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Ozone alters the allergenicity of *Ambrosia artemisiifolia* pollen in a dose-dependent manner

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ABSTRACT

Climate change is causing the spread of common ragweed across Europe, along with rising ground-level ozone (O_3) in semi-urban and rural environments. Although elevated O_3 has been shown to affect pollen-intrinsic allergenic compounds, here we addressed the lack of *in vivo* evidence on how ozone-exposed ragweed pollen modulates allergic responses. Ragweed plants were grown in controlled plant growth chambers under control (40 ppb) or elevated (80 ppb and 120 ppb) O_3 levels. Aqueous pollen extracts (RWE) from control- or O_3-exposed plants were administered *in vivo* in a murine model for allergic airway inflammation (AAI) and employed in a human *in vitro* system of monocyte-derived dendritic cells (DCs), key initiators of the allergic response. Adjuvant factors and metabolites in control- and O_3-RWE were investigated using ELISA and untargeted metabolomics. Compared to control-RWE, 80 ppb O_3 induced a statistically significant enhancement of few AAI parameters, whereas 120 ppb O_3 yielded statistically significant dampening effects on AAI. On the same line, in human DCs isolated from atopic donors, RWE O_3 80 ppb slightly increased, while RWE O_3 120 ppb decreased the expression of maturation markers. Metabolomic profiling revealed pronounced, dose-dependent shifts in pollen primary and secondary metabolites, with moderate stimulation of pro-inflammatory lipid- and protein-derived mediators at 80 ppb, whereas higher O_3 levels (120 ppb) induced metabolic degradation resulting in reduced lipid and protein pro-allergenic compounds. Overall, ozone altered ragweed pollen allergenicity in a dose-dependent manner

Abbreviations: AAI, Allergic airway inflammation; BAL, Bronchoalveolar lavage; DC, Dendritic cell; LPA, Lysophosphatidic acid; LPS, Lipopolysaccharide; LTB4, Leukotriene B4; PALMs, Pollen-associated lipid mediators; PGE2, Prostaglandin E2; RWE, Aqueous ragweed pollen extract; Th2, T helper type 2 cell; Treg, Regulatory T cell.

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through plant physiological responses to oxidative stress, highlighting the interaction between air pollution and plant physiology in shaping pollen allergenicity in a changing climate.

1. Introduction

Ambrosia artemisiifolia (common ragweed) is an herbaceous plant native to North America that has recently been invading Europe as a neophyte. (Storkey et al., 2014) Allergy to ragweed, which manifests with clinical symptoms such as allergic rhino-conjunctivitis and asthma (Jones et al., 2019), is expected to increase due to the plant's intrinsic invasive capacities, climate change, and air pollution. (Lake et al., Mar 2017; Rauer et al., Jun 2021) Ground-level ozone (O_3) is a harmful secondary air pollutant and a major component of "photochemical smog", generated by reactions involving UV radiation, NO_x and volatile organic compounds. Daily average O_3 concentrations over the mid latitudes of the Northern Hemisphere range between 25 and 55 ppb, whereas Mediterranean rural sites are generally higher and expected to increase in future. (Cooper et al., 2014) Its annual average levels, characterized by seasonal cycles, often exceed the EU Air Quality Directive (2008/50/EC). (Eea, 2022) Also, in sunny days characterized by favorable weather conditions, concentrations above 120 ppb are often reached. (Cooper et al., 2014)In addition to the direct effects of O_3 exposure on human respiratory health (Russo et al., Apr 2025), the indirect effects of O_3 affecting the allergenic potential of pollen have also been considered. The impact of O_3 may be concentration-dependent and strongly varies among plant species. (Ribeiro et al., 2014)Whereas *in vitro* fumigation of *Phleum pratense* pollen with 100 ppb O_3 for 4 h causes decreased IgE recognition of allergens due to allergen loss (Rogerieux et al., 2007), in *Betula* pollen ambient O_3 concentrations up to 80 ppb were shown to correlate with an increased amount of the main allergen Bet v 1 and with its allergenicity. (Beck et al., 2013)On the same line, studies in perennial ryegrass and rye crops showed that the allergen amount was upregulated by increased O_3 exposure. (Eckl-Dorna et al., Dec 2010; Masuch et al., Aug 1997) In contrast, ragweed allergen content appears less sensitive to O_3 exposure. In fact, no difference in the expression of the main allergen Amb a 1, a member of the pectate lyase family (Bordas-Le Floch et al., 2015), was found upon O_3 fumigation of ragweed plants up to 80 ppb or upon direct *in vitro* fumigation of ragweed pollen with 100 ppb O_3 for 5 h/day for 7 consecutive days. (Kanter et al., 2013; Pasqualini et al., Oct 2011) Also, the growth and reproduction of ragweed seem insensitive to increased O_3 levels up to 63 ppb. (Ziska, Nov 2002) Yet, pollen allergens alone are not able to trigger allergic responses, but they rather need a plethora of substances contained in pollen grains (e.g. lipid mediators) which act as adjuvants in the development of allergic reactions. (Pointner et al., 2020)Studies in Betula pollen showed that ozone pollen uptake affects its lipid fraction. In ragweed, exposure to O_3 was shown to induce oxidative stress, as evidenced by oxidation of wall components and perturbation of redox homeostasis, and to stimulate the release of pollen-intrinsic NAD(P)H oxidase, suggested to boost the allergenicity of pollen. (Kanter et al., 2013; Pasqualini et al., Oct 2011; Boldogh et al., Aug 2005) Data on the *in vivo* allergenicity of pollen produced from plants subjected to elevated O_3 concentrations are still lacking. In this study, we therefore aimed to elucidate whether exposure of ragweed plants to increasing O_3 levels affects their capacity to induce allergic airway inflammation (AAI). We used pollen collected from ragweed plants exposed to O_3 levels of 40 ppb (Sicard et al., 2017; Vingarzan, 2004) (control) and to elevated O_3 concentrations of twofold (80 ppb) and threefold (120 ppb) the control level during the main phase of vegetative growth and pollen production, using phytotron chambers described previously. (Kanter et al., 2013) The selected O_3 concentrations (40, 80, and 120 ppb) represent a gradient spanning typical background levels, which result in no accumulated exposure above 40 ppb (AOT40 = 0), to elevated concentrations observed during summer pollution peaks. The highest treatment (120 ppb) represents an upper-end exposure scenario applied to capture potential threshold responses of an ozone-tolerant plant species and to investigate mechanistic effects under enhanced ozone stress, resulting in cumulative exposure exceeding the UN-ECE AOT40 critical threshold for vegetation. For this purpose, we used a well-described, adjuvant-free mouse model of AAI (Wimmer et al., Aug 2015) and an established human *in vitro* system of primary dendritic cells (DCs), which are professional antigen presenting cells bridging innate and adaptive immune responses and play a crucial role in allergic sensitization. Human monocyte-derived DCs were chosen as they can provide more statistical power and complementary information on dose–response effects, which cannot easily be obtained from *in vivo* experiments. Moreover, DCs were previously shown to be sensitive to pollen NADPH oxidases. (Rauer et al., Jun 2021; Csillag et al., 2010).

The results of this study show that plant stress physiology must be considered when predicting how O_3 impacts the allergenicity of ragweed pollen, with implications for understanding future allergy risks under rising ozone levels.

2. Methods

Exposure of ragweed plants to increasing O_3 *concentrations*.

Ragweed plants were cultivated as described in Kanter et al. (Kanter et al., 2013) They were grown from seeds to plants in standard soil (Floradur®, Bayerische Gärtnerei Genossenschaft, München, Germany) in walk-in exposure chambers, the "ExpoSCREEN" phytotron in Germany. (Roy et al., Apr 2021) An automated tube system watered the plants daily with 100 mL of water per pot. During the study period, the climatic conditions were simulated on an average seasonal course between May 1st and September 15th for the Munich region. (Kanter et al., 2013) In particular, in the exposure chambers, the day/night temperatures were maintained between 20 and 30°C/10-20°C, with a relative humidity ranging from 30 to 50% to 80–85% (day/night). Light periods were 14.5–16 h daily, providing approximately 500 μmol m^{-2} sec^{-1} PPFD (Photosynthetic Photon Flux Density) with a realistic simulation of climate and solar radiations of UV–Vis-NIR throughout the day (Roy et al., Apr 2021). After 4 weeks of acclimation, plants were either fumigated with ambient O_3 concentration (40 ppb) (Sicard et al., 2017) or enriched O_3 levels (80 or 120 ppb) to simulate cumulative O_3 stress throughout the main phase of vegetative growth and pollen production for *circa* 12.5 weeks. These concentrations reflect typical ambient O_3 levels (~40 ppb) that result in an AOT40 (accumulated ozone exposure above 40 ppb, when irradiation is > 50 W m^{-2}) (Agathokleous et al., 2020) of 0 ppm·h, compared to elevated, yet realistic peak concentrations (80–120 ppb) observed in Europe and North America during favorable photochemical conditions, such as midday summer peaks. (Schultz et al., 2017) Over the exposure period, the estimated AOT40 under elevated O_3 levels was ~ 35–42 ppm·h at 80 ppb, and ~ 70–84 ppm·h at 120 ppb. O_3 exposures occurred continuously during daylight, representing a simplified regime that did not account for diurnal and seasonal variability. (Schultz et al., 2017) Pollen was collected as described before, (Kanter et al., 2013) and continuously from July until the end of August using a modified ARACON system (BETATECH, Ghent, Belgium) covering the male inflorescences, and then stored at −80°C until use. To conduct the murine *in vivo* experiments, aliquots of pollen from various plants within the same exposure conditions were pooled to obtain the required pollen amount for the sensitization protocol. For the *in vitro* model, pollen was collected from single plants. For both the murine *in vivo* and human *in vitro* allergy models, aqueous ragweed pollen extracts (RWE O_3 80 ppb, RWE O_3 120 ppb, and respective controls (ctrl.) RWE O_3 40 ppb) were prepared at a concentration of 10

mg pollen/ml PBS as previously described. (Wimmer et al., Aug 2015).

