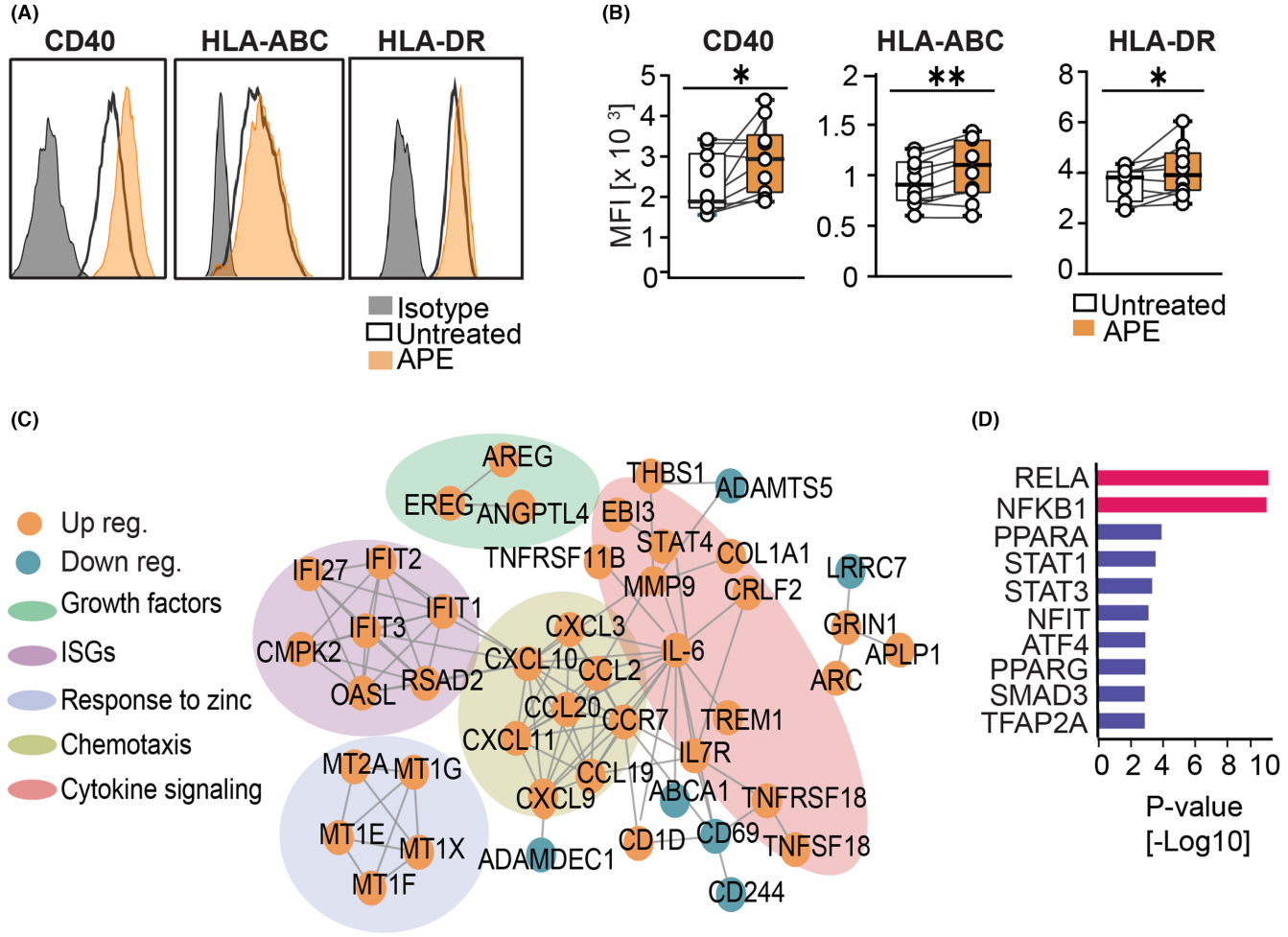

FIGURE 1 APE treatment promotes pro-inflammatory gene signatures in moDC. (A) Flow cytometric analysis of CD40, HLA-ABC and HLA-DR on moDC treated with 3 mg/ml APE for 24 h. (B) Statistical analysis of mean fluorescence intensity (MFI) of surface marker expression. Wilcoxon matched-pairs signed rank test, $^{*}p < .05$, $^{**}p < .0098$, mean ± SD, $n = 10$. RNA expression profiles of moDC treated with APE for 6 or 24 h were analyzed and DEG from both time points were combined. (C) Protein–protein interaction (PPI) network of combined DEG. (D) Transcription factor target analysis of combined DEG.