2.1. Murine sensitization model

Female BALB/c mice aged 6 to 7 weeks were obtained from Charles River (Sulzfeld, Germany). They were housed in pathogen-free conditions in individually ventilated cages (VentiRack, BioZoneGlobal Ltd, Ramsgate, UK) and provided with a standard diet and water *ad libitum.* (Wimmer et al., Aug 2015) Experiments adhered to federal guidelines for the use and care of laboratory animals and were approved by the Government of the District of Upper Bavaria and the Animal Care and Use Committee of the Helmholtz Munich (Approval # 55.2–1-54–2532-156–12, ROB-55.2-2532.Vet_02-18-94).

After one week of acclimation, an adjuvant-free ragweed sensitization protocol was performed as previously described. (Wimmer et al., Aug 2015) In short, mice received 11 consecutive daily intranasal instillations (10 μL/nostril) of RWE O_3 80 ppb, RWE O_3 120 ppb, or respective control RWE O_3 40 ppb (RWE ctrl.). The total protein amount administered was approximately 33 μg. A negative control group received PBS (10 μL/nostril) using the same protocol. Airway hyperresponsiveness was measured 24 h after the final instillation (day 12) in the 80 ppb group only. All mice were sacrificed 24 h after the final instillation (day 12, Fig. 1A, 3A) and bronchoalveolar lavage (BAL) was performed as previously described. (Wimmer et al., Aug 2015) Blood samples were taken before the first instillation (day 0) and at sacrifice. Lung tissue and/or cervical lymph nodes were prepared for flow cytometric analysis of inflammatory and antigen-presenting cells and lung tissue for histology.

2.2. Human blood cell donors and DC stimulations

Isolation, culture, and stimulation of primary cells for this study were approved by the ethical committee of the Medical Faculty of the Technical University Munich (ethics statement code: 54/17 S) and the consultative commission of the Augsburg University Medical School (ethics statement code: 2016–7). Blood samples of healthy non-atopic and atopic donors were collected after written informed consent. Atopy status of blood was determined by measuring total serum IgE and allergen specific IgE by serum ImmunoCAP (Thermo Fisher Scientific). Non-atopic donors were defined by total IgE < 100 kU/L and negative specific IgE against common aeroallergens (trees, timothy grass, mugwort, ragweed, house dust mite, fungal spores, cat). An overview of the cell donors' characteristics is presented in Table S2. Day 5 immature DCs, generated by culture from magnetic activated cell-sorted human $CD14^+$ monocytes, (Gilles et al., 2011) were stimulated with RWE O_3 80 ppb or RWE O_3 120 ppb, or respective RWE ctrl. (RWE O_3 40 ppb), using untreated cells as unstimulated controls.

For detailed information on the analysis of pollen extracts, including metabolomics, *in vivo* and *in vitro* experiments, see online supplementary methods.

2.3. Statistical analysis

Statistical significance of the *in vivo* data was tested by unpaired one-way ANOVA or by two-way ANOVA with Tukey's post-hoc test. *In vitro* data were analyzed by one-way ANOVA (paired) or paired mixed-model ANOVA with post-hoc pair-wise comparisons (RWE ctrl. vs RWE O_3 80 ppb or 120 ppb). Metabolites in Figs. 6 and 7 (A-D) were analyzed by unpaired Mann-Whitney test. Analysis and graphs were performed in GraphPad Prism 8.4.1. Results were considered statistically significant if $p < 0.05$.

For metabolomic analysis, all variables were mean-centered and unit variance (UV)-scaled prior to multivariate analysis. Principal Component Analysis (PCA) was initially employed as an unsupervised exploratory method to assess data structure, identify potential outliers, and visualize sample clustering. Subsequently, three Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) models were generated to identify metabolites that significantly discriminate among the following group comparisons: (1) (40 ppb O_3) ctrl. 1 vs 80 ppb O_3; (2) (40 ppb O_3) ctrl. 2 vs 120 ppb O_3; (3) a three-class model including a control pool (1 + 2), 80 ppb, and 120 ppb O_3 samples. For each model, Variable Importance in Projection (VIP) scores and loading values were extracted to identify the metabolites that contributed most strongly to group separation. Model performance was evaluated by five iterations of 5-fold cross-validation. To assess potential overfitting, cross-validated analysis of variance (CV-ANOVA) was performed. The quality of each model was summarized by the proportion of explained variance in the response variable (R^2Y) and the cumulative predictive ability (Q^2cum). In parallel, univariate analyses were conducted using t-tests or Wilcoxon rank-sum tests (when data were not normally distributed), with Benjamini-Hochberg false discovery rate (FDR) correction. Results were summarized (Table S4), and significant metabolites were visualized by volcano plots and heatmaps in R. Enrichment of specific compounds annotated by the 'Multidimensional stoichiometric compound classification' (MSCC) approach (see Supplementary methods) was evaluated by hypergeometric testing using the R function '*phyper*', with right-tailed p-values for overrepresentation and left-tailed for underrepresentation, corrected by FDR. Significant metabolites identified through both multivariate and univariate analyses were visualized using volcano plots, highlighting those with both high fold change and low adjusted p-values. Additionally, different Heatmaps were generated to illustrate the relative abundance patterns of discriminatory metabolites across groups, thereby revealing metabolite classes that were upregulated or downregulated in specific conditions. The metabolomic data were analyzed using SIMCA (version 13.0.3, Umetrics AB) and RStudio (2024.12.1 Build 563, © 2009–2025 Posit Software, PBC).

3. Results

3.1. Doubling ambient O_3 levels on growing ragweed plants enhances only a few parameters of pollen-induced AAI in vivo

To evaluate the effects of RWE 80 ppb O_3 on AAI, we used an established allergy mouse model (Wimmer et al., Aug 2015) (Fig. 1A) and compared the response to RWE 40 ppb ctrl. Eleven daily intranasal instillations with RWE O_3 80 ppb induced a mild BAL cellular infiltration, similar to the one elicited by RWE ctrl.; only BAL lymphocyte numbers were significantly increased in the RWE O_3 80 ppb-treated group compared to both PBS and to its control 40 ppb O_3 ($p < 0.01$, Fig. 1B). Additionally, RWE O_3 80 ppb displayed a significant increase in total serum IgE compared to day 0 ($p < 0.05$). In contrast, in RWE ctrl. total IgE was only slightly increased (Fig. 1C, top). Amb a1-specific IgG_1 levels were only slightly increased, and to a similar extent in both RWE groups compared to PBS control (Fig. 1C, bottom). Along the same lines, histopathological scores of PAS-stained lungs (Fig. 1D-G) and measurements of airway hyperresponsiveness (Fig. 1H) revealed a statistically significant increase in both RWE-treated groups compared to PBS control, with no differences between the two RWE groups. Flow cytometric analysis of lung tissue retrieved 24 h after the last intranasal instillation revealed a similar, slight increase in eosinophils in both RWE groups, in line with the BAL data (Fig. 2A). Since dendritic cells are considered key sentinels of the immune system (Pointner et al., 2020; Liu et al., Nov 2021), we evaluated percentages of dendritic cells in the lungs of the three experimental groups. We detected an increased rate of $CD11b^+$ DCs in RWE O_3 80 ppb compared to RWE ctrl. ($p < 0.05$; Fig. 2B, left) and a mild, non-significant increase in plasmacytoid DCs in both RWE groups compared to PBS (Fig. 2B, right). FACS data from cervical lymph nodes showed only slight variations in the percentage of Tregs and in both DC populations in the three experimental groups (Fig. 2C, D). Lastly, the level of the pro-inflammatory cytokines IL-2, IL-17A and TNF-α in BAL supernatant was generally low but similarly increased in both RWE-treated groups compared to PBS (Fig. 2E). Taken

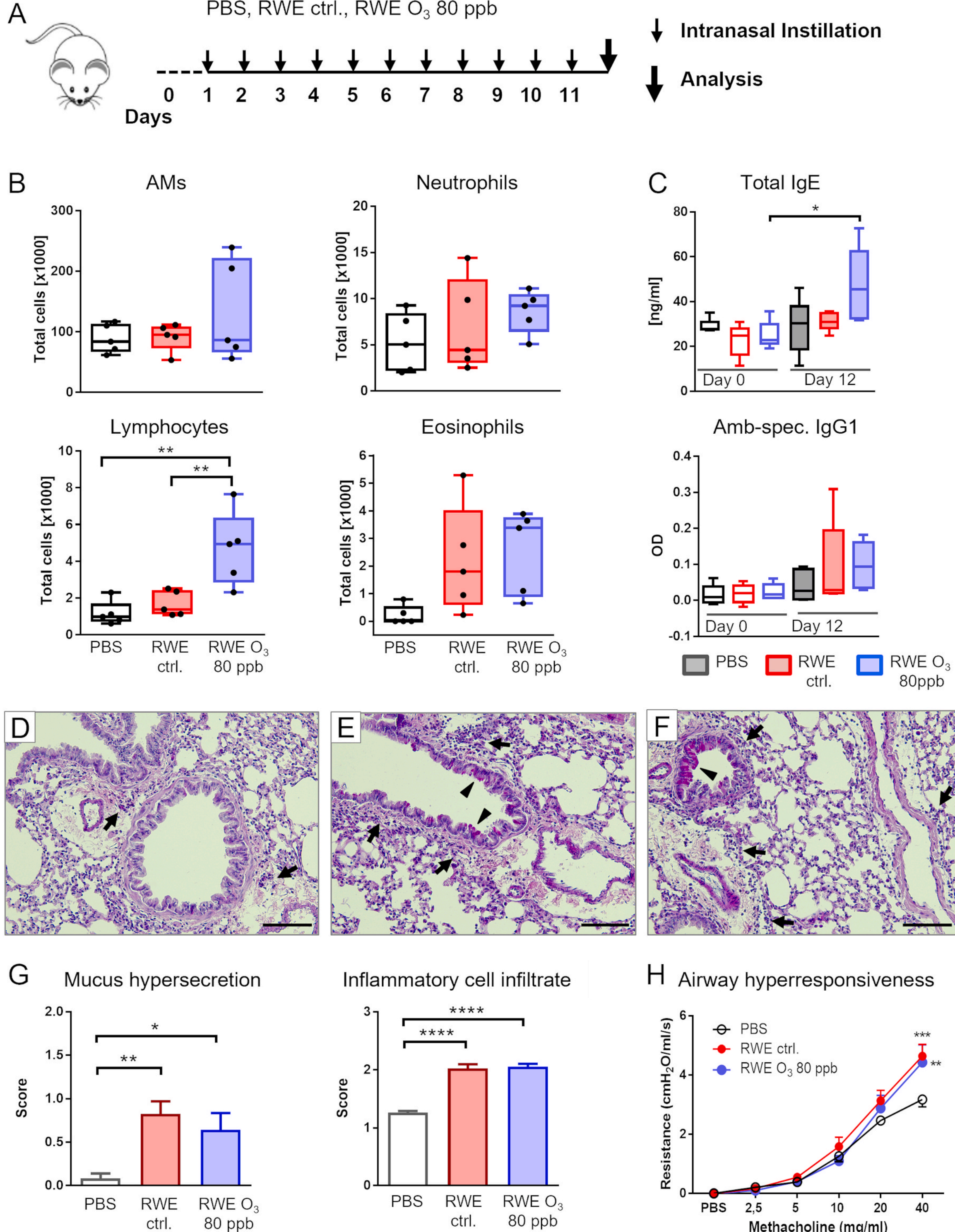


Fig. 1. *Pollen of ragweed plants grown under 80 ppb O_3 levels has slightly enhancing effects on allergic inflammation in vivo*. Female BALB/c mice received 11 daily intranasal instillations of PBS, RWE O_3 80 ppb or respective control RWE O_3 40 ppb and were analyzed on day 12. (A) Experimental setup. (B) BAL cell analysis. (C) Total IgE levels (top) and Amb-spec. IgG1 levels (bottom) were measured in mouse sera at day 0 and at the end of the experiment. (D-F) Representative PAS-staining of lung sections from mice instilled with the following: (D: PBS, E: control-RWE O_3 40 ppb, F: RWE O_3 80 ppb). Arrows: inflammatory infiltrate; arrowheads: mucus hypersecretion; scale bar: 100 µm. (G) Histological scores. (H) Airway hyperresponsiveness was measured 24 h after the final intranasal exposure. A-H: n = 5 mice/group; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ vs PBS at the same methacholine concentrations for airway hyperresponsiveness, or as indicated. Representative data of two independent experiments; one-way ANOVA or two-way ANOVA [for (C) and (H)] with Tukey's post-hoc test. AMs, alveolar macrophages.

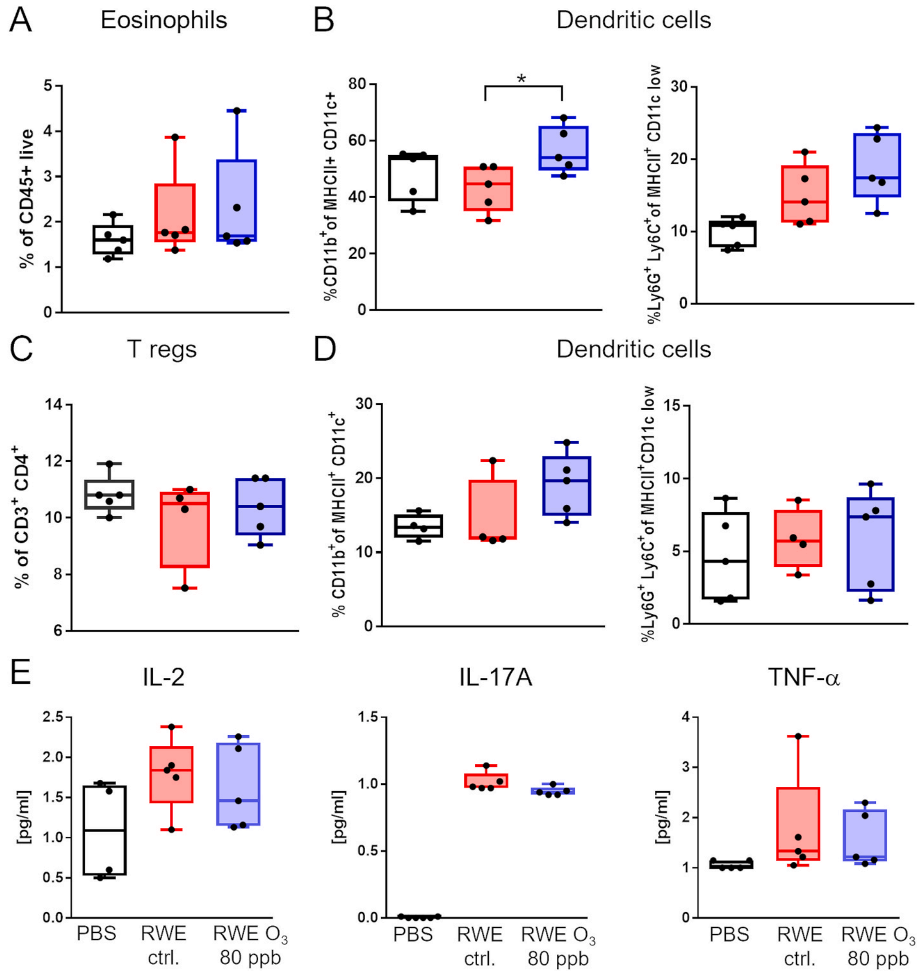


Fig. 2. *Pollen of ragweed plants grown under 80 ppb O_3 levels has slightly enhancing effects on allergic inflammation in vivo.* Female BALB/c mice received 11 daily intranasal instillations of PBS, RWE O_3 80 ppb or respective control RWE O_3 40 ppb and were analyzed on day 12. (A) Flow cytometric analysis of eosinophils and dendritic cells in lung (A-B) and of regulatory T cells (T regs) and dendritic cells in cervical lymph nodes (C-D). (E) Cytokine levels in BAL fluid. A-E: n = 5 mice/group; one-way ANOVA with Tukey's post-hoc test; $*p < 0.05$.

together, in our murine allergy model doubling ambient O_3 concentration up to 80 ppb had only minor effects on the allergic response compared to RWE control, as evidenced by a mild, although statistically significant increase in only some isolated parameters.

3.2. Tripling ambient O_3 levels on growing ragweed plants dampens pollen-induced AAI in vivo

We then assessed the impact of RWE O_3 120 ppb compared to RWE ctrl. in the same murine allergy model (Fig. 3A). RWE ctrl. group showed increased BAL lymphocytes and eosinophils compared to PBS ($p < 0.01$). Conversely, RWE O_3 120 ppb exhibited a reduced cellular response, with lymphocytes and eosinophil recruitment to the lung not significantly different from PBS (Fig. 3B). Serum total IgE levels displayed a statistically significant increase similarly in all groups at day 12 compared to day 0, but more strongly in RWE ctrl. (Fig. 3C, top). Accordingly, Amb a1-specific IgG_1 was significantly increased only in RWE ctrl. ($p < 0.05$) and not in RWE O_3 120 ppb (Fig. 3C, bottom). Histopathological analysis of PAS-stained lungs revealed a statistically significant increase in mucus hypersecretion and perivascular and peribronchiolar inflammatory cell infiltration in both RWE-treated groups compared to the PBS control. Nevertheless, the former parameter showed a statistically significant decrease in RWE O_3 120 ppb compared to RWE ctrl., while the latter decreased without reaching statistical significance ($p = 0.05$; Fig. 3D-G). Flow cytometric analysis of cervical lymph nodes retrieved 24 h after the final intranasal instillation showed decreased Treg percentages in RWE ctrl. ($p < 0.05$), while in RWE O_3 120 ppb Treg percentages were similar to those in the PBS group (Fig. 4A). Furthermore, RWE ctrl. lymph nodes showed a statistically significant higher percentage of $CD11b^+$ DCs, whereas in lymph nodes from RWE O_3 120 ppb $CD11b^+$ DCs were only slightly elevated compared to PBS (Fig. 4B, left). Similarly to the results obtained with RWE O_3 80 ppb (Fig. 2B, right), plasmacytoid DCs were only slightly increased in both RWE-treated groups compared to PBS (Fig. 4B, right). Lastly, analysis of pro-inflammatory cytokines IL-2, IL-17A and TNF- α in BAL supernatant revealed a slight increase of IL-2 and IL-17A in RWE ctrl. compared to PBS but not in RWE O_3 120 ppb (Fig. 4C). TNF-α levels were unchanged across all groups (Fig. 4C). Overall, tripling ambient O_3 levels during ragweed plants growth dampens the pollen-induced AAI.