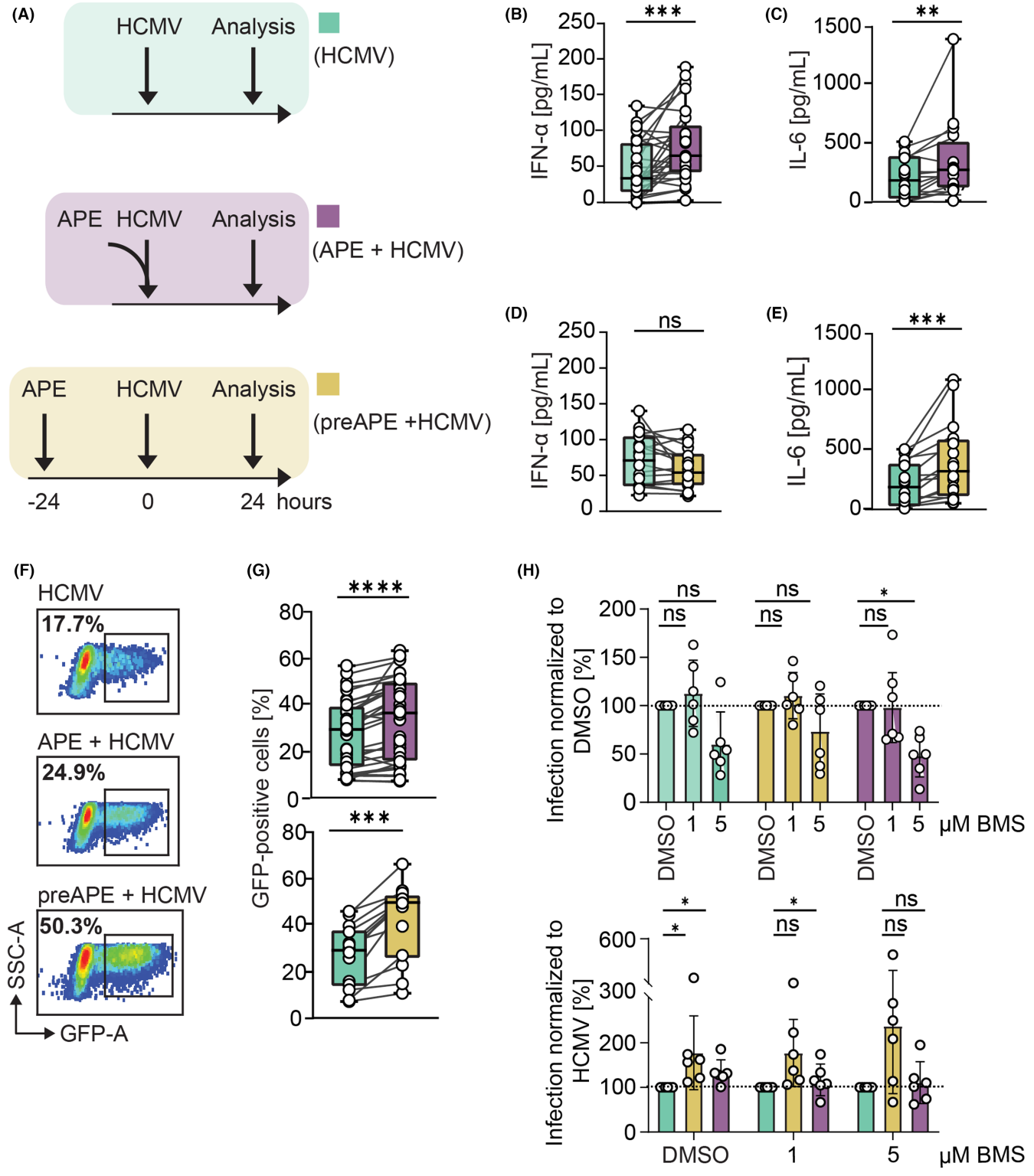

FIGURE 2 APE treatment enhances susceptibility of moDC to HCMV infection. (A) moDC were either exposed to HCMV, co-treated with APE and HCMV (APE + HCMV), or pre-treated with APE for 24 h and then infected with HCMV (preAPE + HCMV). IFN-α and IL-6 content in supernatants of (B and C) APE + HCMV treated and (D and E) preAPE + HCMV treated moDC. (F) GFP expression and (G) percentages of GFP-positive cells were analyzed. (H) moDC were treated with HCMV and APE in the presence of DMSO or 1 μM and 5 μM BMS. Wilcoxon matched-pairs signed rank test, $^{*}p < .05$, $^{**}p < .0078$, $^{***}p < .0007$, $^{****}p < .0001$, data represent mean ± SD. $n = 6–32$, each dot represents moDC from an independent donor.