3.3. Evaluation of the inflammatory response profile in human dendritic cells

To translate our murine findings to humans, we employed a previously established *in vitro* model based on human monocyte-derived DCs (Rauer et al., Jun 2021) and examined how increasing concentrations of the different RWEs analyzed in murine experiments affected DC's surface marker expression and cytokine and chemokine secretion. In DCs isolated from non-atopic donors, both RWE O3 80 ppb and RWE O_3 120 ppb promoted weaker maturation marker induction compared to their

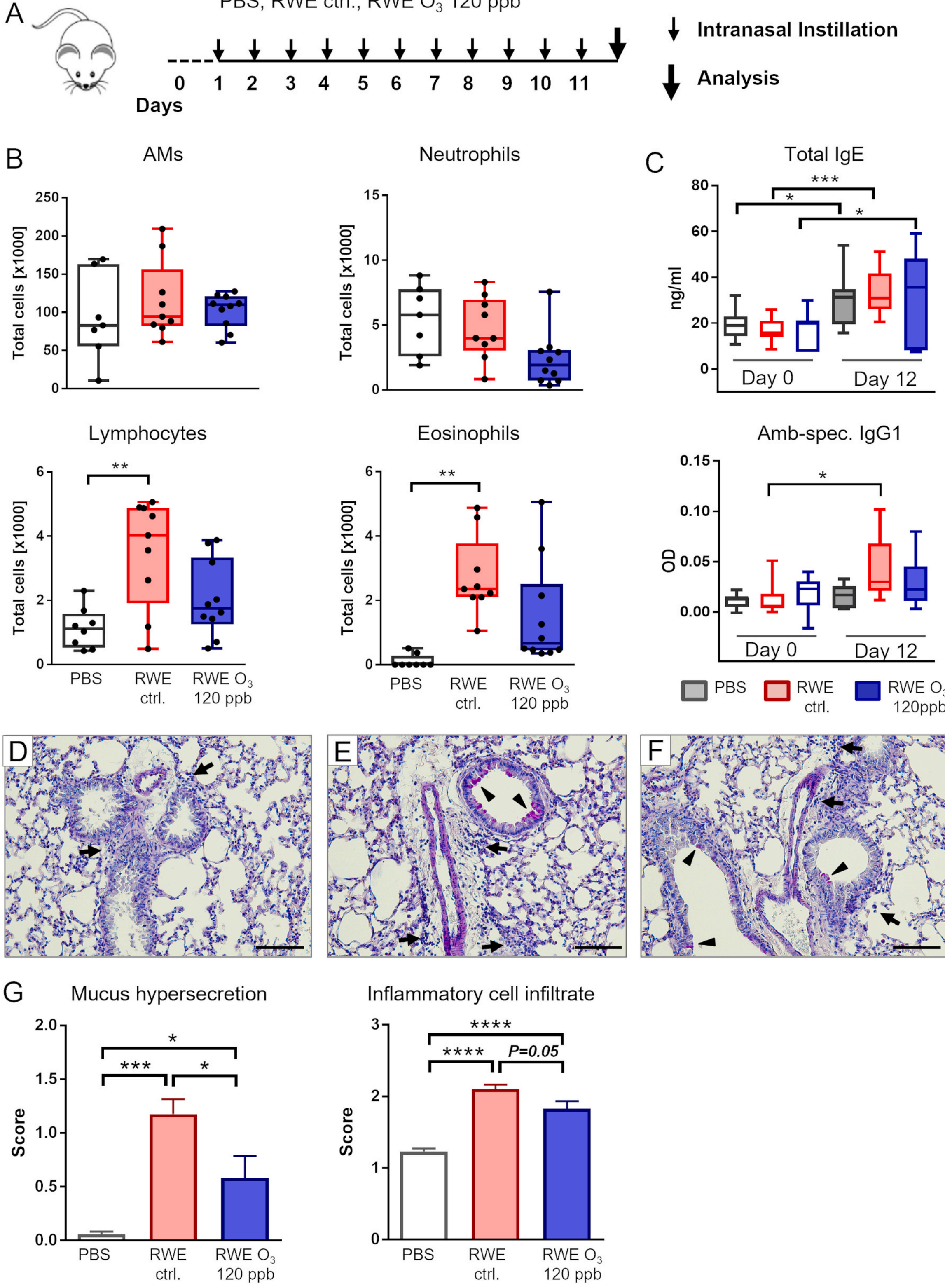


Fig. 3. *Pollen of ragweed plants grown under 120 ppb O_3 levels has dampening effects on allergic inflammation in vivo.* Female BALB/c mice received 11 daily intranasal instillations of PBS, RWE O_3 120 ppb or respective control RWE O_3 40 ppb and were analyzed on day 12. (A) Experimental setup. (B) BAL cell analysis. (C) Total IgE levels (top) and Amb-spec. IgG1 levels (bottom) were measured in mouse sera at day 0 and at the end of the experiment. (D-F) Representative PAS-staining of lung sections from mice instilled with the following: (D: PBS, E: control-RWE O_3 40 ppb, F: RWE O_3 120 ppb). Arrows: inflammatory infiltrate; arrowheads: mucus hypersecretion; scale bar: 100 μm. (G) Histological scores. B-C: n = 7–10; G: n = 5 mice/group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ as indicated; one-way ANOVA or two-way ANOVA [for (C)] with Tukey's post-hoc test. AMs, alveolar macrophages.

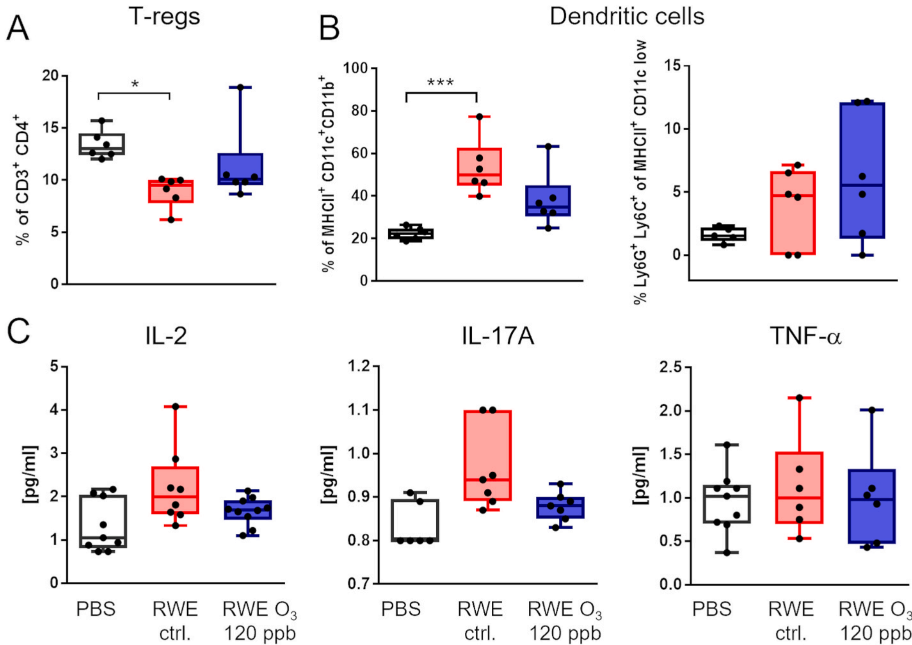


Fig. 4. *Pollen of ragweed plants grown under 120 ppb O_3 levels has dampening effects on allergic inflammation in vivo.* Female BALB/c mice received 11 daily intranasal instillations of PBS, RWE O_3 120 ppb or respective control RWE O_3 40 ppb and were analyzed on day 12. (A-B) Flow cytometric analysis of regulatory T cells (T regs) and dendritic cells in cervical lymph nodes. (C) Cytokine levels in BAL fluid. A-B: n = 6; C: n = 6–10 mice/group; one-way ANOVA with Tukey's post-hoc test; $^{*}p < 0.05$, $^{***}p < 0.001$.

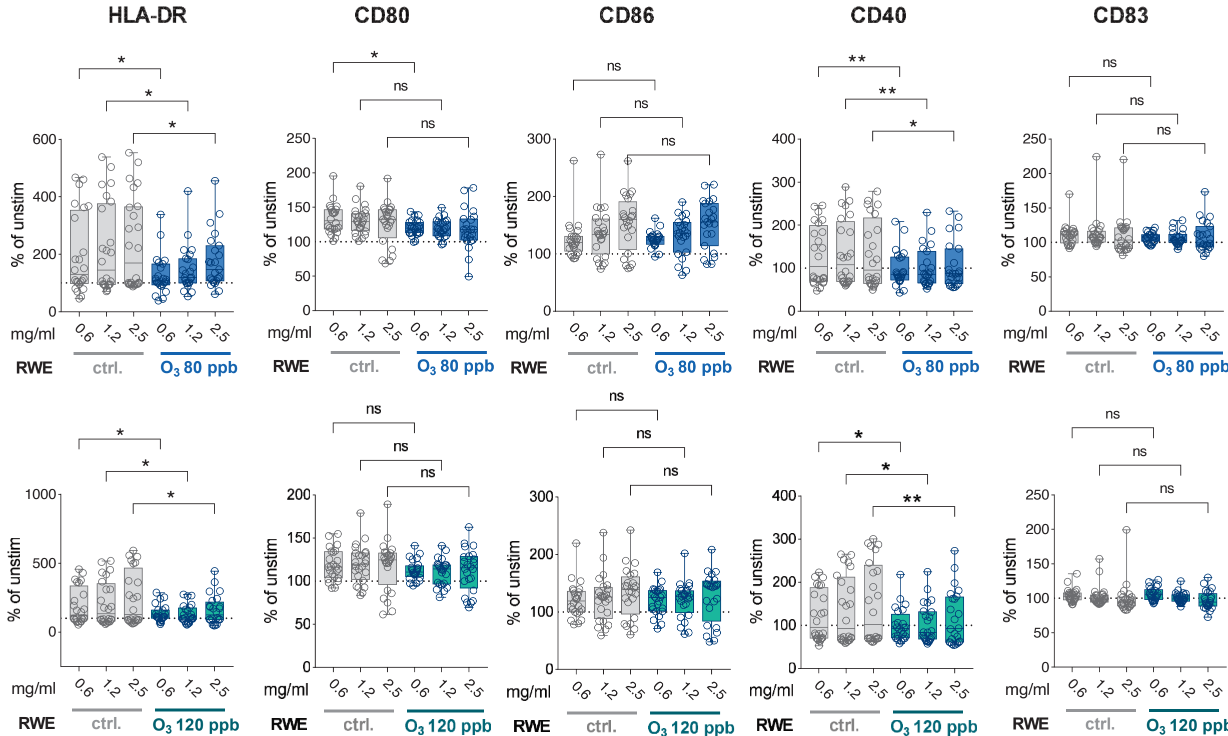
respective RWE ctrls. Specifically, CD40 expression decreased at all concentrations ($p < 0.05$; $p < 0.01$) and CD80 only at the lowest concentration for 80 ppb O_3 ($p < 0.05$), (Fig. 5A). HLA-DR expression was also weaker in response to both RWE O_3 80 ppb and RWE O_3 120 ppb compared to the respective RWE ctrls. ($p < 0.05$), (Fig. 5A). In contrast, DCs from atopic donors showed different results after stimulation with RWE O_3 80 ppb and RWE O_3 120 ppb compared to their respective RWE ctrls. Indeed, RWE O_3 80 ppb increased CD40 expression, although only at the lowest concentration, compared to RWE ctrl. ($p < 0.05$), while RWE O_3 120 ppb slightly decreased CD40 expression, though this was not statistically significant (Fig. 5B). Moreover, CD80 expression, which did not differ between RWE O_3 80 ppb and RWE ctrl., showed a statistically significant decrease in DCs stimulated with RWE O_3 120 ppb compared to RWE ctrl. (Fig. 5B). No differences in IL-10, IL-6 or TNF-α expression were found after DC stimulation with the RWEs, regardless of the O_3 treatment regime (Fig. S3).

3.4. Pollen extract composition reveals a few candidate substances that may contribute to altered allergenic potential of O_3-exposed ragweed pollen

Sensitization to pollen results from complex interactions between the host innate immune system and pollen allergens, as well as pollen-derived adjuvants. (Pointner et al., 2020) To determine which pollen component might mediate the O_3-induced modulatory effects on ragweed pollen, we first analyzed the major allergen Amb a1 in RWE. Similarly to the total protein content in RWE, the level of Amb a 1 in RWE was not significantly influenced by O_3 fumigation (Fig. 6A, 7A), although there was a trend of higher Amb a 1 level in RWE O_3 120 ppb compared to its RWE ctrl. (Fig. 7A). In addition to allergens, pollen grains release lipid mediators which possess pro-inflammatory and immunomodulatory properties and play a role in the allergic sensitization. (Pointner et al., 2020; Wimmer et al., Aug 2015; Traidl-Hoffmann et al., May 2002; Traidl-Hoffmann et al., 2005) RWE O_3 80 ppb had significantly higher pollen-associated lipid mediator (PALM)-leukotriene (LT)B_4 concentration than its RWE ctrl. ($p < 0.05$), whereas neither PALM-prostaglandin (PG)E_2 nor adenosine concentrations differed between the RWEs (Fig. 6B). Conversely, the lipid mediator concentrations did not differ between RWE O_3 120 ppb and its RWE ctrl. (Fig. 7B). Due to the importance of endotoxin in pollen-induced allergic sensitization (Oteros et al., 2019), we also quantified lipopolysaccharide (LPS) in the pollen samples, and no differences in the LPS content were observed between all RWEs used in this study (Fig. 6C, 7C). Lastly, since O_3 is a phytotoxic air pollutant known to induce oxidative stress in both, foliage and pollen (Pasqualini et al., Oct 2011; Langebartels et al., 2000; Zhao et al., 2017), we measured pollen intrinsic NADPH oxidase activity in our samples. Whilst 80 ppb O_3 did not cause an increase in NADPH oxidase, a slight increase in NADPH oxidase was observed in pollen from plants fumigated with 120 ppb O_3 compared to their respective ctrls. (Fig. 6D, 7D). Conversely, O_3 fumigation even at 120 ppb did not affect pollen viability (45.1 ± 13.7 vs 40.7 ± 22.1 of the respective ctrl. 40 ppb, mean ± SD, n = 8–9 plants).

To comprehensively explore shifts in primary and secondary metabolites in ragweed pollen in response to O_3 fumigation, we performed untargeted metabolomics of RWEs. Across all pollen samples, we detected 2,505 metabolite-related mass features, which we classified into chemical classes using the MSCC approach (Table S3). (Rivas-Ubach et al., 2018) This classification method, based on elemental composition, can accurately group compounds (>98%) into broad categories, including carbohydrates, lipids, protein-related compounds (i.e., amino acids and small peptides), secondary metabolites, amino sugars, and nucleotides. PCA and OPLS-DA analysis revealed significant differences in pollen metabolomes across O_3 exposure levels ($R^2Y(cum) = 1$; $Q^2(cum) = 0.79$ and CV-ANOVA, $p = 0.022$), particularly between the 80 ppb and 120 ppb treatments and their respective ctrls. (Fig. S4).

To gain further insight into metabolites underlying the altered allergenic potential, we initially focused on those metabolites that differed between pollen samples of plants exposed to 80 ppb or 120 ppb O_3 compared to their respective 40 ppb ctrls. (OPLS-DA, VIP > 1.5; $R^2Y(cum) = 1$; $Q^2(cum) = 0.87$ and CV-ANOVA, $p = 0.012$ for 80 ppb and $R^2Y(cum) = 0.96$; $Q^2(cum) = 0.65$ and CV-ANOVA, $p = 0.071$ for 120 ppb respectively; Figs. 6-7 E). Cluster heatmap analysis showed that RWE O_3 80 ppb replicates formed a distinct cluster from 40 ppb ctrls. (Fig. 6E). This separation was caused by the upregulation at 80 ppb of



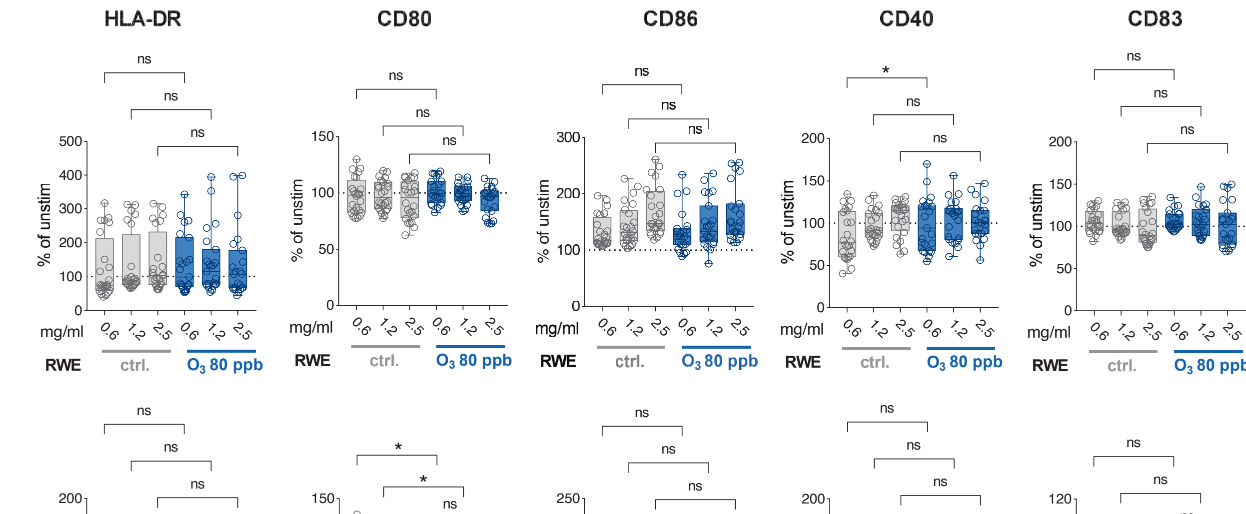


Fig. 5. *Pollen of ragweed plants grown under elevated O_3 levels induce mild alterations in maturation markers in human monocyte-derived DCs.* Monocyte-derived DCs were isolated from peripheral blood mononuclear cells from non-atopic (A) or atopic (B) donors, stimulated for 24 h with pollen extracts and analyzed for maturation markers by flow cytometry. n = 36–42 independent experiments using cells from 13 different donors (7 non-atopic; 6 atopic); one-way ANOVA (paired) or Mixed Model ANOVA (paired) with post-hoc pair-wise comparisons (Ctrl. vs. O_3); dashed lines: unstimulated control. *$p < 0.05$, **$p < 0.01$.