cells (DC) critically contribute to. Therefore, here we addressed whether treatment of human DC with birch pollen affects their gene expression profiles, their innate antiviral responses, and their susceptibility to viral infection. In accordance with earlier studies,[3] treatment of monocyte-derived DC (moDC) with aqueous pollen extract (APE) downregulated LPS induced IL-12p70 responses in a

dose-dependent manner (Figure S1A). The strongest inhibition was detected at a concentration of 3 mg/ml APE, which we also used in the subsequent experiments. APE stimulation of moDC for 24 h moderately induced the surface expression of the DC maturation markers CD40, HLA-ABC, and HLA-DR (Figure 1A, B). Bulk RNA sequencing of moDC after APE treatment for 6 and 24 h revealed ample transcriptional changes (Figure S1B). Protein–protein interaction (PPI) network analysis of the combined differentially expressed genes (DEG) from both time points highlighted genes involved in pro-inflammatory innate immune functions such as type I interferon (IFN) signaling and IL-6 expression (Figure 1C). The transcription factors NF-κB and RELA were identified as potential upstream controllers of APE-regulated genes (Figure 1D). At the protein level, APE-treated moDC did not produce any measurable IFN-α, and they did not significantly increase IL-6 production (Figure S1C, D). Thus, our results indicate that APE treatment of moDC promotes a pro-inflammatory prone status at the transcriptional level, but has mild effects on the expression of pro-inflammatory cytokines at the protein level.

To investigate whether APE treatment enhances pro-inflammatory cytokine responses upon virus infection, we utilized the β-herpesvirus human cytomegalovirus expressing the fluorescent marker GFP (HCMV). This virus is known to infect various different subsets of the myeloid cell lineage, including moDC.[4] Upon exposure of moDC with HCMV together with APE (APE + HCMV) (Figure 2A), protein secretion of IFN-α and IL-6 was significantly enhanced compared to moDC treated with HCMV alone (Figure 2B, C). Interestingly, pre-incubation with APE for 24 h followed by HCMV exposure (preAPE + HCMV) did not have an impact on IFN-α production, whereas it still augmented IL-6 expression (Figure 2A, D, E and S2A, B). Notably, upon the various treatments, moDC did not produce detectable levels of anti-inflammatory cytokines, such as IL-10, TGF-β, or IL-23 (Figure S2C). Thus, APE treatment of moDC promotes a pro-inflammatory milieu upon HCMV infection.

To address whether APE affects viral infection, we quantified percentages of GFP expressing moDC by flow cytometry. Upon exposure of moDC to APE + HCMV, percentages of GFP-positive moDC, that is, cells that support viral gene expression, were significantly enhanced (Figure 2F, G) and the release of viral progeny was increased (Figure S2E). PreAPE + HCMV treatment further increased the percentage of GFP-positive moDC and the amount of viral progeny (Figures 2F, G, S2D, F). Thus, APE-driven changes in gene signatures of moDC provide a favorable environment for HCMV infection and replication, despite an enhancement in pro-inflammatory cytokine responses.

NF-κB signaling, which was induced by APE treatment of moDC, was reported to enhance HCMV infection.[5] Pharmacological inhibition of NF-κB by BMS-345541 (BMS) treatment reduced percentages of GFP expressing moDC after HCMV exposure (Figure 2H, upper panel), suggesting that NF-κB signaling is important for efficient HCMV infection of moDC. Moreover, BMS treatment prevented the increase of GFP expressing cells in APE + HCMV treated moDC, but not in preAPE + HCMV treated moDC when compared with HCMV treatment alone (Figure 2H, lower panel). Thus, our results indicate that APE + HCMV treatment augments HCMV infection in an NF-κB-dependent manner.

Warmer temperatures and expanding urbanization increase the release of birch pollen into the air and also enhance the amount of immune stimulatory mediators contained in pollen.[3] This in turn could increase the risk of herpesvirus infection and reactivation in sensitized and non-sensitized individuals. A recent study demonstrated that HCMV and Epstein–Barr virus were the two most abundant viruses in the lung of asthma patients and that the presence of these viruses correlated with the severity of the disease.[6] As asthma patients are especially sensitive to the effects of pollen, the pollen-induced enhancement of HCMV infection that we report here might have even more severe implications for such high-risk patients than for healthy individuals.

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CONFLICT OF INTEREST

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