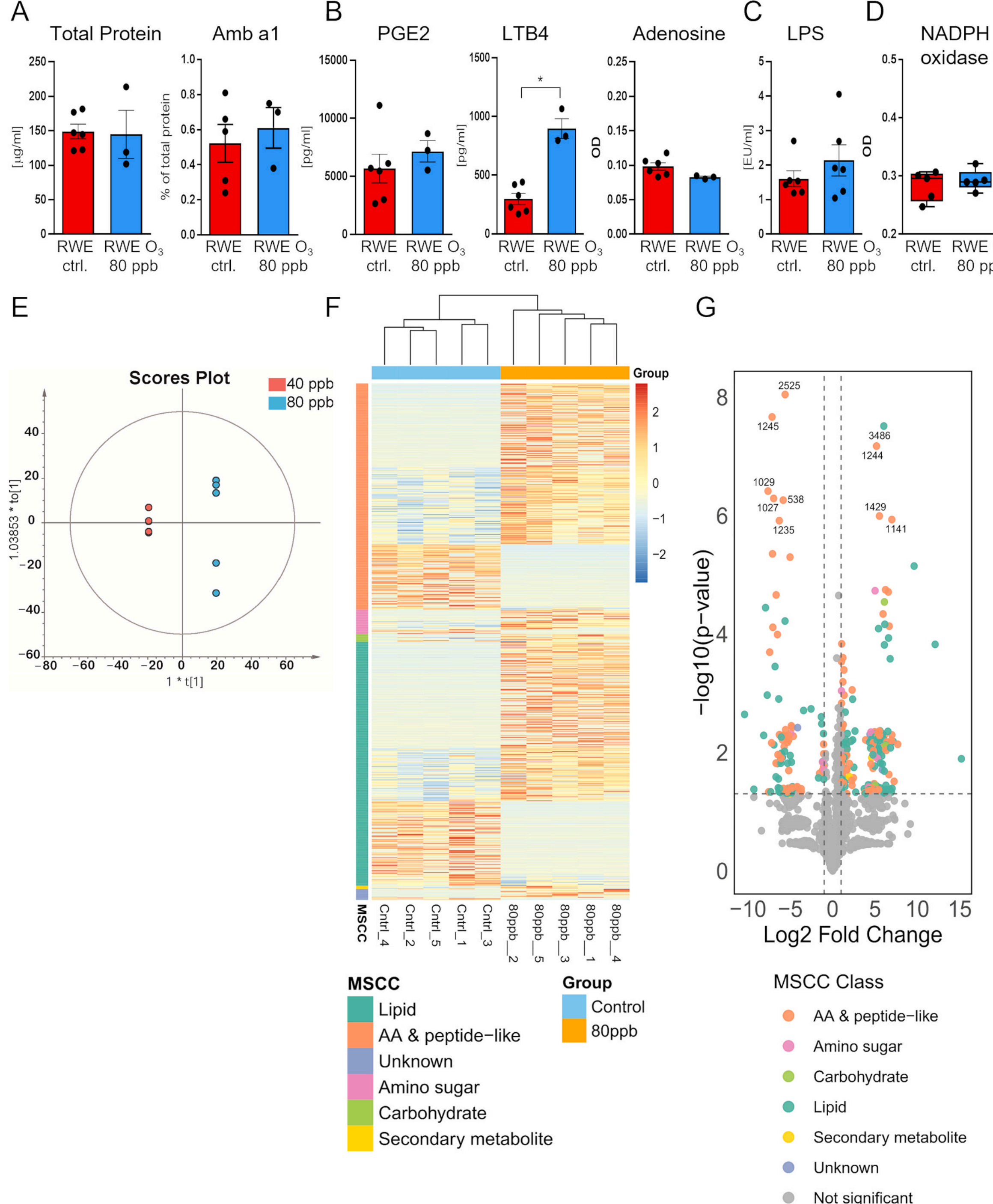


Fig. 6. *Metabolome analysis of RWEs reveals differentially expressed clusters of compounds in RWE O_3 80 ppb.* (A-D) Total protein and Amb a 1 concentration, PGE2, LTB4, adenosine, LPS and NADPH oxidase measured in extracts of single plants (n = 3–6); unpaired Mann-Whitney test. (E) OPLS score plot of RWE O_3 80 ppb vs respective RWE O_3 40 ppb ctrl. (F) Heatmaps of metabolites in RWE O_3 80 ppb vs respective ctrl. Rows represent individual metabolites, annotated by the multi-dimensional stoichiometric compound classification (MSCC); columns represent biological replicates. Metabolite intensities were *z*-score normalized; colors indicate the relative abundance with blue (−2) and red (+2) corresponding to 2 SD below and above the mean, respectively. MSCC classes are color-coded as indicated in the legend. (G) Volcano plot of metabolite changes between RWE 80 ppb O_3 and respective ctrl. Each point represents a metabolite. Significant metabolites (|log10 fold change| > 1 and $p < 0.05$) are colored according to MSCC. Non-significant metabolites are in grey. The top 10 significant metabolites are labelled by ID (see Table S4). PGE2, prostaglandin E2; LTB4, leukotriene B4; LPS, lipopolysaccharide. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

two major metabolite groups: (i) protein-related compounds (285 features), including amino acids (e.g. doubling levels of tryptophan, ID = 39; IDs refer to Table S4) and several di- and tripeptides (Table S4); (ii) lipids (282 features), including C_{18} oxylipins such as the putatively identified 12(13)Ep-9-KODE (ID = 581) and α-dimorphecolic acid (syn. 9S-HODE; ID = 657). However, despite their large absolute number (282), lipids were significantly underrepresented in RWE at 80 ppb relative to total detected features (hypergeometric test, FDR-adjusted p = 0.046, Table S5). Among the top 30 metabolites ranked by p-value and log fold change ratios, 10 lipids and 17 protein-related compounds were

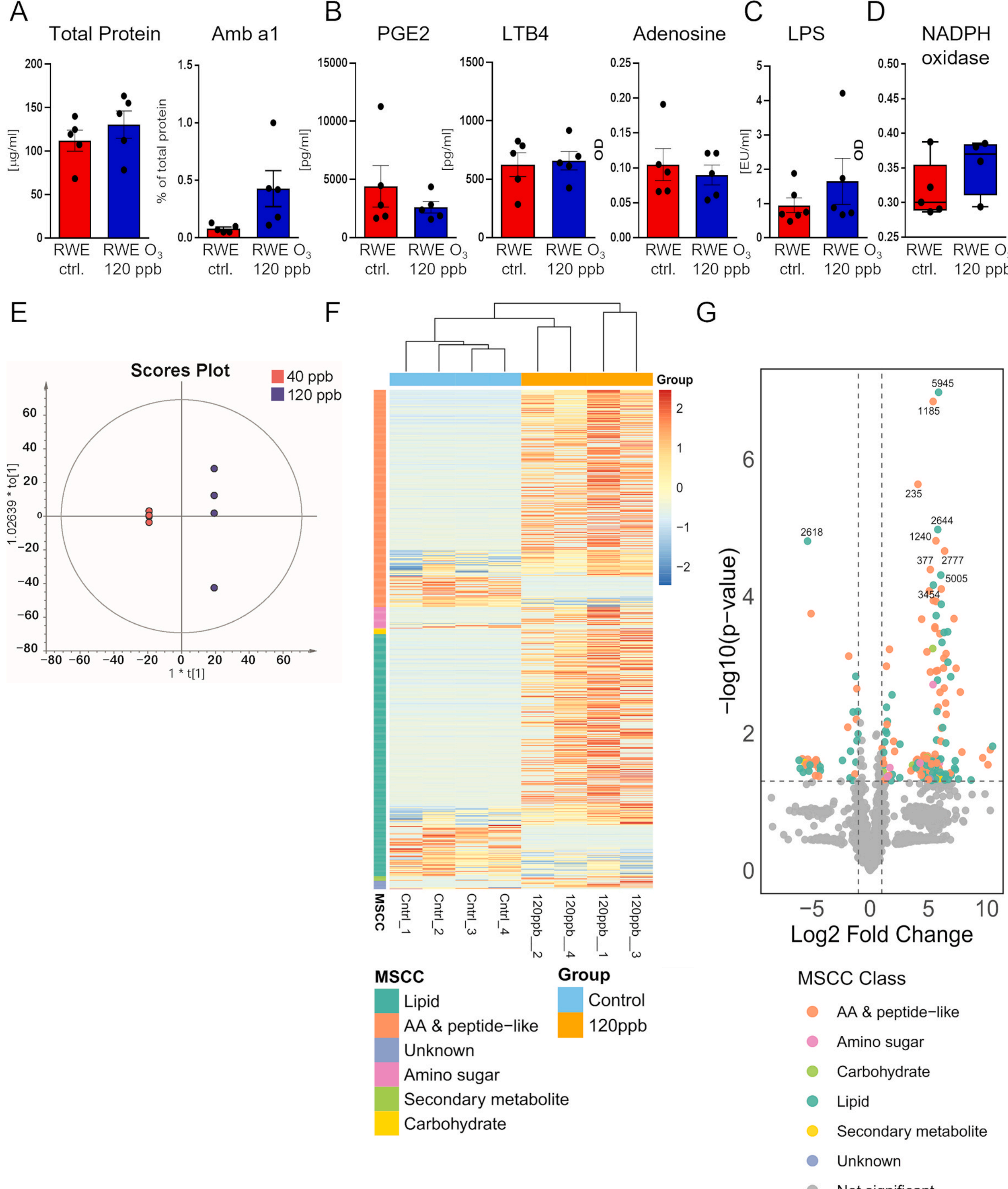


Fig. 7. *Metabolome analysis of RWEs reveals differentially expressed clusters of compounds in RWE O_3 120 ppb.* (A-D) Total protein and Amb a 1 concentration, PGE2, LTB4, adenosine, LPS and NADPH oxidase measured in extracts of single plants (n = 4–6); unpaired Mann-Whitney test. (E) OPLS score plot of RWE O_3 120 ppb vs respective RWE O_3 40 ppb ctrl. (F) Heatmaps of metabolites in RWE O_3 120 ppb vs respective ctrl. Rows represent individual metabolites, annotated by the multi-dimensional stoichiometric compound classification (MSCC); columns represent biological replicates. Metabolite intensities were *z*-score normalized; colors indicate the relative abundance with blue (−2) and red (+2) corresponding to 2 SD below and above the mean, respectively. MSCC classes are color-coded as indicated in the legend. (G) Volcano plot of metabolite changes between RWE O_3 120 ppb and respective ctrl. Each point represents a metabolite. Significant metabolites ($|\log10$ fold change$| > 1$ and $p < 0.05$) are colored according to MSCC. Non-significant metabolites are in grey. The top 10 significant metabolites are labelled by ID (see Table S4). PGE2, prostaglandin E2; LTB4, leukotriene B4; LPS, lipopolysaccharide. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

upregulated, while 8 lipids and 15 protein-related compounds were downregulated (Fig. 6F, Table S4).

At 120 ppb, multivariate analyses revealed a larger overall shift in the pollen metabolome relative to ctrls., as seen by increased dendrogram distances and a higher number of significantly altered metabolites (Fig. 7E-F, Table S4). This shift was characterized by widespread changes affecting both lipids and protein-related metabolites. Compared with ctrls., exposure to 120 ppb resulted in upregulation of 186 features, with roughly equal representation of lipid- and protein-related compounds, whereas only 54 features were upregulated at 80 ppb. Notably,

the number of downregulated features remained comparatively low and similar between exposure levels, indicating a general accumulation of metabolites as O_3 levels increased. Specifically, comparing 120/40 ppb to 80/40 ppb, more lipids (80 vs. 19) and protein-related metabolites (93 vs. 26) were upregulated under 120 ppb O_3, whereas downregulated features were fewer and comparable between 120/40 ppb vs. 80/40 ppb (lipids: 15 vs. 8; protein-related: 14 vs. 15).

When directly comparing metabolites significantly altered at 80 ppb vs. 120 ppb and relative to ctrls. (OPLS-DA; Fig. S4), the O_3 levels altered the composition of unsaturated lipids (Table S4). Fourteen unsaturated lysophosphatidic acid (LPA) derivatives showed significant changes (Benjamini-Hochberg adj. $p < 0.05$, ANOVA), 13 of which were significantly higher at 80 ppb than at 120 ppb, particularly LPA O-19:2 (LogFC = 6.5, adj. $p < 0.01$; ID = 2432) and LPA 27:1 (LogFC = 6.3, adj. $p < 0.01$; ID = 4380). Consistent with the observed enhanced oxidative stress at higher O_3 level, oxygenated lipid and small-molecule derivatives increased at 120 ppb O_3 ('120vsCTRL (UP)', Table S4). These included hydroxy- and oxo-containing lipid species such as ID 1832, a hydroxy-substituted octadecanoid derivative, and ID 1015, putatively annotated as a hydroxy-acyl carnitine, both of which are commonly associated with lipid oxidation and oxidative stress. In parallel, metabolites reduced between 80 and 120 ppb relative to ctrls. ('80vs120_difCTRL(1 + 2) (DOWN)', Table S4) including moderately oxidized lipid mediators and redox-active compounds, notably ID 1268, an ω-hydroxy, keto-substituted degradation product, and ID 4868, a hydroxylated vitamin-D-related conjugate. The concurrent accumulation of highly oxygenated lipid derivatives and depletion of moderately oxidized and antioxidant-linked metabolites at 120 ppb indicated a shift from regulated oxidative signaling at lower O_3 exposure towards oxidative stress and metabolic exhaustion of lipid signaling pathways at higher exposure.

Consistent with enhanced oxidative pressure at 120 ppb, protein-related metabolites constituted the second major group affected, including amino acids and short peptides, likely reflecting increased protein turnover and redox buffering in response to O_3-induced damage. In parallel, several metabolites with antioxidant properties were enriched at 120 ppb, including tocopherol (vitamin E; ID = 2391), vitamin E-related compounds (ID = 652), cysteine-containing tripeptides, carotenoids, phenolics, and osmoprotectant sugars and amino acids such as proline and its derivatives (IDs = 77, 282). These changes indicated a compensatory antioxidant response that accompanied, but did not prevent, the accumulation of highly oxidized lipid derivatives at higher O_3 exposure. Notably, 3,4,4′-trihydroxypirardixanthin (ID = 4871), a resveratrol analogue with reported antioxidant and anti-inflammatory activity (Kohandel et al., Jan 2022), together with other flavonoids (IDs = 2530, 1224, 2492), was significantly upregulated at 120 ppb.

4. Discussion

Ground-level O_3, together with particulate matter and NO_x, is among the most harmful air pollutants, not only for human health, but also for plants, including crops. (Sicard et al., 2017; Lu and Yao, 2023) Notably, O_3 concentrations are generally higher in rural zones than in urban areas, mainly due to depletion by reactions with anthropogenic pollutants, especially nitrogen monoxide (NO), although the annual O_3 averages have been increasing with a faster rate at urban centers. (Paoletti et al., Sep 2014; Sicard et al., Dec 2018) By exposing plants to three O_3 concentrations during daylight throughout most of the plant growth period, we aimed to investigate the effect of increasing levels of O_3 on the allergenicity of Ambrosia. The investigated O_3 concentrations (40, 80, and 120 ppb) cover a range from typical ambient levels to elevated concentrations observed during pollution episodes and heatwaves in Europe and North America. (Schultz et al., 2017) In relation to critical levels for vegetation, cumulative exposure metrics such as the UN-ECE AOT40 threshold of 5 ppm·h are widely used to assess ozone risk under field conditions. While the 40 ppb treatment corresponded to no accumulated exposure above 40 ppb (AOT40 = 0 ppm·h), the elevated O_3 treatments resulted in cumulative AOT40 values of ~ 35–42 ppm·h at 80 ppb, and ~ 70–84 ppm·h at 120 ppb, both exceeding the UN-ECE threshold. For comparison, average AOT40 values in the Northern Hemisphere is ~ 30 ppm·h. (Schultz et al., 2017).

In a previous study, we demonstrated that doubling ambient CO_2 levels – which increases pollen production (Ziska and Rising, 2000) – also enhances the proinflammatory potential of pollen (Rauer et al., Jun 2021). Here, we demonstrated a dose-dependent effect of O_3 on RWE allergenicity in the interplay with the plant response. Our data showed that doubling O_3 concentrations during plant growth (from 40 to 80 ppb) slightly elevated few pollen-induced AAI parameters at a statistically significant level. Apart from an increase in total BAL lymphocytes and in the percentage of $CD11b^+$ DCs in lymph nodes compared to RWE control, all other parameters remained unchanged. Additionally, airway hyperresponsiveness was increased by both RWEs compared to PBS control, but did not differ between RWE O_3 80 ppb and RWE O_3 40 ppb. By contrast, tripling the ambient O_3 concentration up to 120 ppb clearly dampened the inflammatory response of the plants' pollen. This was reflected by reduced lung inflammatory infiltrate and BAL cytokines, as well as decreased mucus hypersecretion and serum immunoglobulin levels. In cervical lymph nodes, we also observed a slight decrease in the percentage of $CD11b^+$ DCs, paralleled by an increase in Tregs, which clearly indicates reduced Th2 responses, contrary to our previous results with elevated ambient CO_2 levels. (Rauer et al., Jun 2021).

CD40, CD80 and CD86 act as important costimulatory molecules on antigen-presenting cells for Th2 cell differentiation. (Li et al., Mar 2016; Suzuki et al., 2018) Direct stimulation of human DCs with RWE pollen extracts yielded different results in non-atopic versus atopic donors. Notably, only the results of the DC stimulations in atopic donors were in line with the mouse data presented in this paper. This is most likely because, similarly to the *in vivo* situation, DCs isolated from atopic subjects are already programmed towards an atopic status. (Smole et al., 2015) These results suggest that, while exposure of ragweed pollen to 120 ppb O_3 has no profound impact on its sensitizing potential, it may reduce its inflammatory potential during allergic recall responses.

In this work, we did not detect significant variations in the major allergen Amb a 1 following O_3 exposure at either concentration compared to control, in good agreement with previous publications (Kanter et al., 2013; Pasqualini et al., Oct 2011). Interestingly, an Illumina-based sequencing transcriptome study on O_3 fumigation of ragweed demonstrated that elevated O_3 concentrations up to 80 ppb induce mild stimulatory effects in various allergen transcripts, whereas O_3 concentrations of 120 ppb induce rather a downregulation of most detected pollen allergen transcripts, similarly to results obtained with grass pollen. (Zhao et al., 2017; Albertine et al., 2014) These studies support a dose-dependent effect of O_3 on allergenicity, which decreases once a species–specific threshold is exceeded, likely reflecting the plant's inability to counteract excessive oxidative stress. In accordance to this concept and with Pasqualini et al (Pasqualini et al., Oct 2011), we observe a slight increase in NADPH oxidase activity only in RWE O_3 120 ppb, although this was not significant compared to RWE ctrl. To identify substances responsible for the biological effects shown in this study, we first analyzed the pollen-derived lipid mediators, which are important modulators of pollen-induced immune response. (Pointner et al., 2020) Our findings show no alterations in PALM-PGE_2 and adenosine concentrations in RWE O_3 regardless of the exposure, but significantly higher levels of PALM-LTB_4 in RWE O_3 80 ppb compared to its ctrl., which may in part explain the slight inflammation-enhancing potential of 80 ppb O_3. (Beck et al., 2013; Gilles et al., 2009) Contrarily, PALM-LTB_4 was stable in RWE O_3 120 ppb.

To further deepen our analysis, we investigated the pollen metabolome. Our metabolomic analysis demonstrates that O_3 alters ragweed pollen chemistry in a dose-dependent and mechanistically relevant manner. At 80 ppb O_3, the pollen metabolome was enriched in protein-

related compounds and lipid mediators, including putative oxylipins and unsaturated LPA-like derivatives. Oxylipins are central components of plant oxidative stress signaling and are responsive to ozone exposure. (Kangasjärvi et al., 2005) LPA derivatives such as LPA O-19:2 and LPA 27:1 have been implicated in airway inflammation through G protein-coupled LPA receptors, which promote cytokine release and activate downstream inflammatory cascades in airway epithelium. (Zhao and Natarajan, Mar 2009; Ackerman et al., 2016) Similarly, oxylipins such as 12(13)Ep-9-KODE and 9S-HODE function as potent bioactive lipids, capable of shaping dendritic cell polarization, granulocyte recruitment, and epithelial damage. (Traidl-Hoffmann et al., May 2002; Gilles et al., 2009; Henricks et al., 1991; Mabalirajan et al., 2013) The presence of these compounds at 80 ppb O_3 is consistent with the modest increases in lung inflammatory cells and IgE observed *in vivo*, as well as with the dendritic cell responses observed *in vivo* and *in vitro* in atopic donors, suggesting that O_3 drives a lipid remodeling in the pollen, which may be responsible for AAI enhancement.

By contrast, tripling O_3 to 120 ppb triggered a more profound, yet qualitatively different metabolic shift. Although a larger number of lipid- and protein-related features were altered overall, the response was characterized by enhanced lipid oxidation and accumulation of small di- and tripeptides, consistent with metabolic depletion and increased protein turnover under oxidative overload. The response at 120 ppb likely reflects a threshold at which oxidative stress exceeds detoxification capacity, triggering damage-related processes resembling acute oxidative injury rather than purely cumulative or acclimatory responses. Indeed, the 120 ppb O_3 signature was dominated by oxidized lipid products (e.g., 13-OxoODE) and a limited set of metabolites with putative antioxidant or redox-buffering roles, including tocopherol- and carotenoid-related metabolites, cysteine-containing peptides, and proline derivatives. Collectively, these changes suggest activation of compensatory redox mechanisms rather than a fully effective antioxidant acclimation response. (Baier et al., 2005; Obata and Fernie, 2012) Nevertheless, various flavonoids and terpenes were still found upregulated at 120 ppb O_3. Flavonoids are polyphenolic secondary metabolites of plants involved in the oxidant stress response, directly scavenging reactive oxygen and nitrogen species. Different flavonoids have been reported to promote a tolerogenic DC phenotype by reducing pro-inflammatory cytokines and costimulatory molecules while increasing PD-L1 and IL-10 expression. (Liu and Jiao, 2025) Among the annotated flavonoids was quercetin, a well-known metabolite reported to inhibit DC activation by reducing TLR4, IRAK4, and NF-kB expression. (Kang et al., 2023) Mechanistically, this could explain the attenuated allergic phenotype *in vivo*: excessive oxidative damage to pollen at 120 ppb may have depleted or destabilized key pro-inflammatory metabolites (e.g., LPAs, oxylipins) in the pollen and concomitantly upregulated tolerogenic substances, thereby limiting the capacity of pollen to drive AAI.

Overall, our results are consistent with a non-linear, potentially biphasic response of O_3 effects on pollen allergenicity. Relative to the control condition (40 ppb), O_3 at intermediate levels (80 ppb) may contribute to allergenic potential by stimulating the production of bioactive lipid and protein-derived mediators with direct pro-inflammatory activity. At higher levels (120 ppb), once a plant-specific oxidative threshold may be exceeded, catabolic and degradative processes appear to prevail, resulting in a reduced release of pro-allergenic metabolites and an increased availability of tolerogenic mediators that dampen the immune response. However, this interpretation should be taken with caution, as the limited number of exposure levels does not allow a definitive characterization of the underlying relationship, which would require a broader O_3 gradient. However, O_3 exposure treatment in the phytotron allowed us to dissect the effects of ozone alone, but limit extrapolation to real-world conditions, where co-pollutants (e.g., NOx, PM, VOCs, and secondary aerosols) may interact chemically and biologically with O_3. These interactions can influence redox homeostasis, lipid peroxidation, and secondary metabolite biosynthesis. (Li et al., Sep 2017) Also, elevated CO_2 is known to reduce stomatal conductance (Paoletti and Grulke, Oct 2005), thus reducing O_3 uptake, and to influence the biosynthesis and emission of plant VOCs (Li et al., Sep 2017), thereby altering plant defense responses under future climate conditions with additional consequences for pollen allergenicity beyond O_3 effects alone. In addition, rising temperatures may act synergistically with O_3 to alter plant stress response under future climate, further affecting the allergenic potential of pollen. This important aspect warrants future studies. Nevertheless, our findings emphasize that both the O_3 dose and plant stress physiology must be considered when predicting how atmospheric oxidants will influence future pollen allergenicity under climate change.

Author statement

All authors accept the responsibility for the content of the manuscript and approve the final version of the manuscript.

Author contributions

FA: Study design and murine experiments, plotting and analysis of data, writing of the first draft of the manuscript, manuscript editing; SU, LM, DR: DC stimulation and analysis; ML, JU: Metabolome analysis and plotting of results; BS, MW: Murine experiments and FACS analysis; AE: Pollen extract preparation, measurement of pollen compounds; UF: Plant exposures; DE, JBW: Supervision of plant exposures; AD: Supervision of statistical analysis of *in vitro* experiments; LA: Analysis of pollen allergens; HB, CT-H: Study design, infrastructure, funding; FF, J-PS, PS-K, CBS-W: Infrastructure, funding; AG: Supervision of metabolome analysis, plotting and analysis of data, manuscript editing; SG: Study design, supervision and funding for *in vitro* experiments, manuscript editing. All: Review of manuscript.

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CRediT authorship contribution statement

Francesca Alessandrini: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization. **Swetlana Urban:** Writing – review & editing, Visualization, Investigation. **Marianna Lucio:** Writing – review & editing, Visualization, Investigation, Data curation. **Jenny Uhl:** Writing – review & editing, Visualization, Data curation. **Benjamin Schnautz:** Writing – review & editing, Methodology, Investigation. **Leopold Moelter:** Writing – review & editing, Investigation. **Denise Rauer:** Writing – review & editing, Investigation. **Maria Wimmer:** Writing – review & editing, Visualization, Investigation. **Annika Eggestein:** Writing – review & editing, Investigation. **Ulrike Frank:** Writing – review & editing, Supervision, Investigation. **Dieter Ernst:** Writing – review & editing, Supervision. **Jana Barbro Winkler:** Writing – review & editing, Supervision. **Athanasios Damialis:** Writing – review & editing, Supervision. **Lorenz Aglas:** Writing – review & editing, Investigation. **Fatima Ferreira:** Writing – review & editing, Resources, Funding acquisition. **Heidrun Behrendt:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Jörg-Peter Schnitzler:** Writing – review & editing, Resources, Funding acquisition. **Philippe Schmitt-Kopplin:** Writing – review & editing, Resources, Funding acquisition. **Carsten B. Schmidt-Weber:** Writing – review & editing, Resources, Funding acquisition. **Claudia Traidl-Hoffmann:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Andrea Ghirardo:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology. **Stefanie Gilles:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2026.110304.

Data availability

The data generated and analyzed in this study are included in this article and in its additional files.

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